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Compatibility and stability of diploids in *Coprinus lagopus*

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1. INTRODUCTION

The pioneer work of Pontecorvo and Roper (Ref. Pontecorvo, 1959) on diploids in the ascomycete *Aspergillus nidulans* has demonstrated how diploids in fungi can be used in analysing linkage and recombination. An unusual and unexplained feature of diploidy in *Aspergillus* is the failure to recover the diploid through meiosis and into the ascospores (Elliott, 1960).

Diploids in the basidiomycete *Coprinus lagopus* (Casselton, 1965) can be tested as in *Aspergillus* and also in a prolonged dikaryotic phase which is peculiar to the basidiomycetes.

The formation of the dikaryon which must precede the formation of the fruiting body and sexual reproduction is under the control of two incompatibility genes *A* and *B*. Diploids have been tested for their production of dikaryons and fruiting bodies using different combinations of *B* alleles in the mating stocks. In such tests it has been possible to make a comparison of incompatibility reactions similar to those which have been carried out in flowering plants (Lewis, 1960), and in consequence to throw some light on the action of the *B* incompatibility gene.

The stability of a diploid nucleus in *Coprinus* can be tested under a variety of conditions, in monokaryotic and dikaryotic mycelium and in fruiting bodies. Great differences in stability have been found and as a result of this a ready method for haploidizing the diploid nucleus has been made available.

2. MATERIALS AND METHODS

(i) *Stocks*

The stocks of *Coprinus lagopus* used to form dikaryons are listed in Table 1. Diploid stocks were synthesized by the method of Casselton (1965) from heterokaryons which were homoallelic for the *A* gene and heteroallelic for the *B* gene, termed common *A* heterokaryon. Auxotrophic mutants used to force the selection of diploids were linked to either the *A* or the *B* gene as indicated in Table 1.

Table 1. *Genotypes of haploid and diploid stocks used to form dikaryons.* adhi-1, chol-1, me-5, paba-1, ad-8 *and* ad-9 *determine requirements for adenine+ histidine, choline, methionine, para-amino benzoic acid and adenine respectively*

(a) Haploid stocks

TC	A_2B_3	wild-type
H9	A_6B_6	,,
12803	A_2B_6	,,
H1	A_5B_5	,,
T6	A_6B_3	,,

(b) Diploid stocks

Diploid I	*me*-5	A_6	+	B_3	*adhi*-1	+
	+	A_6	*ad*-8	B_6	+	*chol*-1
Diploid II	*me*-5	A_6	*ad*-8	B_6	+	+
	+	A_6	+	B_3	*adhi*-1	*chol*-1
Diploid III	*me*-5	A_2	*ad*-8	B_6	+	
	+	A_2	+	B_3	*adhi*-1	
Diploid IV	*ad*-9	A_5	*ad*-8	B_2	+	
	+	A_5	+	B_3	*adhi*-1	
Diploid V	*paba*-1	A_5	+	B_5	*adhi*-1	
	+	A_5	*ad*-8	B_2	+	

(ii) *Experimental procedures*

Details of culturing, general techniques and media have been given by Lewis (1961). The specialized techniques used for resolving dikaryons into their monokaryotic components by means of mycelial chlamydospores and somatic veil cells from fruiting bodies have been described in detail by Lewis (loc. cit.) and Cowan (1964) respectively. In all analyses the *A* and *B* alleles were identified by dikaryon tests with tester stocks of known *A* and *B* constitution.

3. RESULTS

(i) *Dikaryon formation*

Dikaryotic hyphae are produced soon after hyphal anastomosis between compatible stocks and these can readily be distinguished from monokaryotic hyphae by the acute angle of the branches and the characteristic clamp connexions at all cross walls. The two genes *A* and *B* which control dikaryon formation have different functions (Swiezynski & Day, 1960). The *A* gene is responsible for clamp formation and the *B* gene controls nuclear migration. In matings between haploid stocks with similar *B* alleles but different *A* alleles (common *B* matings) clamps are formed but nuclear migration is prevented, the clamps fail to fuse with the adjacent cell (false clamps), and one nucleus is trapped in the false clamp.

Diploid stocks with different *B* allele constitutions have been tested for dikaryon formation with other diploid and haploid stocks giving a variety of common *B* and

fully compatible pairings. Haploid–haploid matings have been made for comparison. Formation of dikaryons has been studied by determining (i) the time taken for dikaryotic hyphae to be produced after mating and (ii) by examination of the clamp connexions.

(*a*) *Speed of dikaryon formation*

Asexual spores (oidia) of diploid and haploid stocks were germinated on minimal medium and isolated at a stage when two or three hyphal branches had formed. Isolates to be mated were placed side by side in the centre of a plate of complete medium (ten plates for each pair), incubated and examined microscopically at 24 hours, 48 hours and 72 hours. The numbers of cultures which had formed dikaryotic hyphae at these times are given in Table 2.

Table 2. *Speed of dikaryon formation in compatible and common* B *matings. Ten replicates were made of each mating*

		Time		
Ploidy	Mating	24 hours	48 hours	72 hours
Haploid × Haploid	$A_6B_6 + A_2B_3$ (compatible)	10	10	10
Diploid × Haploid	$A_6A_6B_6B_3 + A_2B_6$ (semi-common *B*)	4	10	10
Diploid × Diploid	$A_6A_6B_6B_3 + A_5A_5B_5B_2$ (compatible)	0	9	10*
Diploid × Diploid	$A_6A_6B_6B_3 + A_2A_2B_6B_3$ (common *B*)	0	10	10*

* Sectors of dikaryon only.

All haploid–haploid matings and some of the diploid–haploid matings had formed dikaryotic or dikaryotic-like hyphae within 24 hours although most abundantly in the doubly haploid matings. All cultures were fully dikaryotic at 72 hours. In contrast, diploid–diploid matings took longer than 24 hours to produce dikaryotic hyphae and by 72 hours only small sectors of dikaryon were present, the major part of the mycelium being monokaryotic. It is clear that diploid stocks produce dikaryons more slowly than haploid stocks, but the speed at which dikaryons are formed does not distinguish between common *B* and compatible matings.

(*b*) *Clamp connexions*

The types of matings examined and the proportion of false and true clamp connexions in the dikaryons formed are listed in Table 3. Clamps were examined microscopically and random samples of 110 clamps were recorded for each dikaryon. Clamps formed by terminal cells were not included as these may be at varying stages of completion.

In all fully compatible matings whether between haploid, haploid and diploid or between diploid stocks, less than 6% of the clamps were false. Diploid–haploid and diploid–diploid matings classified as semi-common *B* behaved as if fully compatible.

Table 3. *The proportion of true and false clamp connexions in dikaryotic or dikaryotic-like mycelium produced in a variety of fully compatible and common* B *matings*

Ploidy	Mating	Clamps: True	Clamps: False	% False clamps
Haploid × Haploid	$A_2B_3 + A_6B_6$ compatible	106	4	3·6
	$A_2B_3 + A_6B_3$ common *B*	7	103	93·5
Diploid × Haploid	$A_6A_6B_6B_3 + A_5B_5$ compatible	104	6	5·5
	$A_6A_6B_6B_3 + A_2B_3$ semi-common *B*	105	5	4·5
	$A_6A_6B_6B_3 + A_2B_6$ semi-common *B*	105	5	4·5
Diploid × Diploid	$A_6A_6B_6B_3 + A_5A_5B_5B_2$ compatible	104	6	5·5
	$A_2A_2B_6B_3 + A_5A_5B_5B_2$ compatible	105	5	4·5
	$A_6A_6B_6B_3 + A_5A_5B_2B_3$ semi-common *B*	104	6	5·5
	$A_2A_2B_6B_3 + A_5A_5B_2B_3$ semi-common *B*	104	6	5·5
	$A_6A_6B_6B_3 + A_2A_2B_6B_3$ common *B*			
	(i)*	64	46	42·0
	(ii)*	77	33	30·0
	Same after 2 subcultures			
	(i)*	102	8	7·3
	(ii)*	108	2	1·8

* Examination of two dikaryons synthesized from the same diploid strains.

In diploid–diploid common *B* matings at least 30% of the clamps were false in newly formed dikaryons. This is intermediate between a haploid common *B* heterokaryon with 94% false clamps and a true dikaryon with less than 6% false clamps. After subculturing, the number of false clamps decreased and these dikaryons were then indistinguishable from true dikaryons. The significance of the intermediate number of false clamps and the reduction in number with subculturing will be discussed later.

(ii) *Stability of diploid nuclei in diploid–diploid dikaryons*

Previous work, in which dikaryons formed from diploid and haploid stocks were fruited and the basidiospore progeny identified, showed that the diploid component may persist into the fruiting body but more often haploidizes either before or during fruiting body formation (Casselton, 1965). Haploidization occurred in both compatible (e.g. $A_1A_1B_1B_2 \times A_2B_3$) and semi-common *B* (e.g. $A_1A_1B_1B_2 \times A_2B_1$) dikaryons and was not therefore due to incompatibility of common *B* alleles in the latter type of dikaryons. Diploid nuclei were shown to be extremely stable in monokaryons and haploidization in random samples of the asexual spores was rare, approximately 0·5%. It was suggested that instability of diploid nuclei in dikaryons might be due to lack of exact mitotic synchrony of a haploid and a diploid nucleus in the conjugate division. If this is so it would be expected that in a dikaryon in which both nuclei are diploid, these would be stable.

It is possible to test for the stability of diploid nuclei in dikaryotic cells by recovering the two components at three distinct stages, (i) chlamydospores produced by the vegetative mycelium, (ii) somatic veil cells from the immature fruiting body and (iii) basidiospores.

If common *B* diploid matings are not fully compatible (as suggested by the appearance of the clamp connexions) and compatibility is dependent on at least one nucleus losing a *B* allele, it is possible to detect this by testing the component nuclei.

Component nuclei from all dikaryons were identified with respect to *B* alleles, and the description of haploidization which follows refers only to tests on the *B* chromosome. Two complete analyses of components were made and these will be referred to later as they are of considerable importance in the interpretation of the haploidization process.

(*a*) *Chlamydospore analyses*

In Table 4 are the results of chlamydospore resolution of three compatible diploid dikaryons and two common *B* diploid dikaryons. The prediction that diploid nuclei would be stable in a compatible diploid dikaryon is clearly discounted. In both compatible and common *B* diploid dikaryons there was evidence of haploidization. There are however important differences between the two types of dikaryon.

Table 4. B *allele constitution of dikaryon components recovered by the chlamydospore technique*

A. Compatible diploid dikaryons formed between Diploid V ($A_5A_5B_5B_2$) and Diploid I, II (both $A_6A_6B_6B_3$) or III ($A_2A_2B_6B_3$)

	Components in chlamydospores					
	Diploid I, II or III			Diploid V		
Dikaryon	B_6B_3	B_6	B_3	B_5B_2	B_5	B_2
Diploid I + Diploid V	16	1	1	39	—	11
Diploid II + Diploid V	5	3	1	5	1	4
Diploid III + Diploid V	9	1	5	26	—	5
Totals	30	5	7	70	1	20

B. Common *B* diploid dikaryons formed between Diploid III ($A_2A_2B_6B_3$) and Diploid I or II ($A_6A_6B_6B_3$)

	Components in chlamydospores					
	Diploid I or II			Diploid III		
Dikaryon	B_6B_3	B_6	B_3	B_6B_3	B_6	B_3
Diploid I + Diploid III						
(i)	4	—	—	—	30	—
(ii)	—	5	—	7	3	3
Diploid II + Diploid III						
(i)	6	53	—	27	—	10
(ii)	6	32	—	7	—	1
Totals	16	90	—	41	33	14

Whilst both diploid nuclei may persist in common *B* diploid dikaryons (e.g. Diploid II × Diploid III), one component was recovered predominantly as a haploid

derivative. The overall recovery of components which had lost a *B* allele was far in excess of those still heterozygous. Where both nuclear components had haploidized, with the exception of Diploid I × Diploid III (ii), haploid derivatives were compatible and therefore only one *B* type was derived by haploidization from each original diploid nucleus (i.e. B_6 from one and B_3 from the other).

Both diploid components were more easily recovered from compatible diploid dikaryons and, in contrast to common *B* dikaryons, the total number of isolates heterozygous for *B* alleles was nearly three time those which had lost a *B* allele. Moreover, haploidization produced haploid derivatives with either of the two *B* alleles present in each original diploid nucleus since all haploid combinations would be compatible.

Two of the analyses, one each from a compatible and a common *B* dikaryon, were made in more detail to see if haploidization affected the *A* chromosome as well as the *B* chromosome. These more detailed analyses are given in Table 5. It is clear

Table 5. *Detailed analysis of the* A *and* B *chromosome constitution of chlamydospore resolvates from two dikaryons.* B *chromosomes were identified by mating tests on the* B *allele, and* A *chromosomes by auxotrophic markers*

	Components recovered as:			
Dikaryon	Disomic for *A* and *B*	Disomic for *A* only	Disomic for *B* only	Monosomic for *A* and *B*
Diploid I + Diploid V (compatible)				
Diploid I	14	2	2	—
Diploid V	37	11	2	—
Diploid I + Diploid III (common *B*)				
Diploid I	3	—	1	—
Diploid III	—	—	—	30

that the *A* and *B* chromosomes are lost independently. It follows therefore that haploidization is a progressive process and not initiated by the presence of incompatible relationships at the *B* locus, or the result of a somatic meiosis.

The differences between common *B* and compatible dikaryons indicates that once haploidization has begun in a nucleus there is a strong selection in favour of the loss of a common *B* allele so that true dikaryon formation in a common *B* pair must await the loss of a *B* allele from one nucleus.

(b) Veil cell analyses

Veil cells were taken from fruiting bodies produced by three compatible diploid dikaryons and one common *B* diploid dikaryon (two different dikaryons). The results of the analyses are given in Table 6.

The striking feature of all analyses whether veil cells were derived from compatible or common *B* dikaryons is that only one diploid component was recovered as such. Without exception, the second component had lost one particular *B* allele and must

Table 6. B *allele constitution of dikaryon components recovered by the veil cell technique*

A. Compatible diploid dikaryons formed between Diploid V ($A_5A_5B_5B_2$) and Diploid I, II (both $A_6A_6B_6B_3$) or III ($A_2A_2B_6B_3$)

	Components in veil cells					
	Diploid I, II or III			Diploid V		
Dikaryon	B_6B_3	B_6	B_3	B_5B_2	B_5	B_2
Diploid I + Diploid V	—	—	38	1	—	2
Diploid II + Diploid V	—	—	24	4	—	4
Diploid III + Diploid V	8	1	2	—	—	24

B. Common *B* diploid dikaryons formed between Diploid II ($A_6A_6B_6B_3$) and Diploid III ($A_2A_2B_6B_3$)

	Components in veil cells					
	Diploid II			Diploid III		
Dikaryon	B_6B_3	B_6	B_3	B_6B_3	B_6	B_3
Diploid II + Diploid III						
(i)	4	5	—	—	—	8
(ii)	—	—	43	19	—	3

therefore have lost this allele before fruiting body initiation. It can be assumed from the more detailed analyses of chlamydospore isolates that haploidization would have effected loss of other chromosomes also. The results of these veil cell analyses suggest that cells which have two diploid nuclei cannot produce fruiting bodies.

(*c*) *Basidiospore analyses*

The final stage at which the components of a dikaryon can be identified is in the basidiospore progeny. This type of analysis has been confined to compatible diploid dikaryons since it served only to confirm the results of the veil cell analyses. At least 260 germinated spores were isolated for each analysis but due to poor viability only a small number of these produced colonies which could be tested.

Table 7. *Identification of* B *alleles in the basidiospore progeny of three compatible diploid dikaryons formed between Diploid V* ($A_5A_5B_5B_2$) *and Diploid I, II* (*both* $A_6A_6B_6B_3$) *or III* ($A_2A_2B_6B_3$)

	B alleles from Diploids I, II or III		*B* alleles from Diploid V		
Dikaryon	B_6	B_3	B_5	B_2	Disomic*
Diploid I + Diploid V	14	—	1	17	13
Diploid II + Diploid V	14	—	11	14	9
Diploid III + Diploid V	15	10	—	20	28

* Disomic colonies could not be identified further because of universal compatibility.

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Despite the small numbers analysed, the results in Table 7 are in complete agreement with those obtained from veil cell analyses. Both *B* alleles of one component were recovered but only one *B* allele from the other component. This is consistent with the conclusion that fruiting bodies with two different diploid nuclei cannot be formed, and confirms the similar findings of Elliott (1960) with diploid strains of *Aspergillus*.

4. DISCUSSION

Diploid nuclei when present alone in a monokaryon are stable. The same diploid nuclei, when they are in a dikaryon, become highly unstable so that by the time the dikaryotic mycelium can be resolved into its components from chlamydospores at least one and sometimes both nuclei have become haploid. Fruiting bodies with the two diploid nuclei which were present at the formation of the dikaryon have not been found.

The cause of instability of diploid nuclei in dikaryons is not clear. There are two obvious possible explanations which could easily be related. (i) Incompatibility relationships at the *A* and *B* loci result in selection against a common allele and hence in selection for those nuclei which have lost it by haploidization or by any other means. (ii) When the component nuclei differ in ploidy there is a lack of co-ordination during mitotic divisions and the synchrony of division is upset. This would result in loss of lagging chromosomes and finally haploidization.

Both these explanations are discounted by the present studies because it has been shown that in a dikaryon in which both the component nuclei are diploid and moreover fully compatible, nuclear instability is still found. Thus there is no obvious reason for instability and we are left with vague and untestable explanations such as, the synchronous division is so delicately balanced that it is upset by any deviation from the normal haploid condition. Although the cause of instability is unexplained, it does form a useful means of haploidizing diploid nuclei without the segregation and recombination which accompanies meiotic reduction.

As in the case of diploid *Aspergillus* (Pontecorvo & Käfer, 1956; Käfer, 1961), the haploidization process in *Coprinus* appears to be by a progressive loss of chromosomes at mitotic division because aneuploid as well as haploid nuclei are found and recombination of linked genes has not been detected. This is supported by the work of Cowan & Lewis (1966) on the selection of nuclei disomic for one chromosome by the use of recessive methionine suppressors and the evidence obtained from common *A B* fruiting bodies in *Schizophyllum commune* by Middleton (1964). There is no evidence in *Coprinus lagopus* for a type of precocious somatic meiosis postulated in *Schizophyllum* by Crowe (1960), Ellingboe & Raper (1962) and Parag (1962).

The present study was directed to the elucidation of the action of the *B* incompatibility gene. Previous studies in *Schizophyllum commune* (Raper & Oettinger, 1962) and in *Coprinus lagopus* (Casselton, 1965; Cowan & Lewis, 1966), and the present work confirms this, have shown that a B_xB_y disomic or diploid produces a normal and compatible dikaryon with a nucleus which is monosomic for either

B_x or B_y. Raper and Oettinger concluded that the action of the B gene is one of complementation between different alleles to give compatibility rather than one of opposition between like alleles to produce a positive incompatibility as in higher plants. The tests with diploids with both B alleles in common do not support this conclusion, but they do support an oppositional action which is similar to, but not identical with, that in flowering plants. If the action is one of complementation between different alleles, two different diploid cultures with the same B alleles, B_xB_y, should produce a fully compatible dikaryon. The results (Table 3) show that the fusion product is not a typical dikaryon but a common B heterokaryon with many false clamps.

The B gene controls processes involved in (1) the migration of invading nuclei through the mycelium and (2) the completion of the clamp connexion by its fusion with the adjacent hyphal cell (Swiezynski & Day, 1960). An invading nucleus with a compatible B relationship with its host causes a dissolution of the domed cap that covers the simple pore in the cross walls of the mycelium (Giesy & Day, 1965). This dissolution allows the invading nuclei to pass through the pore. Cap dissolution and cell wall dissolution in the completion of the clamp may be two expressions of the same B gene action—*the localized dissolution of cell membranes.*

Examination of clamp connexions (Table 3) shows that two diploid nuclei are fully compatible when there is one B allele in common and one different, e.g. $B_xB_y + B_xB_z$, but incompatible when both alleles are common, e.g. $B_xB_y + B_xB_y$ (although progressive haploidization results eventually in compatible situations of $B_x + B_y$ or $B_xB_y + B_y$ or B_x). This difference can be explained if it is assumed that (1) the product of the B gene is responsible for local membrane dissolution and (2) when the same B gene products are on the two sides of the membrane these are neutralized and no dissolution occurs. This is illustrated in Fig. 1.

That membrane dissolution occurs in both directions in relation to an invading nucleus is shown by the fact that a diploid or disomic B_xB_y and a haploid B_x or B_y form dikaryons by nuclear migration in either direction, e.g. a B_xB_y nucleus can migrate through a B_x or B_y mycelium and vice versa (Casselton, 1965). Furthermore, in a dikaryon between B_xB_y and a haploid B_x or B_y the number of true clamps is not 50% but 100% of a fully compatible dikaryon. It would be expected that the 'invading' nucleus in the clamp would be equally either B_xB_y or the haploid. The results suggest that membrane dissolution occurs irrespective of which nucleus is in the clamp.

The postulated oppositional and neutralization action of the B gene is fully reconcilable with the types of B mutation found by Raper and Parag (Ref. Fincham & Day, 1965; Raper, Boyd & Raper, 1965). Mutations generally lead to loss of B specificity. Such a mutant B allele would lose the power to neutralize a B gene product, either its original or any other and would be self-compatible. The secondary mutation of the B gene, described by Raper *et al.*, 1965, which converts a self-compatible primary mutant to a self-incompatible double mutant could be a mutation causing complete loss of function or even absence of a B gene product. Without a functional B gene product, membrane dissolution would not occur.

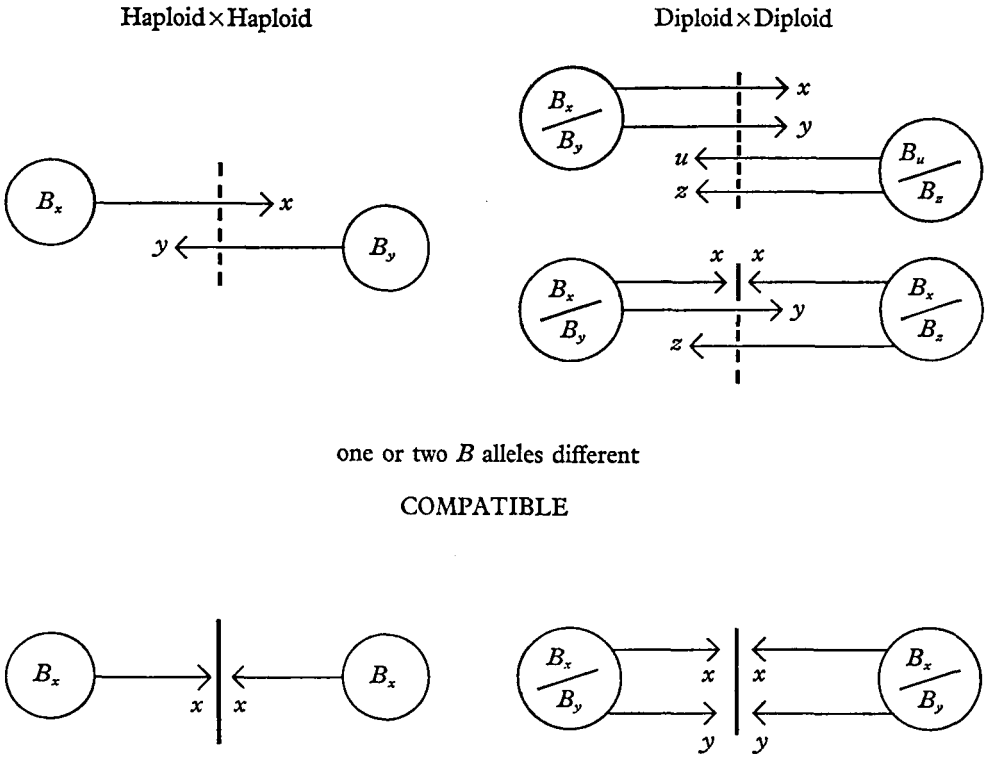

Fig. 1. Illustration of the hypothetical action of the B gene in the dissolution of membranes (for explanation see text).

This hypothesis of B gene action has some features in common with the oppositional hypothesis based upon self-recognition by an antigen–antibody type of reaction for incompatibility in flowering plants. It differs, however, in one important respect: whereas in plants the incompatibility reaction is one between the same gene products to produce a positive inhibition, in *Coprinus* the incompatibility reaction is one between the same gene products which are neutralized and are necessary for compatibility.

If the hypothesis can be substantiated it would have important implications for the interpretation of the two sub-genes α and β which comprise both the A and the B complexes (Ref. Fincham & Day, 1965). These sub-genes are very closely linked in the A complex of *Coprinus* and are between 4 and 17 units apart in *Schizophyllum*. Because the specific incompatibility reaction is the product of the α and β sub-genes, Lewis (1964) has argued from this and from an analogous but slightly different situation in the Gramineae in flowering plants that these two sub-genes encode different polypeptides which associate to produce a dimer that is unique for each combination of sub-genes. This elaborate explanation is now unnecessary but not excluded for

Coprinus. A simpler hypothesis is that the two sub-genes are duplicates as suggested by Raper, Baxter & Middleton (1958) and they have not differentiated as implied by the dimer hypothesis. A possible origin for the α and β sub-genes can be postulated as follows.

The tendency for a disomic or diploid nucleus to haploidize in a dikaryon puts a premium on diploidy in the wild because the dikaryon is the predominant phase. With diploidy arising at the frequency found in *Coprinus*, i.e. 1×10^{-3}–1×10^{-4}, such nuclei will be continually arising in nature. In these there would be a chance of unequal crossing-over to produce a tandem duplication of closely linked genes, e.g. A_xA_y and B_xB_y. Alternatively a non-reciprocal translocation could produce a chromosome with two different alleles on the same chromosome, but at a considerable distance apart. Both of these would be stable. The closely linked duplication would be similar to the α and β sub-genes in *Coprinus* and the non-reciprocal translocation to the more distantly spaced sub-genes in *Schizophyllum*. It is of course possible that in the widely spaced A complex in *Schizophyllum* both arrangements are present: that both the α and β are themselves two units.

SUMMARY

Artificially selected diploids of *Coprinus lagopus* when mated in compatible combinations, either together or with haploids, produce dikaryotic mycelia which are typical of normal haploid–haploid dikaryons. In a diploid–haploid dikaryon, the diploid nucleus is not as stable as when alone in a monokaryon but it can persist through repeated sub-culturing into a fruiting body and eventually through meiosis into the basidiospores. In a diploid–diploid dikaryon either one or the other nucleus becomes haploid so that fruiting bodies with two diploid nuclei are never formed. This fact constitutes a restriction on diploidy in nature and a useful method of reducing diploids to the haploid state.

Matings that might be considered to be incompatible at the B mating gene show a significant difference which is related to the number of B alleles common to the mating colonies. Matings with one B allele in common, e.g. $B_3B_6 + B_2B_3$ produce fully compatible and normal dikaryons. Matings with two B alleles in common, e.g. $B_3B_6 + B_3B_6$ have, at first while the diploid nuclei still persist, the appearance of an incompatible common B haploid heterokaryon. This indicates that the B incompatibility system is based not on a complementary action between different B alleles but on an oppositional action between the same alleles neutralizing the B gene product which is necessary for dikaryon formation.

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