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Heterokaryon formation in *Coprinus lagopus*

By K. M. SWIEZYNSKI* and P. R. DAY
John Innes Horticultural Institution,
Bayfordbury, Hertford, Herts.

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INTRODUCTION

In *Coprinus lagopus* mating type is determined by two independently inherited factors *A* and *B* (Hanna, 1925). Two haploid strains with different alleles at the *A* and *B* loci are compatible and when crossed form a dikaryon characterized by the presence of clamp connexions between adjacent cells. Each cell contains two nuclei, one from each parent mycelium. On suitable media the dikaryon produces fruit-bodies bearing haploid basidiospores.

In the related species *C. fimetarius* strains having a common *B* factor may form heterokaryons (Quintanilha, 1933). The same was also found in *C. lagopus* by Lewis & Day (1958). They further showed that common *B* heterokaryons composed of complementary auxotrophic mutants were prototrophic on minimal medium and possessed hyphal tips containing both types of nuclei. No record is known of common *A* heterokaryons or heterokaryons with both mating-type factors in common, although these have been reported in other basidiomycetes (Papazian, 1950; Raper & San Antonio, 1954; Fulton, 1950).

The present study was undertaken to analyse the formation, stability, growth and fruiting ability of various heterokaryons in *C. lagopus*.

MATERIALS AND METHODS

Four wild-type strains with different mating types were isolated from a single fruit-body growing on manure at Bayford, Herts, in 1956. The four mating types were A_5B_5 (H_1), A_6B_5 (H_2), A_5B_6 (H_6) and A_6B_6 (H_9). Three mutants, originally induced in H_9 by Anderson (1959), namely, G2212 (*chol*-1), G2242 (*ad*-8) and G1905 (*me*-5), were each crossed with H_1 and the other three recombinant mating types were obtained (i.e. A_6B_5 *chol*-1, A_5B_6 *chol*-1, A_5B_5 *chol*-1 etc.). The relative growth-rates of these mutants compared with wild type are given in Table 6. Certain other mutants and wild-type strains with different alleles at *A* and *B* have also been used occasionally.

The presence of particular nuclei in the mycelia of heterokaryons was established either by testing samples for ability to grow on a suitable medium, or by mating

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samples with appropriate tester stocks and scoring clamp formation. Sometimes both methods were used.

All the experiments, unless otherwise stated, were performed at 28° C. in Petri dishes 100 mm. in diameter on Fries (1953) minimal medium and complete medium described by Day (1959). The complete medium was modified by the addition of 1·25 ml. hydrolysed nucleic acid to each litre. Fruit-bodies were produced on sterilized manure.

The terminology of Papazian (1950) and Raper (1953) has been adopted. Heterokaryons with nuclei possessing the same alleles at one or both mating-type loci are called common *A*, common *B* and common *AB* heterokaryons respectively. Heterokaryons in which the alleles are different at both loci are called compatible heterokaryons or dikaryons.

The formation of compatible, common A, *common* B *and common* AB *heterokaryons*

The three mutants (*chol*-1, *ad*-8 and *me*-5) each represented by four strains of different mating types were intercrossed on minimal medium in all forty-eight possible combinations of strains with different growth requirements. The crosses were made by placing inocula of *ca.* 1 mm. side on minimal medium so that they were touching. These were incubated at 40° C. Preliminary experiments had shown that heterokaryon formation occurs more quickly at 40° C. than at 28° C. All combinations of strains (i.e. 12 common *AB*, 12 common *A*, 12 common *B*, and 12 compatible) developed and showed much better growth on minimal than the very limited growth of the parental strains. After 5 days' incubation at 40° C. most combinations had formed colonies at least 15 mm. in diameter and samples of *ca.* 1 mm. side were tested for growth requirements. Two sites in each colony were sampled, and from each site inocula were transferred to three test media: minimal plus choline, minimal plus adenine, and minimal plus methionine. If all three samples from one site grew, both parental nuclei were assumed to be present. If only one sample grew, only one parental nucleus was present and was characterized by the supplemented medium on which growth occurred.

The samples from compatible and common *B* mycelia developed on all media, showing that they contained both parental nuclei. In seven out of the twelve common *AB* crosses, samples from one or both sites gave only one type of nucleus. The same was true of five of the common *A* crosses, showing that if these common *AB* and common *A* combinations were heterokaryons they had areas in which only one type of nucleus was present. The next step was to determine whether both types of nuclei may be found in the same hypha. In combinations of compatible strains the regular formation of true clamps has always been associated with a binucleate condition in our experience. In other common *B* combinations Lewis & Day (1958) have shown that hyphal tip isolates from balanced common *B* heterokaryons give rise to prototrophic growths. The hyphal tip analysis was therefore limited to common *A* and *AB* heterokaryons.

The most vigorous common *A* and common *AB* combinations on minimal were

selected and single hyphal tips of not more than a few cells were transferred to complete medium. Five or ten tips were taken from each of four common A combinations and three common AB combinations. Forty-five out of sixty tips grew into colonies which were then tested for growth on minimal medium. The results from two common AB and two common A combinations which gave rise to colonies showing growth on minimal medium are given in Table 1. In addition,

Table 1. *The growth of hyphal tip isolates from common* AB *and common* A *heterokaryons*

Heterokaryon number	Parental strain requirements	Mating-type factors in common	Isolated	Number of hyphal tips: Growing on complete medium	Number of hyphal tips: Growing on minimal medium
9	*chol/me*	A_6B_5	10	9	9
10	*ad/me*	A_6B_6	5	5	1
18	*chol/me*	A_5	5	2	1
23	*chol/me*	A_6	10	10	10
		Total	30	26	21

all samples from common A heterokaryons able to develop on minimal medium were crossed with appropriate B tester stocks. This test confirmed the presence of two types of nuclei in each example. The same test could not be applied to the common AB combinations. However, one hyphal tip culture derived from heterokaryon No. 10 and four hyphal tip cultures derived from heterokaryon No. 9 were crossed with a compatible wild-type monokaryon. The resulting five dikaryons were fruited and the badisiospores tested for the segregating auxotrophic marker genes present in the original common AB combination. Of twenty basidiospores from the cross with No. 10, ten were prototrophic, eight were adenine-requiring, and two could not be classified owing to poor growth. None were methionine-requiring. The crosses with No. 9 showed poor basidiospore germination, but in general agreed in showing segregation of one or other but not both of the heterokaryon markers. The results suggest that the prototrophic growth of the common AB heterokaryon was not due to the presence of prototrophic nuclei arising by back mutation or somatic recombination and that each dikaryon contained only one type of nucelus from its common AB heterokaryon parent.

Evidence has been presented that two types of nuclei may be present in the same hypha in common A and common AB combinations. We propose to call all prototrophic combinations heterokaryons, but it is clear that many hyphae in these heterokaryons may have only one type of nucleus. The nuclear ratios have not been investigated. The evidence for the existence of common B heterokaryons has already been discussed, and in the succeeding sections of this paper the stability, growth-rates and fruiting ability of these three types of heterokaryon and the compatible heterokaryon will be discussed.

In another paper (Swiezynski & Day, 1960) it is shown that common *A* heterokaryons between mutant or wild-type strains are formed easily on minimal and complete media. The same is true of common *B* heterokaryons if suitable techniques are used. Nuclei are unable to migrate in a mycelium with resident nuclei having the same *B* allele. When two monokaryotic mycelia with common *B* alleles meet, an area of dense mycelium is frequently formed. This dense mycelium is composed partly of the common *B* heterokaryon which is unable to grow through the surrounding monokaryons. When transfers are made from the zone of contact, heterokaryotic hyphae with clamps or false clamps have always been recovered. False clamps fail to fuse with their adjacent cells whether this dense mycelium is formed or not. In some cases only a small proportion of the hyphae show clamps. In the common *B* heterokaryons studied, both true and false clamps were observed, sometimes on the same hyphae. False clamps were also seen on some dikaryons, but much less frequently than on common *B* heterokaryons. No clamps of any kind were seen on common *A* or common *AB* heterokaryons.

The stability of heterokaryons

It was shown in the previous section that in samples of *ca.* 1 mm. side of common *A* and common *AB* heterokaryons sometimes only one type of nucleus could be detected. This finding suggests that these heterokaryons are unstable and readily

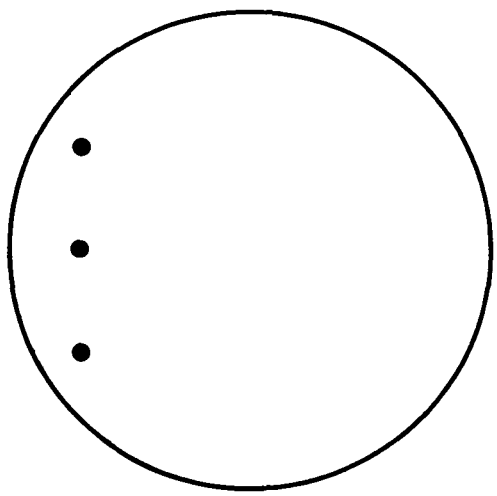

Fig. 1. Positions of the three inocula of a heterokaryon used in tests of stability and growth measurements.

break down to form their component monokaryons. The following experiments were designed to check this possibility.

The heterokaryons were first subcultured to minimal and complete medium in Petri dishes. Each dish received three inocula of *ca.* 2 mm. side, from the same source culture. They were placed as shown in Fig. 1. Compatible and common *B*

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heterokaryons were mainly tested on complete medium which would support the growth of component monokaryons if they were formed by breakdown of the heterokaryons. The five common *A* heterokaryons tested were among the least stable. After the colonies had grown for 15 days samples of 1 mm. side were taken from the margins and subcultured to minimal plus choline, minimal plus adenine and minimal plus methionine. Compatible, common B and common *AB* heterokaryons were sampled at two sites where growth was weakest. Common *A* heterokaryons were sampled at four sites; two from the most vigorous and two from the weakest marginal regions. There were few instances (*ca* 4%) in which two samples from one site grew while the third did not, showing that the distribution of nuclei was usually homogeneous within each site.

A summary of the results is given in Table 2. Both types of nuclei were recovered in all samples of compatible and common *B* heterokaryons. Very few of the common *AB* heterokaryons gave samples with both nuclei present, while from 45 to

Table 2. *Stability of heterokaryons on minimal and complete media*

	Number analysed		Nuclei recovered	
Heterokaryon	Crosses	Samples	Both	One
		on minimal medium		
Compatible	2	4	4	—
Common *B*	2	4	4	—
Common *A*	5	20	17	3
Common *AB*	8	16	4	12
		on complete medium		
Compatible	12	24	24	—
Common B	12	24	24	—
Common *A*	5	20	9	11
Common *AB*	11	22	2	20

85% of the common *A* heterokaryon samples gave both nuclei. In common *A* and common *AB* heterokaryons both nuclei were recovered more frequently in samples from minimal than in samples from complete medium.

A further experiment was carried out to see if the medium may influence which nuclei are lost in the five common *A* heterokaryons. At the same time a further check was made on the stability of ten common *B* heterokaryons. Each heterokaryon was inoculated as in Fig. 1 to two plates of minimal medium each supplemented with a requirement of one of the two component nuclei. It was expected that on a medium able to support growth of only one component of a heterokaryon the other component would be the one to be lost most frequently.

After 15 days' incubation samples of *ca.* 1 mm. side were taken from the colony margins and transferred to the three kinds of supplemented minimal medium. A summary of the results is given in Table 3.

Where only one type of nucleus was recovered, with two exceptions, this nucleus was the one with requirements covered by the source medium. The

Table 3. *The stability of common* A *and common* B *heterokaryons on supplemented media*

Type of heterokaryon	Number analysed: Crosses	Number analysed: Samples per cross	Component requirements	Media supplemented with: Choline: Nuclei recovered in samples: both	Choline: *chol*	Choline: *ad*	Choline: *me*	Adenine: both	Adenine: *chol*	Adenine: *ad*	Adenine: *me*	Methionine: both	Methionine: *chol*	Methionine: *ad*	Methionine: *me*
Common *A*	2	4	*chol/ad*	5	1	2	×	5	—	3	×				
	2	2	*chol/me*	2	2	×	—					—	—	×	4
	1	4	*ad/me*					3	×	1	—	1	×	—	3
Common *B*	3	2	*chol/ad*	5	1	—	×	4	—	2	×				
	3	2	*chol/me*	6	—	×	—					6	—	×	—
	4	2	*ad/me*					8	×	—	—	4	×	—	4
			Total	18	4	2	—	20	—	6	—	11	—	—	11

exceptions, two adenine-requiring samples obtained from a common *A* heterokaryon between *chol*-1 and *ad*-8 growing on minimal plus choline, were both from the same heterokaryon.

When each cross in Table 3 was analysed separately it was found that in all five common *A* heterokaryons and in one common B heterokaryon either nucleus could be lost depending on the kind of medium.

Several experiments of this kind have been made on a small scale with similar results. An interesting example was provided by a common *A* heterokaryon colony growing on complete medium one area of which had only adenine-requiring nuclei and another area only choline-requiring nuclei. Occasionally one type of nucleus was recovered with a requirement not satisfied by the medium, as in the

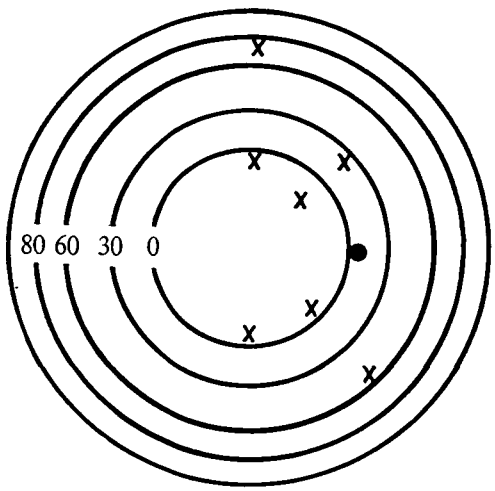

Fig. 2. Positions of the established colony margin 0–80 hours after addition of common *A* monokaryon inoculum, ●. The seven sampling points are shown X.

above example with adenine nuclei in a common *A* heterokaryon. Our findings show that the selective effect of the medium is not great and does not always operate in the direction expected.

In common *B* heterokaryons the loss of one type of nucleus was less frequent, and the resulting monokaryon was usually found as a definite sector. Such sectors would be expected as there is no migration of common *B* nuclei. In common *A* heterokaryons the loss of one type of nucleus was frequently observed, not in sectors but in areas, usually visible macroscopically as regions with weaker growth. This fact indicates that migration of common *A* nuclei may be restricted or delayed. The problem was more closely analysed in two series of experiments involving wild-type strains. In the first series an inoculum of one strain was placed at the edge of an established 4-day-old colony of a different strain, between 35 and 40 mm. in diameter, growing on complete medium. Seven points shown in Fig. 2 were selected for sampling, and from them samples of *ca.* 1 mm. side were taken after 30, 60, 80, 100 and 120 hr. and after 10 days from the time of adding the

inoculum. The samples were tested with appropriate stocks which would only be dikaryotized by the nuclei of the added strain. Migrating nuclei never disappeared from an area they had once reached. Thus the loss of nuclei of one type in a common A heterokaryon is due not to elimination of nuclei from a definite place but to the fact that they are unable to establish themselves in new growth. Such new growth may be mainly initiated by hyphae containing only one type of nucleus. In a second series of experiments, common *A* heterokaryons between wild-type stocks, in which it was known which nucleus came from the established colony and which nucleus had migrated, were inoculated as in Fig. 1. Four different combinations were available ($A_5B_5 \times A_5B_6$, $A_5B_6 \times A_5B_5$, $A_6B_6 \times A_6B_5$ and $A_6B_5 \times A_6B_6$). After 8 days' incubation hyphal tips were isolated from the margins of the colonies and the colonies they gave rise to were checked for the presence of one or both nuclei by mating with appropriate tester stocks. The results are presented in Table 4. Both nuclei were seldom recovered together, but in 30% of the cases the nucleus which remained was the migrant one.

Table 4. *Hyphal tip analysis of common* A *heterokaryons*

Allele in common	Number of tips isolated	Type of nucleus		
		Both	Resident	Migrant
A_6	26	1	16	9
A_5	26	1	18	7

We find that the common *A* heterokaryon frequently has hyphal tips and areas of mycelium in which only one nucleus, resident or migrant, is present. These monokaryotic areas are free from the other nucleus, whose migration therefore seems to be limited. We propose to call this phenomenon secondary limitation of migration. It is a property of established common *A* heterokaryons, and we would distinguish it from primary limitation, which prevents or retards the establishment of heterokaryons.

Rate of growth of various heterokaryons

All forty-eight combinations of marker strains described on p. 115, all combinations of the original wild-type strains and all single marker strains and wild types were inoculated to Petri dishes as in Fig. 1 and their growth measured every 3 days. The experiment was run in two series, one on minimal and the other on complete medium. On each plate the distance from the inoculum to the colony margin (radius) of each of the two lateral colonies was measured. This method does not take into account variation in the density of growth which could result in colonies with the same absolute growth-rate having different radii.

Most colonies of compatible and common *B* heterokaryons were able to cover the entire plate after prolonged incubation up to 18 days. Other colonies and

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most common *A* and common *AB* heterokaryons ceased to develop after initial normal growth. The marginal hyphae of these colonies were often found to be abnormal when inspected under the microscope. Colonies either remained in this state or after a few days commenced new but usually weaker growth.

Average values of the largest radii from each plate, measured after 9 days for the various groups of heterokaryons and parental strains, are presented in Tables 5 and 6. Table 7 shows the variation in colony size within and between groups of

Table 5. *Average maximal radius (mm.) of various heterokaryons after 9 days of growth*

Requirements of parental strains	Number of heterokaryons tested	Compatible	Common *B*	Common *A*	Common *AB*
		Complete medium			
chol/ad	4	32	40	27	25
chol/me	4	41	45	34	38
ad/me	4	39	36	26	23
Average		37	40	29	29
Wild type	1	69	48	37	—
		Minimal medium			
chol/ad	4	25	25	12	17
chol/me	4	28	27	20	28
ad/me	4	30	26	16	14
Average		28	26	16	20
Wild type	1	42	38	30	—

Table 6. *Average maximal radii (mm.) of parental strains after 9 days*

	Choline	Adenine	Methionine	Average	Wild type
Complete medium	34	34	34	34	49
Minimal medium	9*	3	7	6	47

All four mating types of each parental genotype were measured.

* Growth of choline requiring mutants on minimal medium is very faint.

heterokaryons and between colonies on the same plate. The variation between groups is presented directly. The variation within groups is expressed as the standard deviation (between plates) of each group. Each component of the group was the average of the two radii measured on each plate. The variation within plates is presented as the average difference between the radii of the largest and smallest colony on each plate.

The data for wild-type and for mutant strains are presented separately. All possible combinations were analysed. The four wild-type common *A* heterokaryons each have a different mother strain (as indicated on p. 121), and, of the six compatible heterokaryons, four have different mother strains and in two the

Table 7. *Growth variation in heterokaryons*

Type of mycelium	Wild-type strains: Number measured	Wild-type strains: Average radius (mm.)	Wild-type strains: Standard deviation between plates	Wild-type strains: Average differences between colonies on same plate	Auxotroph strains: Number measured	Auxotroph strains: Average radius (mm.)	Auxotroph strains: Standard deviation between plates	Auxotroph strains: Average differences between colonies on same plate
				Complete medium				
Parental strains	4	48	4·1	0·2	12	31	8·2	4·8
Compatible	6	67	12·3	2·8	12	44	14·3	4·3
Common *B*	2	46	1·4	4·0	12	39	7·5	4·3
Common *A*	4	34	5·0	4·7	12	26	7·0	4·5
Common *AB*	–	—	—	—	12	27	10·2	2·7
				Minimal medium				
Parental strains	4	44	4·3	0·7	12	5*	3·3	1·9
Compatible	6	40	13·6	10·5	12	24	9·1	7·7
Common *B*	2	38	8·1	1·5	12	24	7·5	3·2
Common *A*	4	27	3·8	5·5	12	14	6·1	3·4
Common *AB*	–	—	—	—	12	16	9·7	7·0

* Faint growth of auxotrophic mutants on minimal medium.

mother strains were not determined. The direction of mating was not taken into account in the mutant strain combinations. Also, in common *B* combinations between wild-type strains it could not be determined.

The following conclusions may be drawn from the data presented in Tables 5–7:

1. Common *A* heterokaryons grow less vigorously than their corresponding parental strains. With wild-type strains the difference is obvious on complete and minimal medium, but with mutant strains it is visible only on complete medium.

Common *A* heterokaryons appear to grow more slowly on minimal medium than common *AB* heterokaryons (Table 5).

2. Common *B* heterokaryons are approximately as vigorous as compatible heterokaryons and much more vigorous than common *A* heterokaryons.

3. The compatible heterokaryon usually grows fastest, but there are two exceptions:

(*a*) On minimal medium the wild-type compatible heterokaryons are not as vigorous as the haploid component strains and are much less vigorous than when grown on complete medium (Table 7).

(*b*) On complete medium compatible heterokaryons show the greatest variation in vigour (Table 7); indeed, among the twelve compatible heterokaryons there were two which showed less growth than the average growth of their parental monokaryons.

Fruit-body formation

The ability of the heterokaryons to produce fruit-bodies has been tested in two kinds of experiments:

The first experiments began as an attempt to induce mutations at the *A* or *B* mating-type loci. Mixed complementary auxotrophic strains with common *A* or common *B* factors were grown on minimal medium in Petri dishes and irradiated with ultra-violet light in various doses. The Petri dish cultures were then transferred to flasks of dung and incubated. Any mutations leading to full compatibility were expected to give rise to a prototrophic dikaryon and hence to a fruit-body when inoculated to dung.

In the other experiments heterokaryons between mutant and wild-type strains and wild-type monokaryons were inoculated to dung flasks without treatment.

The data from these experiments were supplemented with data obtained from current laboratory work with wild-type strains and mutants in compatible heterokaryons.

The results obtained are summarized in Table 8. No haploid strains produced fruit-bodies, but the number tested was limited to four wild-type strains, each in two replications. Compatible heterokaryons invariably produced fruit-bodies after 8–15 days. Common *B* fruit-bodies were obtained several times, most frequently from untreated heterokaryons, and common *A* fruit-bodies were obtained three times, two from treated and one from untreated heterokaryons.

Spores were analysed in tetrads from two common B fruit-bodies, one from a

treated heterokaryon, the other from an untreated one. Both gave normal segregation for A alleles. There was also evidence of the segregation of a new B reaction in both fruit-bodies.

Table 8. *Fruit-body formation by various mycelia*

Heterokaryon	Number of combinations	Total number of flasks	Number of flasks fruited	Days kept	Days to fruit
Compatible	9	21	21	—	8–15
Common B treated	10	33	1	40–90	12
Untreated	5	11	7	40–90	10–35
Common A treated	4	20	2	50–90	33–43
Untreated	10	13	1	50–60	17
Monokaryons	4	8	0	50	—

Spores from all three common A fruit-bodies obtained were analysed. None of the spores from the first fruit-body could be germinated. The second fruit-body (A_6B_5 *me*-5 × A_6B_6 *ad*-8) was contaminated and only four spores were recovered. Three were A_5B_6, and the fourth produced clamp connexions spontaneously. Twenty-nine spores were analysed from the third fruit-body ($A_5B_5 \times A_5B_6$, wild types untreated); five were A_5B_5 and six were A_5B_6. The remaining eighteen cultures all produced clamps spontaneously, but, except for two, could be assigned to B_5 or B_6 since they were only able to dikaryotize one of these testers. All eighteen cultures were able to dikaryotize the A_5 tester.

When examined again, the culture with clamps from the second fruit-body also behaved in this way and was assigned to B_5.

The new A and B reactions found suggest that fruiting may be a result of mutation in the heterokaryons.

DISCUSSION

From the foregoing data we are now able to draw a fairly clear picture of heterokaryon formation in *C. lagopus*. All types of heterokaryon—compatible, common A, common B and common AB—may be formed and, as was shown elsewhere (Swiezynski & Day, 1960), nuclear migration occurs in matings between strains with common A factors as well as in compatible matings. Two important features of these heterokaryons emerge: (1) that different B alleles are necessary for nuclear migration, and (2) that different A alleles are necessary for synchronous nuclear division and clamp formation. These features imply a functional difference between the two loci determining mating type.

C. lagopus shows many similarities with other tetrapolar basidiomycetes. Migration of nuclei in common A matings resulting in the formation of common A heterokaryons has been observed in *Schizophyllum commune* (Raper & San Antonio, 1954; Snider & Raper, 1958) and in *Cyathus stercoreus* (Fulton, 1950). In the

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latter fungus, Fulton has claimed that the B locus controls nuclear migration. The common *A* heterokaryon of *S. commune* is similar to that of *C. lagopus* in showing weaker growth than a dikaryon and in giving rise mainly to monokaryotic colonies when subcultured from single hyphal tips (Papazian, 1950; Raper & San Antonio, 1954). The apparent discrepancies between *C. lagopus* and *S. commune*: that the common *A* heterokaryon of *S. commune* is sterile, does not show genetic complementation, and tends to produce morphological mutants (heterokaroytic mutagenesis), may be dependent on the genetic background and unconnected with the *A* locus itself.

Common *B* heterokaryons with pseudo-clamps have been described in *Coprinus fimetarius* by Quintanilha and in *Cyathus stercoreus* by Fulton. They are probably produced in many other forms (Nobles *et al.*, 1957). In *C. stercoreus* nuclear migration does not occur in common *B* matings. In *S. commune* the common *B* heterokaryon has only been analysed to a limited extent (Papazian, 1950). It appears to be difficult to obtain and has no clamps.

Examples of common *AB* heterokaryons are scarce. They have been tentatively described in *S. commune* (Raper & San Antonio, 1954, and in *C. stercoreus*, *loc. cit.*), in which they are described as having some uninucleate cells with false clamps.

From the foregoing discussion there are indications that the differentiation in function between the *A* and *B* loci which we have postulated in *C. lagopus* may be found in other basidiomycetes. However, this idea must be tested extensively before valid generalizations may be made.

Our analysis of the various heterokaryons reveals some further features. Thus, although nuclear ratios in common *A* heterokaryons of *Coprinus* have not been determined, the selective action of supplemented media (Table 4) shows that the relative proportions of component nuclei may change in either direction. The response of common *B* heterokaryons to such selection pressures is very limited.

A comparison of the growth rates of the heterokaryons is instructive. It shows that under some circumstances the prescribed nuclear ratio of compatible heterokaryons places them at a disadvantage compared with their component monokaryons. (Compare Tables 6 and 7, growth on minimal). We also find that the standard deviation of 'colony radius' shown in compatible heterokaryons is greater than that of other heterokaryons. One reason for these findings could be that if nutrient demands exceeded the capacity of the substrate to supply them, growth would slow or stop. The same thing would also happen if some harmful metabolic products were produced too rapidly. Dissociation into component monokaryons might result in continued if less vigorous growth. On the other hand, the poorer growth of common *A* heterokaryons may be due to unbalanced nuclear ratios.

We have demonstrated that common B heterokaryons may produce fruitbodies in *C. lagopus*. These have also been noted in *C. fimetarius* (Quintanilha, 1933). Fruiting in common *A* heterokaryons has not been reported before in this

organism and we know of no account in any other tetrapolar basidiomycete. Preliminary studies of the products of these fruit-bodies have proved the heterokaryotic nature of the mycelia on which they were borne, since we have shown that two *A* factors segregate from the common *B*, and two *B* factors segregate from the common *A* fruit-bodies. In both the expected lack of segregation of the original homozygous allele was complicated by the appearance of new properties which have so far not been explained.

SUMMARY

1. The four possible kinds of heterokaryon of *Coprinus lagopus* with no, one or both mating-type factors in common (dikaryon, common *A*, common *B* and common *AB*) were produced. Analysis of hyphal tips of common *A* and common *AB* heterokaryons has shown that both nuclei may be present in the same hypha.

2. All four heterokaryons are prototrophic when synthesized from two auxotrophic components with different requirements.

3. When synthesized in this way compatible heterokaryons were stable in all tests, but the other heterokaryons showed different degrees of stability. Common *B* heterokaryons were the most stable and rarely gave rise to monokaryotic mycelia. Dissociation of the common *A* and the common *AB* heterokaryon into either component took place much more easily.

4. Comparisons of the growth-rates of wild-type heterokaryons on complete medium show that common *A* heterokaryons are less vigorous, and dikaryons more vigorous than their monokaryon components. On minimal medium both compatible and common *A* heterokaryons are less vigorous than their wild-type monokaryon components. The possible reasons for this are discussed.

5. Fruit-bodies have been obtained from both common *A* and common *B* heterokaryons. Both types showed normal segregation at the heterozygous locus (*B* or *A*), but showed in addition the segregation of new reactions at the 'homozygous' locus.

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