# EVIDENCE FOR PARTIAL HYBRID CLEISTOTHECIA IN *ASPERGILLUS RUGULOSUS*

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ABSTRACT.—Modification of an established technique for the identification of hybrid cleistothecia in *Aspergillus rugulosus* has led to the detection of partial hybrid cleistothecia. Partial hybrid cleistothecia contain asci which develop following the fusion of identical nuclei as well as asci which develop following the fusion of genetically dissimilar nuclei. As a result, the expected distribution of segregating genes is obscured. Random ascospore analyses from cleistothecia which contain only asci developing following the fusion of genetically dissimilar nuclei, yield the expected distribution of segregating genes and substantiate the existence of partial hybrids.

In the course of investigations with *Aspergillus nidulans*, Hemmons, et al. (1953) were able to determine by random ascospore analyses of a particular cross that approximately 15 percent of the cleistothecia in the cross contained asci of more than one kind, i.e., they yielded colonies giving a yellow to green conidial color ratio that was significantly different from the expected 1:1. Cleistothecia which contain selfed and crossed asci are termed "partial hybrid" cleistothecia. In the cross of *Aspergillus rugulosus* discussed in this paper, 15 percent of the cleistothecia analyzed were hybrid. However, only 4 percent of cleistothecia examined contained asci of more than one kind. This paper provides evidence for the existence of partial hybrid cleistothecia in *Aspergillus rugulosus*.

## MATERIAL AND METHODS

The source of the isolate of *Aspergillus rugulosus* used as well as the techniques for the selection and identification of mutants have been given (Coy and Tuveson, 1964).

The minimal medium employed throughout this investigation was Czapek's solution agar (Difco). The concentration of supplements added to the medium for identification of nutritional markers have been presented (Tuveson and Garber, 1959). The complex medium used was potato dextrose agar supplemented with 5 percent yeast extract (Difco). For the dry weight experiments, the agar was filtered out of the complex medium and the liquor was then supplemented as required and sterilized. Thirty ml volumes of this liquid were pipetted into Petri dishes and inoculated with approximately 100 ascospores/plate. After 4 days incubation, the resulting mycelium was collected or dried, weighed filter paper. The paper plus mycelium was then dried for 24 hours in an oven maintained at 60° C and then reweighed. The total weight minus the weight of the filter paper was used as a measure of growth under the nutritional conditions employed.

All cultures were grown in incubators maintained at a temperature of 37° C.

To distinguish cleistothecia containing asci of more than one kind (partial hybrids) from those containing asci of selfed origin which failed to show segregation of the markers distinguishing the parentals (only the yellow marker or only the

green marker being recovered) or those cleistothecia giving yellow and green in a 1:1 ratio, 0.1 ml aliquots of 3 ml saline solutions containing single crushed cleistothecia were plated; preserving the major part of each suspension in the refrigerator. The suspensions from which the aliquots had given the expected or aberrant ratios for color markers after two days incubation were further plated on non-selective medium (complex medium + 0.5 mg/ml lysine) on a scale sufficient for the complete analysis of all segregating genes.

## Experimental Results and Discussion

Table 1 presents data showing the results of random ascospore analyses of a cross in *Aspergillus rugulosus* in which two cleistothecia gave a greatly decreased number of ascospores producing colonies having the color characteristics of one of the parentals. This phenomenon had not been detected in previous analyses when loopfuls of ascospore suspensions were streaked onto complex medium to establish hybridity (Coy and Tuveson, 1964), but became apparent when 0.1 ml aliquots of saline containing crushed cleistothecia were dallied onto plates for the analyses described here.

Of the 44 cleistothecia harvested from the cross represented in Table 1, seven were determined to be hybrids. Of these seven, two gave highly distorted color ratios. These two cleistothecia were analyzed as to color and auxtrophy of ascospores with two cleistothecia whose ascospores produced colonies giving the expected color ratio. In such analyses, each random ascospore represents an independent observation as it is taken from a large population. The results of these analyses are presented.

Each entry represents a separate cleistothecium. The numbers, 2 and 5, represent the number of days each crushed cleistothecium was refrigerated in saline. These periods of refrigeration apparently had no effect on the viability of the ascospores.

Partial hybrids 2 and 5 each gave an excess of wild type alleles for the markers of one parent. Those spores requiring para-amino-benzoic acid (pab), isoleucine (iso), and lysine (lys) are much fewer in number than expected. In contrast, those requiring proline (prol) are in great excess. Each of these markers is expected to yield a 1:1 ratio of prototrophic to deficient colonies. The appropriate statistical procedure for assessing the significance of a deviation from a 1:1 ratio would be the Chi square test. In testing the goodness of fit to a 1:1 ratio, both of the "partial" hybrids gave highly significant deviations.

No such deviation from the expected 1:1 ratio was found in hybrids 2 and 5. These cleistothecia gave mutant to wild type alleles with approximately equal frequencies of every marker with the exception of the proline marker. Ascospores requiring this amino acid are recovered with a reduced frequency. Experimental evidence indicates that the proline mutant is inhibited by the presence of lysine in the medium. Dry weight determinations for a Y, -prol (green, -proline) mutant grown on liquid potato dextrose agar plus yeast extract (PDA + YE); PDA +

TABLE 1.—Random Ascospore Analysis of "Partial" Hybrid and Hybrid Cleistothecia in *Aspergillus rugulosus*. Cross: yellow, -isoleucine, -lysine, -para-amino-benzoic acid, non-producer[a]/green, -proline, producer.

| | | Wild-type alleles recovered at each locus analyzed | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | green conidia | +iso | +pab | +prol | +lys | producer | Total no. colonies analyzed | X no. colonies/ plate[b] |
| Partial hybrid 2 | obs | 138 | 146 | 140 | 74 | 145 | 151 | 208 | 33.4 |
| | exp | 104 | 104 | 104 | 104 | 104 | 104 | | |
| | $X^2$ | 22.2 | 33.8 | 24.8 | 17.2 | 32.2 | 42.4 | | |
| Partial hybrid 5 | obs | 158 | 159 | 152 | 27 | 157 | 128 | 182 | 44.1 |
| | exp | 91 | 91 | 91 | 91 | 91 | 91 | | |
| | $X^2$ | 86.2 | 89.0 | 72.2 | 76.8 | 83.6 | 27.4 | | |
| Hybrid 2 | obs | 102 | 102 | 89 | 130 | 119 | 98 | 203 | 36.1 |
| | exp | 101.5 | 101.5 | 101.5 | 101.5 | 101.5 | 101.5 | | |
| | $X^2$ | 0.004 | 0.004 | 3.0 | 16.0 | 6.4 | 0.2 | | |
| Hybrid 5 | obs | 96 | 97 | 88. | 114 | 91 | 98 | 195 | 48.6 |
| | exp | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | | |
| | $X^2$ | 0.04 | 0.004 | 1.8 | 5.4 | 0.8 | 0.004 | | |

[a] Non-producer = inability to synthesize an as yet unidentified antibiotic.
[b] Mean number of colonies on complex medium plates from which random colonies were picked for analysis.

YE / 0.5 mg/ml of isoleucine; and PDA + YE / 0.5 mg/ml lysine support this hypothesis. The mean values for the dry weights on these three media were respectively: .2539 gm, .2505 gm, and .2405 gm. The probability that the dry weight of the mycelium from these treatments differed significantly was calculated using a "T" test on the means. The difference in dry weight of the mutant grown on the lysine supplemented medium as compared to the mycelial weights obtained from the other two experimental conditions was statistically significant at the 5 percent level suggesting inhibition of growth of the proline mutant by lysine. Another noticeable deviation is found in the prototrophs for lysine in hybrid 2. These are somewhat in excess; however the Chi square value, 6.4, is of borderline significance. Its probability is less than one in twenty, but greater than one in a hundred. It does not approach the deviations observed in the case of the partial hybrid cleistothecia. Further, in hybrid 5 no such deviation is observed suggesting the deviation for the lysine marker in hybrid 2 is random.

In each case where the green to yellow color ratio was other than 1:1, the green predominated. Although to date few partial hybrids with these markers have been found, there is good evidence from the general growth patterns of the respective mutants that the green color would be expected to predominate. The green mutant produces abundant cleistothecia after 7-10 days incubation. This indicates that the green mutant is capable of selfing. However, the yellow mutant selfs very poorly and produces few cleistothecia even after extended periods of incubation. At best the mycelium of the yellow mutant yields only scattered clusters of cleistothecia. It is assumed that the green mutant, being capable of selfing, enters partial hybrid formation readily. Analyses of individual asci have not been undertaken, but such analyses may give additional information as to the formation of partial hybrids. Nevertheless, it is apparent that not all the hybrid cleistothecia formed in *Aspergillus rugulosus* represents true hybrids. Some must be the product of at least two nuclei.

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