NOTES AND COMMENTS

HYPHAL FUSIONS IN THE BASIDIOMYCETE *COPRINUS LAGOPUS* SENSU BULLER. I. SOME EFFECTS OF INCOMPATIBILITY FACTORS

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SUMMARY

Six classes of hyphal fusion are found in *Coprinus lagopus*. The frequency of fusion is temperature dependent with a high Q_{10} . The frequency of fusion is quite high in non-compatible matings, particularly in certain strains, but significantly lower in common-*B* matings. The effects of the incompatibility factors on fusion frequency in compatible and common-*A* matings are less clearcut. A minor role in regulating fusion frequency is attributed to the incompatibility factors. A lowered frequency of fusion may result from the formation of relatively inactive hybrid enzymes.

1. INTRODUCTION

Coprinus lagopus sensu Buller and *Schizophyllum commune* are tetrapolar basidiomycetes in which mating is controlled by two independent factors termed *A* and *B*. There is a multiple series of each factor (cf. Fincham and Day, 1971). Matings between monokaryons differing in both factors are compatible and lead to production of characteristic, clamped, dikaryotic mycelia. Where mated monokaryons have one factor in common the morphogenetic sequence leading to the establishment of a dikaryon is part-disrupted. The later features of this sequence (Dick, 1965; Raper, 1966; Raper and Raper, 1968) are, as far as is known, common to both fungi. The *A* factor controls the association and conjugate division of nuclei as well as clamp initiation; the *B* factor controls nuclear migration prior to establishment of the dikaryon and also clamp cell fusion (Papazian, 1950; Raper and San Antonio, 1954; Parag and Raper, 1960; Swieczynski and Day, 1960; Raper, 1966). When both factors are common, plasmogamy and nuclear exchange are limited to the zone of contact.

Until recently (Ahmad and Miles, 1970a) it was assumed (Buller, 1933; Dick, 1965; Raper, 1966) that hyphal fusions between confronting monokaryotic mycelia, a prerequisite for nuclear exchange and subsequent events, occurred randomly, did not strictly constitute a part of the incompatibility sequence and were thus not subject to genetic control by the incompatibility factors. The results of Ahmad and Miles specifically implicated the *A*-factor in the control of hyphal fusion frequency in *Schizophyllum*, *A*-factor homozygosity lowering this frequency. This paper describes an investigation of fusion frequency in *Coprinus* and suggests some means by which fusion may be controlled (Smythe, 1972).

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2. MATERIALS AND METHODS

The strains used were H1, H2, H6, H9, 12803 and 5906 with mating types A5B5, A6B5, A5B6, A6B6, A2B6 and A2B5 respectively. They are described by Day (1959, 1960, 1963) and Day and Anderson (1961).

The complete medium of Fries (1953) was used throughout. This was solidified with 2 per cent. Oxoid Agar No. 3 and autoclaving was at 121° C. for 15 min. Cultures were grown in petri dishes filled to a depth of 5 mm. of medium and incubated for 5-7 days at the temperature of the experiment.

The following procedure was used for making preparations in which hyphal contacts could readily be observed and scored as positive or negative with respect to fusion. A new, sterilised scalpel blade was used to cut a strip 10 mm. long by 2 mm. wide from near a colony front and at approximately right-angles to the radius of growth. Appropriate pairings of such inocula were placed growth-side-down, parallel to one another and 4-5 mm. apart on a flamed 22 mm. square coverslip. This was inverted on to a 76 by 26 mm. microscope slide on which two such preparations could easily be accommodated. Each slide was placed in a 9-cm. petri dish lined with filter paper wetted with sterile distilled water, but supported on a platform of several small pieces of broken glass to prevent flooding. Dishes were incubated at at 28° C. or 37° C. for up to 30 hours, incubation time being varied according to strain so that growth was obtained which was neither too sparse nor too vigorous. On removal, slides and coverslips were cautiously blotted dry before observing with a compound microscope. Possible contacts were located under low power ($\times 100$) then checked under oil immersion ($\times 1000$) to confirm contact and ascertain whether fusion had occurred. All contacts between approaching hyphae, located by working systematically from one end of the preparation, were scored provided they could be traced to their origins. A minimum of eight preparations was scored for each mating.

The effects of mating type on the frequency of hyphal fusions were analysed with the aid of contingency tables and application of the χ^2 -test as a test of independence and of homogeneity (Fisher, 1970).

3. RESULTS

(i) *Classes of fusion*

Numerous contacts were observed in all matings examined but the relative frequencies of fusions in the different matings varied (tables 1 and 2). The majority of fusions (87.25 per cent.) were of types described by Buller (1933) and were either *tip-to-peg* (54.04 per cent.), *peg-to-peg* (26.57 per cent.) or *tip-to-tip* (6.64 per cent.). The remaining fusions (12.75 per cent.) fell into three classes. *Tip-to-side* fusions (0.72 per cent.) superficially resembled those of Ahmad and Miles (1970*b*), but as they definitely did not involve peg formation they might better be called *tip-to-hypha* fusions. *Appression* fusions (9.52 per cent.) were recorded where a hyphal tip deflected on touching the side of a second hypha and grew parallel to it and partly or wholly in contact with it, and part or parts of the adjacent walls broke down. Similarly, *cross-over* fusions (2.51 per cent.) were recorded where two hyphae grew across one another at an angle, continuing beyond the point of contact, and adjacent walls broke down at the point of contact. In none of the above cases did observations support the idea that fusion was the result of attracted growth.

TABLE 1
Effect of temperature on hyphal fusion frequency in selected matings

Mating	Temp. °C.	Contacts	Fusions	Fusions per cent.
Non-compatible				
H2 × H2	28	421	8	1.9
	37	594	86	14.5
H6 × H6	28	470	1	0.2
	37	476	36	7.6
Compatible				
H2 × H6	28	456	13	2.9
	37	509	96	18.9

TABLE 2
Fusion frequencies in non-compatible, compatible and hemi-compatible matings at 37° C.

Mating	Contacts	Fusions	Fusions per cent.
Non-compatible			
H1 × H1	524	22	4.2
H2 × H2	594	86	14.5
H6 × H6	476	36	7.6
H9 × H9	448	12	2.7
12803 × 12803	433	23	5.3
5906 × 5906	427	72	16.9
Compatible			
H1 × H9	474	21	4.4
H2 × H6	509	96	18.9
H2 × 12803	419	46	11.0
Common-A			
H1 × H6	471	1	2.1
H2 × H9	724	66	9.1
12803 × 5906	429	37	8.7
Common-B			
H1 × H2	451	14	3.1
H6 × H9	400	2	0.5
H2 × 5906	463	36	7.8
Analysis:			
Compatible or hemi-compatible <i>v.</i> mean of corresponding non-compatible		χ^2	P(d.f. = 1)
Compatible			
H1 × H9 <i>v.</i> (H1 × H1) + (H9 × H9)/2		0.63	0.30-0.50
H2 × H6 <i>v.</i> (H2 × H2) + (H6 × H6)/2		12.34	< 0.001
H2 × 12803 <i>v.</i> (H2 × H2) + (12803 × 12803)/2		0.26	0.50-0.70
Common-A			
H1 × H6 <i>v.</i> (H1 × H1) + (H6 × H6)/2		25.62	< 0.001
H2 × H9 <i>v.</i> (H2 × H2) + (H9 × H9)/2		0.13	0.70-0.80
12803 × 5906 <i>v.</i> (12803 × 12803) + (5906 × 5906)/2		1.46	0.20-0.30
Common-B			
H1 × H2 <i>v.</i> (H1 × H1) + (H2 × H2)/2		14.92	< 0.001
H6 × H9 <i>v.</i> (H6 × H6) + (H9 × H9)/2		15.66	< 0.001
H2 × 5906 <i>v.</i> (H2 × H2) + (5906 × 5906)/2		12.50	< 0.001

(ii) *Effect of temperature on fusion frequency*

The first experiments were designed to test the importance of controlling temperature when determining fusion frequencies. Fusion frequencies were measured at 28° C. and 37° C. for the two non-compatible matings and one compatible mating. There was a significantly greater number of fusions at the higher temperature in all the matings (table 1). This contrasted with the results of Ahmad and Miles (1970a) and therefore the treatment of these authors, who added the data between temperatures when testing for differences between crosses, could not be followed.

(iii) *Fusion frequencies in non-compatible, compatible and hemi-compatible matings*

Results are summarised in table 2. Hyphal fusion frequencies in non-compatible matings of *Coprinus* were much higher than in similar matings of *Schizophyllum*. Fusion frequency was also a strain-dependent property and H2 and 5906 could be regarded as high fusion frequency strains. Since the non-compatible group was heterogeneous the statistical method of Ahmad and Miles (1970a), which depended upon intra-group homogeneity, could not be applied. The analysis given was instead based on the means of the appropriate pairings of the tabulated fusion frequencies for the non-compatible matings (cf. table 2). This was legitimate since no significant heterogeneity was found in any series of repeat pairings of the strains used.

Of the compatible matings only H2 × H6 showed a significantly elevated fusion frequency. This could be partly attributed to the inclusion of the high fusion frequency strain, H2, in the mating although the comparisons made do attempt to allow for this. Of the common-*A* matings only H1 × H6 showed a significantly lowered fusion frequency, while the fusion frequencies in the other two matings were elevated by different amounts. There thus appears to be an elevating effect associated with the inclusion of high fusion frequency strains in these crosses and the action of loci other than the *A* and *B* loci should not be ruled out. Fusion frequency in all common-*B* matings was significantly lowered, even in the mating H2 × 5906 which included two high fusion frequency strains. Therefore the effect of *A*-factor heterozygosity in lowering fusion frequency seems unambiguous.

4. DISCUSSION

In *Coprinus* *A*-factor heterozygosity lowers the frequency of hyphal fusion whereas in *Schizophyllum* (Ahmad and Miles, 1970a) *A*-factor heterozygosity increases fusion frequency. This would appear to be the first instance of a difference in action between the mating-type factors in the two organisms. The effect of *B*-factor heterozygosity in *Coprinus* is inconsistent and requires further investigation. However, the effect of *A*-factor heterozygosity was measured with respect to the non-compatible matings and is small relative to the large differences among matings of the same compatibility type. These differences are probably due to the action of loci other than *A* and *B* and suggest a secondary role for the mating-type loci in determining fusion frequency, which would also explain the absence of a difference in fusion between compatible and non-compatible matings compared to the large differences within these compatibility types. The minor role of the *A* and *B* loci in *Coprinus* is in marked contrast with the situation in *Schizophyllum* where

Ahmad and Miles (1970a) showed that genetic factors other than mating-type had no effect on hyphal fusion.

No simple model will at present explain the control of hyphal fusion in *Coprinus*. The failure to observe teleomorphosis, which would suggest hormonal induction of fusion, under the conditions described and the high Q_{10} for the fusion process indicate primary enzymatic control whether initiated before, at or after hyphal contact. The nature of the enzyme(s) involved is yet unknown. Raper (personal communication) believes the incompatibility factors exert their control through an active process. The relatively high fusion frequency in non-compatible matings suggests constitutive formation of the necessary enzyme(s) from sub-units which, in common-*A*, common-*B* and certain common-*AB* matings might form relatively inactive polymers. Varying levels of fusion frequency could relate to the different activities of hybrid enzymes. In some ways this resembles an aggregation model (Kuhn and Parag, 1972), but in which the aggregates are not necessarily active.

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