SOME ASPECTS OF BIPOLAR HETEROTHALLISM
IN FOMES CAJANDERTI KARST.

by

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A Thesis Submitted to the Faculty of the
DEPARTMENT OF PLANT PATHOLOGY
In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE
In the Graduate College
THE UNIVERSITY OF ARIZONA

1970
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APPROVAL BY THESIS DIRECTOR

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Professor of Plant Pathology

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Date
ACKNOWLEDGMENTS

The author wishes to express her deepest gratitude for the invaluable guidance and counsel of Dr. Robert L. Gilbertson, Professor of Plant Pathology, University of Arizona, under whose direction this study was conducted. Thanks are also extended to Cliff A. Harris for his continuing interest and advice, and to Miss Adela Saucedo for her help in preparing the manuscript.
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ABSTRACT

Fomes caianderi Karst. is a widely distributed member of the Polyporaceae which causes a cubical brown rot on conifer logs and stumps. It was previously reported to be heterothallic, bipolar and to possess multiple allelomorphs at the locus for heterothallism.

In this research, studies of the sexuality of this fungus were carried out. Thirty-six representative monokaryons, isolated from 18 dikaryotic stocks that represented the North American distribution of the species, were used. Pairings between these 36 isolates were made. From the results it was possible to estimate the number of multiple allelomorphs at the locus for heterothallism, and roughly determine the randomness of their distribution. Monokaryotic and dikaryotic fruiting, and pairing reactions were also studied. In the latter category, the behavior of di-mon pairings was of special interest. Dikaryotization of the component monokaryon was found to occur in this work, but had previously been reported not to occur for this species.
INTRODUCTION

*Fomes cajanderi* Karst. is a heterothallic, bipolar basidiomycete belonging to the family Polyporaceae. This species is circumboreal in the coniferous forest regions of the northern temperate zone. It is commonly found on conifer logs and stumps, in which it causes a cubical brown rot.

The species was first demonstrated to be bipolar and to possess multiple allelomorphs at the locus for heterothallism over thirty years ago by Mounce and Macrae (1937) as an ancillary feature of their investigation of the genetic differences between this species and the closely related *F. roseus* (Alb. and Schw. ex Fr.) Karst. With the exception of some work on pairing reactions of genetically distinct mycelia (Adams and Roth, 1967), however, very little has been done since. Bipolar sexuality, in general, seems to have been largely ignored in recent years.

Therefore, in light of the need for further investigation, the primary purpose of this study was to elucidate in greater detail various aspects of the sexuality of *F. cajanderi*. Since clamp connections form regularly in the dikaryon and fruiting can be induced in the laboratory, this fungus was considered ideal for study as a representative bipolar species. Those areas which were studied include:

1) Confirmation of its bipolar nature and genetic distinction from *F. roseus*

2) Geographical distribution of the allelomorphs
3) Estimation of the number of allelomorphs at the locus for heterothallism

4) Dikaryotic and Monokaryotic fruiting

5) Pairing Reactions
Heterothallism in the Hymenomycetes was first reported by Ben-saude (1917) and Kniep (1920) working independently with Coprinus fimenterius and Schizophyllum commune, respectively. Both species were of the type termed tetrapolar by Kniep and had compatibility factors at two independently segregating loci. Bipolarity, possessing compatibility factors at a single locus only, was first reported by Vandendries (1923). Many members of the Hymenomycetes and Gasteromycetes are now known to be heterothallic. The relative distribution in these groups is approximately 55% heterothallic and tetrapolar, 35% heterothallic bipolar and 10% homothallic (Whitehouse, 1949a).

These figures may be misleading, however. A nonrandom factor favoring heterothallism is reflected in the available data. Being easier to work with, the fleshy polypores and agarics, which are mostly heterothallic, tend to be preferred experimental organisms. Studies of these species predominate in the literature. More recently, a detailed study of the resupinate Hymenomycetes (Boidin and Lanquetin, 1965) has shown the frequency of homothallism among a sample of 150 species in this group to be 26%. This is a much higher figure than that based on data taken primarily from the sources just mentioned. Whether or not this is true of other, less well known groups remains to be seen, but the question certainly deserves clarification.

Multiple allelomorphs at the loci for heterothallism were first discovered in S. commune (Kniep, 1920, 1922). Shortly thereafter,
Vandendries (1923) and others reported "multiple allelomorphs heterothallism" in several bipolar species. It is now commonly thought that all heterothallic species in the Hymenomycetes possess multiple factors at their compatibility loci.

Whitehouse (1949b), using the available data, estimated the number of allelomorphs at the loci for heterothallism in the heterothallic Hymenomycetes as a whole to be of the order of magnitude of 100 per locus. Raper, Krongelb, and Baxter (1958), thoroughly investigating a large worldwide sample of isolates of S. commune, obtained estimates for the number of allelomorphs at each locus which were in essential agreement with Whitehouse. No comparable study dealing with a bipolar species had as yet been undertaken prior to the work reported here. It should also be noted that the analysis of the data of Raper et al. differed from that of Whitehouse in that they used a method developed by Wright (1937) and previously applied to estimation of the number of self-sterility alleles in clover by Bateman (1947).

There has been some controversy in the past over the significance of the geographical distribution of compatibility factors. Whitehouse (1949b) felt that their distribution must be homogeneous throughout the population due to the extreme efficiency of spore dispersal in the Hymenomycetes. Indeed, Raper et al. (1958) found no evidence to the contrary in their analysis of a large world-wide sample of allelomorphs of S. commune. The topic undoubtedly deserves more intensive statistical and ecological study but, as it has been pointed out before, the enormity of such an analysis is almost prohibitive. Some simple observations on the subject have, nevertheless, been made in this study.
MATERIALS AND METHODS

Eighteen stock dikaryons were used in the greater part of this study, fifteen of which were obtained from the Forest Disease Laboratory, U. S. Forest Service, Beltsville, Maryland, and 3 of which were obtained from specimens in The University of Arizona Mycological Herbarium. These isolates represented the North American range of the species.

The following cultures of *F. cajanderi* were obtained from the Forest Disease Laboratory, U. S. Forest Service, Beltsville, Maryland.

Mad. LP-66--rot isolate from lodgepole pine (*Pinus contorta* Dougl.) pulpwod, submitted by Thilmany Paper Co., to Forest Products Laboratory, Madison, Wisc., received April 6, 1969.

MD-14--rot isolate from creosoted "southern yellow pine" utility pole in service in Ohio, collected by Vaughn, isolated at FPL by Catherine G. Duncan, Recd., April 8, 1954.


MD-350--rot isolate, western red cedar (Thuja plicata Donn) telephone pole in service 9 years at Ann Arbor, Michigan, isol. Catherine G. Duncan, Aug. 4, 1959. Madison isolate.


The following cultures of Fomes cajanderi Karst. were obtained from the University of Arizona Mycological Herbarium.

PDK-100--sporophore tissue isolate, on ponderosa pine, near Shuway, Coconino County, Ariz., coll. P. D. Keener.

RLG-6696--sporophore tissue isolate, on black spruce (Picea mariana (Mill.) B.X.P.), Whitecourt, Alberta, Canada, coll. R. L. Gilbertson, August 21, 1966.

The isolate of *Fomes roseus* 'Alb. et Schw. ex Fr.') Karst. was obtained from the following specimen.


All cultures were grown on 1.5% malt extract medium (Nobles, 1965) at room temperature.

Isolates were made from herbarium specimens in the following manner. The basidio carp was broken in two and the newly exposed surface flamed. Small cubes, approximately 2-3 mm³, were removed from the interior, flamed, and placed on agar slants. Extensive mycelium developed in 1-2 weeks.

It was found that, with most isolates, a dikaryon grown in a petri dish fruited in 3-4 weeks. Inversion of the petri dish resulted in a spore print being deposited on the lid. A non-quantitative dilution method was used to obtain single spore isolates. A drop of sterile, distilled water was placed on the spore print. A loop of the resulting spore suspension was removed and introduced into a tube 1/8 full of sterile distilled water. This generally produced a suitable concentration of spores. Three tubes of melted agar each received a loop of the dilute spore suspension and were poured into empty sterile petri dishes. Germination occurred in 3-4 days. To ensure that only one spore at a time was picked up, the plates were viewed under a dissecting scope while removing individual germinating spores in small blocks of agar with a scalpel. The blocks were placed on agar slants. An extensive monokaryotic mycelium developed within 14 days. Wet mounts in phloxine or cotton blue were
usually made of each single spore isolate to confirm, by the absence of clamp connections, its monokaryotic condition before use in further work.

Stock culture RLG 7942 was used to confirm the bipolar nature of *F. cajanderi* and its genetic isolation from *F. roseus*. Nineteen monokaryons were obtained and paired in all possible combinations (Fig. 1). These and all subsequent pairings were made in 60 x 15 mm plastic petri dishes, "over-filled" to prevent premature drying. Three to four days after the two mycelia had met (approximately 2 weeks), a wet mount of hyphae from the interface was made. The presence of clamp connections indicated a compatible mating and was designated by a "+' sign, and the absence of clamps, or an incompatible mating, was designated by a "-" sign. Except for RLG 7942, only 6 to 9 monokaryons were isolated from each dikaryotic stock. Pairings were made between these 6-9 monokaryons to distinguish the mating types of each stock. A series of 36 representative monokaryons was then obtained. It was then necessary to determine if there was any duplication of allelomorphs in the sample. To this end, representative monokaryons from every other stock dikaryon, and the outcomes scored "+' or "-" as was appropriate.

A series of di-mon pairings was made in the course of the investigation of pairing reactions. Stock dikaryon FP 103788 was paired with each of the representative monokaryons obtained from the other 17 stock dikaryons. The pairings were made in small plastic petri dishes, and incubated at room temperature for 3 weeks. At the end of this period, hyphae from near the mycelial margin of the monokaryotic component most distant from the dikaryotic mycelium was examined for the presence of clamp connections. This was done to preclude the possibility of having
invading dikaryotic hyphae originating from the component dikaryon present in the portion of the mycelium examined. Further, taking hyphae from the mycelial margin insured the examination only of growth occurring after possible dikaryotization, and, hence, only of that part of the original "monokaryon" expected to have clamps, if dikaryotization had occurred.

An investigation of possible fruiting induction by a species of *Penicillium* was carried out in the following manner. Sterile discs of cellophane were placed on the surface of malt extract medium in petri dishes. The *Penicillium* sp. was allowed to grow on the cellophane until it had covered a circle approximately 1 inch in diameter. The disc and fungal hyphae were then carefully removed and *F. cajanderi* was allowed to grow directly on the agar. The plates were then checked regularly for the production of fruiting structures. The isolates used in this experiment were obtained from fresh fruiting bodies on a dead Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) stump on Mount Lemmon, Coronado National Forest, Pima County, Arizona. They were part of a linear series of 14 isolates (ML-A through ML-N) made from fruiting bodies taken at one foot intervals on the stump, all of which had previously failed to fruit.
RESULTS

Confirmation of Bipolar Nature of \textit{F. caianderi}

Determination of the type of heterothallism that a given Hymenomycete species may possess is accomplished by a set of pairings. When several monokaryons derived from single spore isolates of the same stock dikaryon are paired in all possible combinations, the pattern of compatible and incompatible matings that is observed indicates whether the species is bipolar or tetrapolar. Homothallic species, of course, do not require this. A bipolar pattern is one in which all of the monokaryons fall into two groups on the basis of their sexual behavior. Each member of a group is incompatible with all other members of the same group, and compatible with all members of the other group. This, of course, is what would be expected from the segregation of compatibility factors at a single locus during meiosis and basidiospore formation in the dikaryon.

The results of the 171 pairings of nineteen monokaryons, from dikaryotic stock RLG 7942, in all possible combinations, were a typical bipolar pattern (Fig. 1), more easily seen after rearrangement into the two compatibility groups (Fig. 2). The allelomorphs represented by the two groups were designated 1 and 2, respectively. All pairs of allelomorphs similarly distinguished in later work were designated 3, 4; 5, 6; etc., in a like manner. These data confirmed what Mounce and Macrae (1937) had previously reported, that \textit{Fomes caianderi} was a heterothallic, bipolar species.
Fig. 1. Results of pairing 19 single spore isolate, obtained from stock dikaryon RLG-7942, in all possible combinations.
Fig. 2. Results as in Fig. 1, rearranged to show the two compatibility groups, a typical bipolar pattern.
Genetic Isolation of *F. cajanderi* and *F. roseus*

The genetic isolation of *F. cajanderi* and *F. roseus* was also investigated by Mounce and Macrae (1937), since the two species are quite similar morphologically and their distinction on this level had been somewhat inconclusive. Their work was repeated in this study with exactly the same results. Representative $A_1$ and $A_2$ monokaryons of *F. cajanderi* were each paired with eight monokaryotic isolates obtained from single spores of *F. roseus* stock dikaryon RLG-6954. All pairings were incompatible (Fig. 3). Since complete interfertility is the rule between monokaryons representing different mating types from different isolates of the same species, and some interfertility would be found even if both compatibility factors were duplicated in the isolate identified as *F. roseus* it is reasonable to assume, as did Mounce and Macrae, that data such as these represent pairings between monokaryons of two different species. In view of this, the fact that there was at least partial interfertility in all subsequent sets of pairings made between monokaryons isolated from different dikaryotic stocks, identified as *F. cajanderi*, was sufficient proof that no errors in identification had been made.

Geographical Distribution

As previously stated, to determine the number of compatibility factors present in the sample, it was necessary to pair the 36 monokaryotic isolates in all possible combinations. When the 630 pairings necessary to accomplish this were examined, nineteen incompatible pairings, or factor replications, were observed (Fig. 4). Out of a possible maximum of 36, 25 separate and compatible allelomorphs were found (Table 1). Of
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tr>
<td>$A_1$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_2$</td>
<td></td>
<td></td>
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Fig. 3. Results of pairing 8 single spore isolates, from *F. roseus* stock dikaryon RLG-6954 with two monokaryons of *F. cajanderi* (RLG-7942), carrying compatible mating factors, $A_1$ and $A_2$, respectively.
Figure 4. Results of mating the 36 monokaryotic isolates in the sample in all possible combinations.
Table I. Mating factors found in the sample.

<table>
<thead>
<tr>
<th>Stock</th>
<th>Isolate</th>
<th>Allelomorph</th>
<th>Location</th>
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<tbody>
<tr>
<td>RLG-7942</td>
<td>1</td>
<td>A₁</td>
<td>Arizona</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>A₂</td>
<td></td>
</tr>
<tr>
<td>RLG-8165</td>
<td>3</td>
<td>A₃</td>
<td>New Mexico</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>A₄</td>
<td></td>
</tr>
<tr>
<td>Flo-243-A</td>
<td>5</td>
<td>A₅</td>
<td>Maine</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>A₆</td>
<td></td>
</tr>
<tr>
<td>PDK-100</td>
<td>7</td>
<td>A₇</td>
<td>Arizona</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>A₈</td>
<td></td>
</tr>
<tr>
<td>Aho 71-23</td>
<td>9</td>
<td>A₉</td>
<td>Oregon</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>A₁₀</td>
<td></td>
</tr>
<tr>
<td>ME-34</td>
<td>11</td>
<td>A₁₀</td>
<td>California</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>A₅</td>
<td></td>
</tr>
<tr>
<td>RLG-6696</td>
<td>13</td>
<td>A₁₁</td>
<td>Alberta, Canada</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>A₁₂</td>
<td></td>
</tr>
<tr>
<td>MD-206</td>
<td>15</td>
<td>A₁₃</td>
<td>Wisconsin</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>A₁₄</td>
<td></td>
</tr>
<tr>
<td>MAD, 529</td>
<td>17</td>
<td>A₁₅</td>
<td>Wisconsin</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>A₁₆</td>
<td></td>
</tr>
<tr>
<td>MD-350</td>
<td>19</td>
<td>A₁₃</td>
<td>Michigan</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>A₁₆</td>
<td></td>
</tr>
<tr>
<td>MD-14</td>
<td>21</td>
<td>A₁₃</td>
<td>Ohio</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>A₁₄</td>
<td></td>
</tr>
<tr>
<td>FP-70798</td>
<td>23</td>
<td>A₁₇</td>
<td>Washington</td>
</tr>
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<td></td>
<td>24</td>
<td>A₇</td>
<td></td>
</tr>
<tr>
<td>FP-104407</td>
<td>25</td>
<td>A₁₈</td>
<td>Maryland</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>A₁₉</td>
<td></td>
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<tr>
<td>FP-71244</td>
<td>27</td>
<td>A₂₀</td>
<td>Wisconsin</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>A₂₁</td>
<td></td>
</tr>
<tr>
<td>MAD, LP-6</td>
<td>29</td>
<td>A₂</td>
<td>Wisconsin</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>A₂₂</td>
<td></td>
</tr>
<tr>
<td>MD-183</td>
<td>31</td>
<td>A₁₃</td>
<td>Oregon</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>A₂₃</td>
<td></td>
</tr>
<tr>
<td>FP-104132</td>
<td>33</td>
<td>A₂₄</td>
<td>Maryland</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>A₁₄</td>
<td></td>
</tr>
<tr>
<td>FP-103788</td>
<td>35</td>
<td>A₂₅</td>
<td>Maryland</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>A₁₃</td>
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</table>
these, one occurred five times, two occurred three times, three occurred twice, and the remaining 19 only once.

The geographical origins of the isolates which carried replicated compatibility factors were as follows:

**Occurring 5 times:**
- Wisconsin-Michigan-Ohio-Oregon-Maryland

**Occurring 3 times:**
- Maine-Oregon-Wisconsin
- Wisconsin-Ohio-Maryland

**Occurring twice:**
- Arizona-Washington
- Maine-California
- Arizona-Wisconsin

There was no obvious departure, either in the above listing or the accompanying map (Fig. 5), from expectation based on the assumption of random distribution of mating factors. A test for randomness was, nevertheless, made (Raper, et al., 1958).

The 36 monokaryotic isolates fell naturally into 2 groups that reflected a significant discontinuity in the species distribution; namely, the eastern and western coniferous forest regions. Although these two regions are actually continuous farther north, the central plains separates them for much of their length. In terms of effective spore dispersal, the overriding reason for initially hypothesizing random distribution, a significant ecological barrier would seem to be presented by the plains region. The proposed grouping is thus justified.
Fig. 5. Map showing the distribution of the 25 allelomorphs found in the sample. They are grouped in the pairs in which they originally occurred in the stock dikaryons.
The ratio of the number of replications to the total number of pairings of isolates from within a region was compared to the analogous ratio for pairings between isolates from different regions (Table 2). There was no difference in the ratios at the 5% level of significance. Therefore, the data did not contradict the assumption of random distribution.

**Estimation of the Number of Alleломorphs**

Knowing the frequency of factor replication in the sample, it is possible to estimate the number of factors in the population.

The general nature of the problem can be stated as follows: given a series of $n$ factors present in the population, and an experimental procedure of random pairing, what is the probability of getting an identical pair? This has been shown to be

$$p = \frac{1}{n} + n \frac{2}{p}$$

where $p$ is the probability of replication, and $\frac{2}{p}$ is the variance of the factor frequencies (Wright and Dobzhansky, 1941). The ratio of replications to the total number of pairs is equal to $p$ for a sufficiently large sample and an estimate of $n$ is thus possible, if $\frac{2}{p}$ is known.

In the present case, since there is no evidence to the contrary, it is assumed that the compatibility factors are present in equal frequency. Further, as has already been shown, the data do not contradict a hypothesis of random distribution of factors. Under these two assumptions, Wright's equation becomes

$$p = \frac{1}{n}$$

and a first approximation estimate of $n$ can be made.
Table II. Comparison of the frequencies of replications in pairings of isolates from within and between the Eastern and Western coniferous forest regions.

<table>
<thead>
<tr>
<th>Region</th>
<th>No. of pairings</th>
<th>No. of replications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-regional</td>
<td></td>
<td></td>
</tr>
<tr>
<td>East</td>
<td>160</td>
<td>10</td>
</tr>
<tr>
<td>West</td>
<td>120</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>280</td>
<td>11</td>
</tr>
</tbody>
</table>

\[ P_1 = \frac{11}{280} \]

<table>
<thead>
<tr>
<th>Region</th>
<th>No. of pairings</th>
<th>No. of replications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-regional</td>
<td></td>
<td></td>
</tr>
<tr>
<td>East-West</td>
<td>380</td>
<td>8</td>
</tr>
</tbody>
</table>

\[ P_2 = \frac{8}{380} \]

\[ P_1 = P_2 \text{ at 5% level of significance} \]
For a sequence of statistically independent experiments having two possible outcomes, one of which has a low probability, \( p \), of occurring, the probability that this event will occur \( K \) times out of \( N \) experiments is

\[
p = e^{-p} \frac{p^K}{K!}
\]

where \( \sim Np \) and \( \frac{p}{K} \) is moderate in magnitude. That is, the number of times that the improbable event occurs is Poisson distributed (Feller, 1957).

In this study, a sequence of statistically independent pairings was made. For each pairing there were two possible outcomes, compatibility or incompatibility; and the probability of an incompatible outcome was small \( (p=19/630) \). The number of events, \( N \), was 630, and \( \sim Np=19 \) was moderate. Thus the conditions are satisfied and the number of replications is Poisson distributed.

This means that, as others have noted (Bateman 1947, Stevens 1942), the use of the standard error to compute confidence limits is incorrect here. The fiducial limits employed by these authors are the appropriate one. The error of estimation is large as the calculations for this case demonstrate:

\[
p = 1 = 19 \quad \text{with 5\% limits of } 11.61 \text{ and } 29.51
\]

\[
n = 33 \quad \text{with 5\% limits of } 54 \text{ and } 21.
\]

It should be noted that the concepts of random pairing and statistical independence are somewhat strained here since there is an element of predictability inherent in the experimental procedure. That is, once a replication is observed, the outcomes of a certain number of other pairings become predictable events. However, since this lack of independence
is a function of the order in which the pairs are examined, it is con-
venient to consider all pairings as though they were independent. Any
attempt to eliminate this non-randomness would be experimentally prohib-
itive.

Dikaryotic Fruiting

The stock dikaryons used in this study were quite variable in
their vegetative characteristics. This variability was not restricted
to vegetative growth, however. Although distinct, well pigmented pore
surfaces developed in all but two cases, the entire fruiting structures
varied from flat, irregular "patches" to almost spherical outgrowths,
developing at the periphery of the mycelial mat (Fig. 6). Stocks MAD-
LP6 and FP 71244, were atypical in that the pore structure had degenerated
to small, circular, crust-like areas of exposed hymenium. There was
little or no pigmentation in either case, but FP 71244 produced numerous
droplets of thick pinkish exudate in the vicinity of the fruiting area.

There were several dikaryotic isolates on hand that never fruited,
but fruiting ability did not appear to be related to time in culture. In
fact, two stocks used in this study that fruited most readily, MAD 529 and
FP 71244, had been in culture since 1920 and 1936, or for periods of 49
and 33 years respectively.

Several isolates that had at first failed to fruit were noticed
to do so when contaminated with an unidentified Penicillium sp. This led
to consideration of the possibility that the contaminant induced dikaryo-
tic fruiting in F. cajanderi, a possibility suggested by the recent report
of a similar phenomenon involving monokaryotic fruiting in S. commune
Figure 6. Typical Dikaryotic fruiting in an $A_2 \times A_6$ dikaryon.
(Leonard and Raper, 1969). The cellophane disc technique was employed here. All four isolates (ML-C, ML-B, ML-E, & ML-F) used in this case had previously never fruited. Only one, ML-C, did fruit after being grown on the pretreated medium. These results were so inconclusive that, due to time limitations, the subject was pursued no further. However, it was observed that, in the one case where fruiting did occur, it occurred in a short period of time, about three weeks, and that general vegetative growth was more vigorous.

**Monokaryotic Fruiting**

Two of the 36 monokaryons obtained during the course of this investigation were found to produce monokaryotic fruiting structures. They were isolates 12 (A5) and 23 (A17). In both cases, subculturing did not attenuate this ability, and all transfers promptly fruited after sufficient mycelial development, or about one week. The other 34 monokaryons were never noted to fruit spontaneously after, in some cases, over a year in culture.

Of great interest was the fact that both fruiting isolates produced completely sterile fruiting structures. Repeated attempts were made to find basidia or basidiospores in sections of monokaryotic fruiting bodies of various ages, and there never appeared to be even a vestigial hymenium present. Further, inversion of several plates of both monokaryons for up to three weeks failed to result in the formation of a spore print.

Isolate 12 always produced copious amounts of a thick pinkish exudate on the pore surface, similar to that produced by stock dikaryon FP 71244 which was also abnormal in its fruiting behavior (Fig. 7).
Figure 7. Monokaryotic fruiting in A5 (ME-34). - Note drops of exudate.
A second situation where monokaryotic fruiting was noted to occur was in some pairings of incompatible isolates. These pairings involved monokaryons that had not fructed alone, and both members of the pair were always affected (Fig. 8). These fruiting structures also appeared to be quite sterile.

**Pairing Reactions**

During the course of this investigation it was noted that there were various macroscopic changes in the appearance of mycelia in both compatible and incompatible pairings. This made it possible to identify compatible and incompatible matings by visual inspection in many cases. However, these changes were seldom obvious before the second week of incubation. Incompatible pairings were found to be of two types; 1- macroscopically incompatible, and 2- macroscopically compatible. The former was characterized by the classical features of such pairings, observed by other workers in several species (Adams and Roth, 1967; Verral, 1937). A distinct line of demarcation formed between the two mycelia, accompanied by discoloration of the medium below the line and changes in growth and pigmentation of the immediately adjacent hyphae of both mycelia (Fig. 8, 10, 11). Expression of the aversion reaction was stronger with time. The second type, however, displayed little or no discoloration of the medium and the hyphae of the two mycelia appeared to intermingle freely with no signs of aversion or changes in gross morphology. Repeated microscopic examination of hyphae from the interface regions of pairings of this type as much as 30 days old failed to reveal the presence of clamp connections. It was, therefore, necessary to
Figure 8. Monokaryotic fruiting induction in two incompatible pairings -
a. (Left) A_{13} (MD-206) x A_{13} (MD-350); (right) MD-183-4 x MD-183-6, two single spore isolates of stock dikaryon MD-183.
b. Obverse of above, showing discoloration of medium along interface.
Fig. 9. Two sets of matings between single-spore isolates obtained from a single stock dikaryon. An incompatible pairing that was macroscopically compatible occurred in each set, without contradiction. 

a) MD-14-3x7 was macroscopically compatible; in 4x7 there was an aversion reaction. 

b) MD 350-2x5 was macroscopically compatible; an aversion reaction occurred in 3x5.
Figure 10. Matings of single-spore isolates of stock dikaryon MD-350.
Figure 11. Incompatible matings—(top) A₆ (Flo-253-A) x A₅ (Aho-61-23); (bottom) two plates of A₅ (Flo-243-A) x A₅ (ME-34) component is fruiting and the other is not. The irregular margin of the A₅ (ME-34) isolate resulted in an irregular line of demarcation.
conclude that these pairings were also incompatible. This finding was further strengthened by the fact that, in two cases, these pairings fitted without contradiction into bipolar mating patterns. They occurred in the course of pairing several single spore isolates from two stock dikaryons (MD-14 and MD-350) in order to distinguish the two mating types in each (Fig. 9 & 12). Their apparent incompatibility could be checked against the other outcomes in the resultant bipolar patterns. It should also be noted that discoloration does not always occur in aversion reactions in other species as noted in *Fomes pini* by Roth (1952).

Unless the macroscopic incompatibility reaction was quite strong, a wet mount of hyphae was still made for confirmation. This was due to the fact that, whereas most monokaryotic isolates of *F. cajanderi* had colorless hyphae regardless of the pigmentation of the parent dikaryon, many dikaryotic stocks developed pink or brown mycelia and/or caused discoloration of the medium. The compatible pairing of two unpigmented monokaryons often resulted in the formation of a pigmented or pigment producing dikaryons growing at the interface. This situation had the appearance of a weak incompatibility reaction.

It was impossible to consider fruiting structure production as a criterion for determination of interfertility. The two monokaryons that regularly produced monokaryotic fruiting structures in culture usually also did so in the course of a pairing, compatible or not (Fig. 11). Furthermore, pairing incompatible monokaryons, that normally did not form monokaryotic fruiting structures, sometimes induced both members of the pair to do so. This resulted in the superficially contradictory juxtaposition of a clear, heavily discolored, line of demarcation and large areas
Figure 12. Matings of single-spore isolates of stock dikaryon MD-14. The last plate in the bottom row is macroscopically compatible.
of pink pore surface on the same plate. To add to the confusion, compatible matings were not always followed by dikaryotic fruiting on the plate. Thus, this characteristic was valueless as a macroscopic indicator of compatible pairings.

Compatible matings, on the other hand, could sometimes be recognized by the gross morphology of the "component monokaryon-dikaryon-component monokaryon" complex. In these cases, wedges of the more vigorous dikaryotic mycelium growing out from the earliest point of contact of the component mycelia, occupied the interface region. This was only obvious when the rate of growth or mat appearance of the dikaryon differed significantly from those of its components. The phenomenon was most often noted when one of the component monokaryons had a very slow growth rate. This created a situation in which the newly formed dikaryon had more medium available for unhampered growth, allowing differences in gross morphology to be clearly expressed. Unfortunately, the more frequent case, a pairing of two relatively vigorous monokaryons, led to the plate being almost entirely covered by monokaryotic tissue before significant growth of the dikaryon could occur. Any otherwise visible differences in morphology were thus obscured.

After identification of the allelomorphs in the sample, the dikaryon constituent of the di-mon pairings was found to carry allelomorphs A₁₃ and A₂₅. Due to replications within the sample, pairing (A₁₃ × A₂₅) with each of the first 34 monokaryotic isolates implied that several redundant pairings were made.

Examination of hyphae near the mycelial margin of the component monokaryon, after three weeks of growth, revealed the presence of clamps
in all but four pairings (Fig. 13). Three dikaryotized monokaryons pro-
duced abnormal, contorted clamps. Of the three, two were \((A_{13} \times A_{25}) \times A_6\) pairings, and one was an \((A_{13} \times A_{25}) \times A_2\) pairing. Since \(A_6\) was found three times in the sample, a third pairing of the same type was available for comparison. This pairing did not appear to be dikaryotized at all, although an inordinate number of pseudoclamps were observed. In fact, it was possible several times to observe pseudoclamps at several consecutive septa along a hypha in this pairing. In the case of the \((A_{13} \times A_{25}) \times A_2\) pairing, there was, similarly, a second pairing of the same type in the series. This one was dikaryotized, and produced quite normal clamp connections.

In all but two of the di-mon pairings, lines of demarcation were evident at the interface, though the strength of the reaction was variable. For both pairings in which there was no aversion, the monokaryotic component was dikaryotized.

Of the four pairings in which dikaryotization failed to occur, one, \((A_{13} \times A_{25}) \times A_6\), has already been mentioned. The other three, involving monokaryons carrying an \(A_3\), \(A_9\) and \(A_{18}\) allelomorph, respectively, shared with the former one common feature, extreme desiccation of the medium on which the mycelia were growing. Thus, the adverse effect of environment, low humidity in this case, on clamp connection incidence, as previously mentioned, cannot be discounted here. The significance of these four pairings in interpreting data should be correspondingly low in light of this knowledge.
Figure 13. Di-mon pairings. The upper mycelium on each plate is the dikaryon ($A_{13} \times A_{25}$). The strength of the aversion reaction is variable. The monokaryon component were (from right to left): $A_{24}$; $A_{13}(MD-14)$ upper row; $A_{14}(FP 104132)$; $A_{21}$ lower row. All were dikaryotized.
DISCUSSION

An estimate of 33 for the number of allelomorphs in the population may seem low in comparison to the analogous values for several tetrapolar species (Eggerston, 1953; Raper et al., 1958; Whitehouse, 1949b). The outbreeding potential, however, differs little from that of *S. commune*, for both loci of which n was much greater. The value for *S. commune* was 98.15%, and for *F. cajanderi* 96.97%. In other words, for n=33, the fraction of compatible matings out of the total number of possible combination for *F. cajanderi* is:

\[
\frac{1-\frac{1}{n}}{33} = \frac{1-\frac{1}{33}}{0.9697}
\]

A tetrapolar mating system, although reducing the maximum inbreeding level to 25%, also decreases the possible number of fertile matings within the whole population for an average n at both loci, relative to the same fraction in a bipolar system for the same value of n. Thus, to achieve an approximately equal outbreeding efficiency, the values of n at both loci must be somewhat greater for tetrapolar forms. It should be noted that bipolar systems support an inherent maximum inbreeding level of 50%. Nevertheless, in terms of the critical parameter, outbreeding efficiency, it is seen that a bipolar species would be expected to have fewer allelomorphs at the single locus for heterothallism than a comparable tetrapolar form, since fewer are necessary to reach the same level of outbreeding efficiency.
The behavior of the monokaryotic isolates with respect to fruiting was somewhat similar to that of *Lenzites trabea* Pers. ex Fr., and *Tyromyces undosus* Murr., both bipolar, heterothallic species. In these cases, monokaryons either did or did not fruit, and if fruiting did occur, it did so regularly and predictably (Barnett and Lilly, 1947; Brotzman and Gilbertson, 1967). However, in contrast to *F. cajanderi*, both these species do produce basidia and basidiospores.

The complete sterility of the monokaryotic fruiting structures, while unusual, is thought to be merely an extreme case of the more common situation, in which fruiting bodies of monokaryons produce fewer and less viable spores than the dikaryon. This is also in accord with the prevalent concept of monokaryotic fruiting as an attribute of little or no biological significance in nature (Raper, 1960).

The apparent induction of monokaryotic fruiting during the course of certain incompatible pairings is believed to be the first incident of its kind to be reported. That the phenomenon occurs at all has interesting ramifications with respect to current ideas concerning the relationship between compatibility factors and fruiting body development. The sexual process in Basidiomycetes, although poorly understood due to its complexity, has been assumed to proceed via the reciprocal regulation of consecutive steps mediated through the cytoplasm and/or by diffusable substances. It has also been shown that genic control of fruiting is at least partly independent of the compatibility factor locus (Leonard and Raper, 1969; Raper, 1960). Thus, the induction of monokaryotic fruiting during an incompatible pairing would seem to indicate that the critical disruption in the sexual sequence due to incompatibility may occur at
more than one point, and, in some cases, not before a certain amount of reciprocity, independent of the compatibility factor(s) present, has taken place between the two mycelia.

It has previously been reported that dikaryotization of the component monokaryon of a di-mono pairing did not occur in _F. cajanderi_ (Adams and Roth, 1967). Their conclusions were based on their failure to observe clamp connections in hyphae taken from the monokaryotic side of the interface. From the symmetry of the growth pattern of such pairings, it is probable that they were examining tissue which was the result of growth occurring prior to the meeting of the two mycelia. Formation of clamps would not be expected in that portion of the "monokaryon" mycelium, even if dikaryotization had taken place. In the present study, hyphae from near the mycelial margin was examined. These hyphae, formed for the most part after the meeting of the two mycelia, would be expected to form clamps had dikaryotization occurred. Clamps were found, and it now can be said that dikaryotization of monokaryons by dikaryons in _F. cajanderi_ not only can occur, but seems to be the general rule. The fact that an apparent aversion reaction occurred in most cases is not irreconcilable with the phenomenon of dikaryotization, since, in most cases, two dikaryons sharing only one compatibility factor were produced and aversion would be expected. In the four cases where an (A_{13} X A_{25}) X A_{13} pairing was made, dikaryotization occurred, thus producing two dikaryons with the same compatibility factors on the same plate. However, definite aversion reactions were noted in all four pairings. If aversion is indeed an index of differences in genetic make-up, then it must be concluded that genes other than those at the locus for heterothallism are involved. This is not contradicted
by the evidence here, since the four $A_{13}$ isolates, while having a compatibility factor in common, came from entirely different sources, and undoubtedly differed in many other respects genetically.
SUMMARY

In this study some aspects of the sexuality of Fomes cajanderi Karst. have been investigated. The results obtained may provide the basic information necessary to conduct further investigations with this organism. The findings are:

1) The multiple-allelomorphs at the locus for heterothallism to be randomly distributed in the North American population of the species. The number of allelomorphs is estimated to be 33.

2) Monokaryons can produce fruiting structures, but these are completely sterile.

3) The induction of fruiting in monokaryons, and possibly dikaryons, occurs in a manner that indicates the existence of complex physiological and genetic mechanisms governing the sequence of events in the sexual cycle. Furthermore, the use of fruiting induction as a means of studying these mechanisms suggests itself as a fruitful approach to the problem.

4) There are macroscopic characteristics of both compatible and incompatible pairings of monokaryons that generally allow them to be distinguished by visual inspection.

5) Dikaryotization of monokaryons by dikaryons can occur in this species, apparently with high frequency, thus providing an additional possible mechanism for producing new recombinant types in the field.

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LITERATURE CITED


