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Genetical analysis of methionine suppressors in *Coprinus*

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INTRODUCTION

The gene has long been considered as a unit of recombination and also as a physiological unit defined by a complementation test in the double heterozygote. Until some fifteen years ago a gene was thought to be a unit that could have several different alleles which would not complement one another in the double heterozygote and which did not recombine. There was thought to be an absolute correlation between the absence of recombination and absence of complementation. But this is no longer true. Work with the fine analytical techniques now available has shown that low frequencies of recombination are common between alleles that do not complement; but nevertheless low percentages of recombination are still more frequently accompanied by absence of complementation than by its presence. Furthermore, many examples of complementation between some pairs of alleles with extremely low recombination are known in several systems and organisms (Pontecorvo, 1958; Catcheside & Overton, 1958; Giles, 1958; Case & Giles, 1960).

The other well-established correlation, that is between recombination of more than fractional amount and complementation, has so far no true exception. The highest recombination between genes that do not complement in a double heterozygote is 0·5% in *Drosophila pseudoobscura* (Koske & Maynard Smith, 1954), and this is exceptionally high (Pontecorvo, 1958). With higher frequencies of recombination complementation is the rule.

The present study of *Coprinus lagopus* of genes which suppress a mutant requiring methionine has revealed the first real exception to this rule. Two genes 28 units apart do not complement. Thus all the combinations of genetic structure as measured by recombination and physiological function as revealed by complementation are now known.

It would be interesting to consider whether the present example is a rare exception or whether it becomes general and follows the path of previous rare exceptions, such as pseudoalleles and complementation between alleles.

MATERIAL

The cultures were derived originally from fruiting bodies collected in the wild. They were identified as *C. lagopus*. Three wild-type stocks have been used in the experiments, but for the detailed analysis of the suppressors of the *me*-1 locus, two stocks only have been used. Details of these stocks are given in Table 1.

Table 1. *Mating type, methionine loci and origin of the stocks*

		Mating type	Methionine locus	Origin
Wild type	H_5	$A_5\ B_6$	+	Bayford
Methionine mutant	PR2301	$A_2\ B_3$	*me*-1	UV
	PR2205	$A_3\ B_3$	*me*-2	UV
	CR525	$A_3\ B_3$	*me*-3	UV
	P831	$A_3\ B_3$	*me*-4	UV
	G1905	$A_6\ B_6$	*me*-5	UV
	PR7905	$A_5\ B_5$	*me*-6	UV
	M1	$A_6\ B_6$	*me*-7	UV

Life-cycle

Coprinus lagopus has a life-cycle which is fairly typical of its group of fungi, the hymenomycetes, but differs from other groups of fungi (see Fig. 1). The sexual spore produced from meiosis gives rise to a monokaryotic mycelium. This mycelium

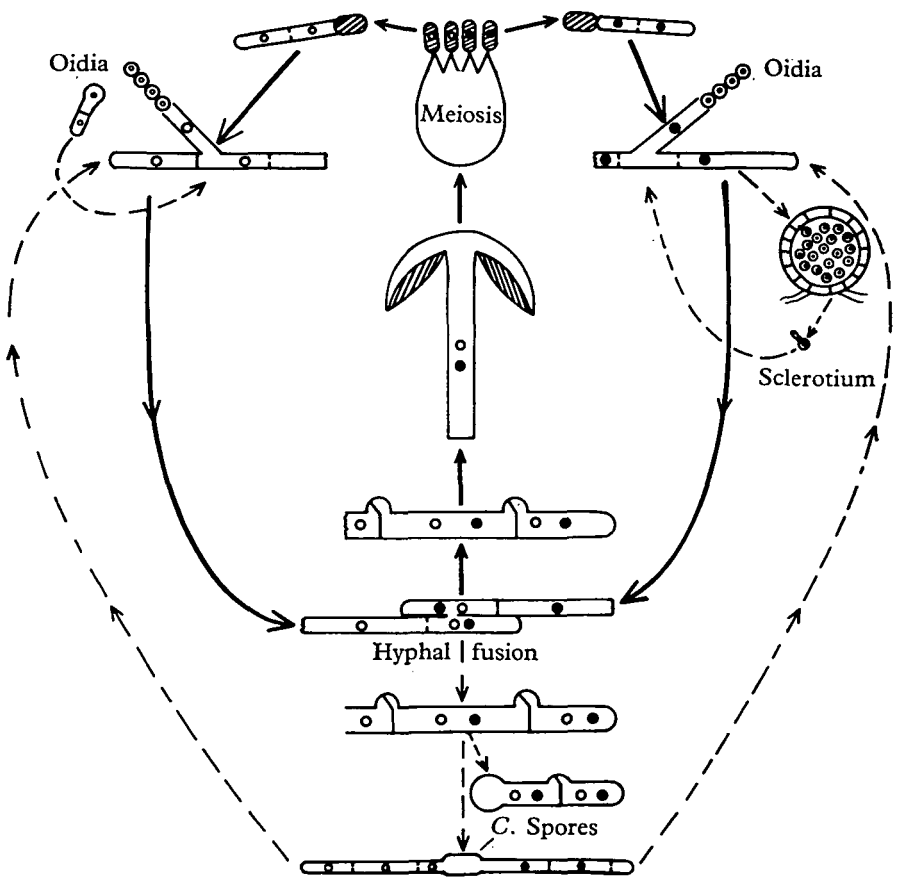

Fig. 1.

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produces abundant uninucleate asexual spores, oidia. Pairs of monokaryons of the right mating types fuse by hyphal anastomosis and produce a dikaryotic mycelium which regularly has two nuclei, one from each parent, in each cell. The mycelium of these two types is very distinct, and this is a factor of some importance in the present studies. The monokaryon has thin mycelium, 3 μ in diameter, has normal transverse cross walls, branching which is at a wide angle, 45–80°, produces asexual oidia and rarely if ever produces fruiting bodies. The dikaryon has thick mycelium, 7 μ in diameter, has clamp connexions at the crosswalls which protrude from the hyphae, branching which is acute-angled at about 20°, does not produce asexual oidia, but produces sexual fruiting bodies (see Plate I (*a*) and (*b*)). The growth rate of the dikaryon is about twice as fast as that of the monokaryon. The dikaryon produces large asexual spores, chlamydospores which are somewhat variable in shape and size but which fall into two main classes: (1) round spores which germinate with a single hypha bearing clamp connexions and are dikaryotic, and (2) long spores which germinate with a hypha from each end, not bearing clamp connexions, and are monokaryotic. These spores are useful for resolving the otherwise stable dikaryon into its component monokaryons without the intervention of nuclear fusion and meiosis (see Plate I (*c*) and (*d*)).

Mating is controlled by two incompatibility loci *A* and *B*, each of which has a large multiple allelic series. A compatible mating leading to the establishment of a dikaryon is obtained between cultures which have different alleles at both the *A* and *B* loci. Unstable heterokaryons can be produced when forced by complementary auxotrophic mutants between stocks having the same *B* alleles but differing at the *A* locus. These produce incomplete clamp connexions—'pseudoclamps'—which readily distinguish them from dikaryons.

The fruiting body in which nuclear fusion occurs followed immediately by meiosis, produces basidiospores in unordered tetrads. A generation can take 10 days under favourable conditions.

METHODS

Culture medium and conditions of culture

The minimal medium found to be most satisfactory for *C. lagopus* is L. Fries' medium (Fries 1953). The nitrogen source is ammonium tartrate and asparagine and the only supplement needed is thiamin. Complete medium includes 0·75 g. casein hydrolysate, 0·75 g. yeast extract, 1·13 g. malt extract and 1·5 ml. nucleic acid hydrolysate per litre. For the production of sexual fruiting bodies sterile horse-dung, 25 g. in half-pint milk bottles, is used. *C. lagopus* will fruit on minimal medium, but the fruiting bodies are small and with some stocks they take much longer to develop than on dung. For mycelial growth cultures were incubated at 37° C.; for fruiting, the cultures were initially incubated at 37° C. for 2 days and then at 26° C.

Selection of methionine-independent prototrophs

Uninucleate oidia of the methionine mutant stocks were obtained in large numbers from tube slant cultures, which were 5 days old. The oidia were suspended in water

by pouring sterile water into the tubes and raking the surface of the agar. The suspension was filtered through a loose fibre-glass filter to remove mycelial fragments, and plated onto solid minimal medium at approximately 5×10^6 per plate. Control plates, for obtaining the percentage germination, were made by plating 200 oidia on solid complete medium. After 4 days at 37° C. the plates were counted for colonies, and isolations were made from the minimal plates to tubes of minimal medium.

Testing the prototrophs

The prototrophs were isolated onto minimal tubes by taking one mycelial transplant from each colony on the minimal plate. The few ungerminated oidia which would be inadvertently transferred were disregarded because they would not germinate in the minimal tubes and would soon be overgrown by the prototrophs. All prototrophs were tested for the type of mutation which had occurred by mating on complete medium each prototroph to a *me*-1 stock with suitable mating-type alleles; for example, stock LR4, $A_5\, B_6$ *me*-1. The dikaryon so formed was then tested on minimal medium by transferring a small piece of agar with dikaryotic hyphae. If the dikaryon requires methionine for growth dikaryotic hyphae grow out from the transplant block into the minimal medium 0·2 to 1·0 mm. only. They then stop growing, and if one or both of the components of the dikaryon can grow on minimal medium, then monokaryotic hyphae grow out from the dikaryon. This can be distinguished without any doubt by the absence of clamp connexions and the wide-angled branching and thin hyphae as contrasted to the clamp connexions, and the acute-angled branching of the dikaryon (see Plate I (*e*) and (*f*)).

If the dikaryon does not require methionine, then it will grow at the normal 5 mm. per day. This test for growth on minimal medium gives an immediate discrimination between certain kinds of mutants. Normal growth on minimal medium of the test dikaryon can result from a back-mutation to a wild allele of the *me*-1 gene

Table 2. *Dikaryon test of prototrophs after mating with* me-*1 and testing growth of dikaryon on minimal medium*

Growth on minimal medium	Dikaryon: Known tester nucleus	Dikaryon: Unknown tester nucleus	Conclusions
+	*me*-1 +	+ +	Back mutation to wild allele of *me* locus
+	*me*-1 +	*me*-1 *Sup*	Mutation to dominant suppressor
−	*me*-1 +	*me*-1 *sup*	Mutation to recessive suppressor
−	*me*-1 +	*me*-1′	Back mutation to recessive 'wild' allele at *me* locus

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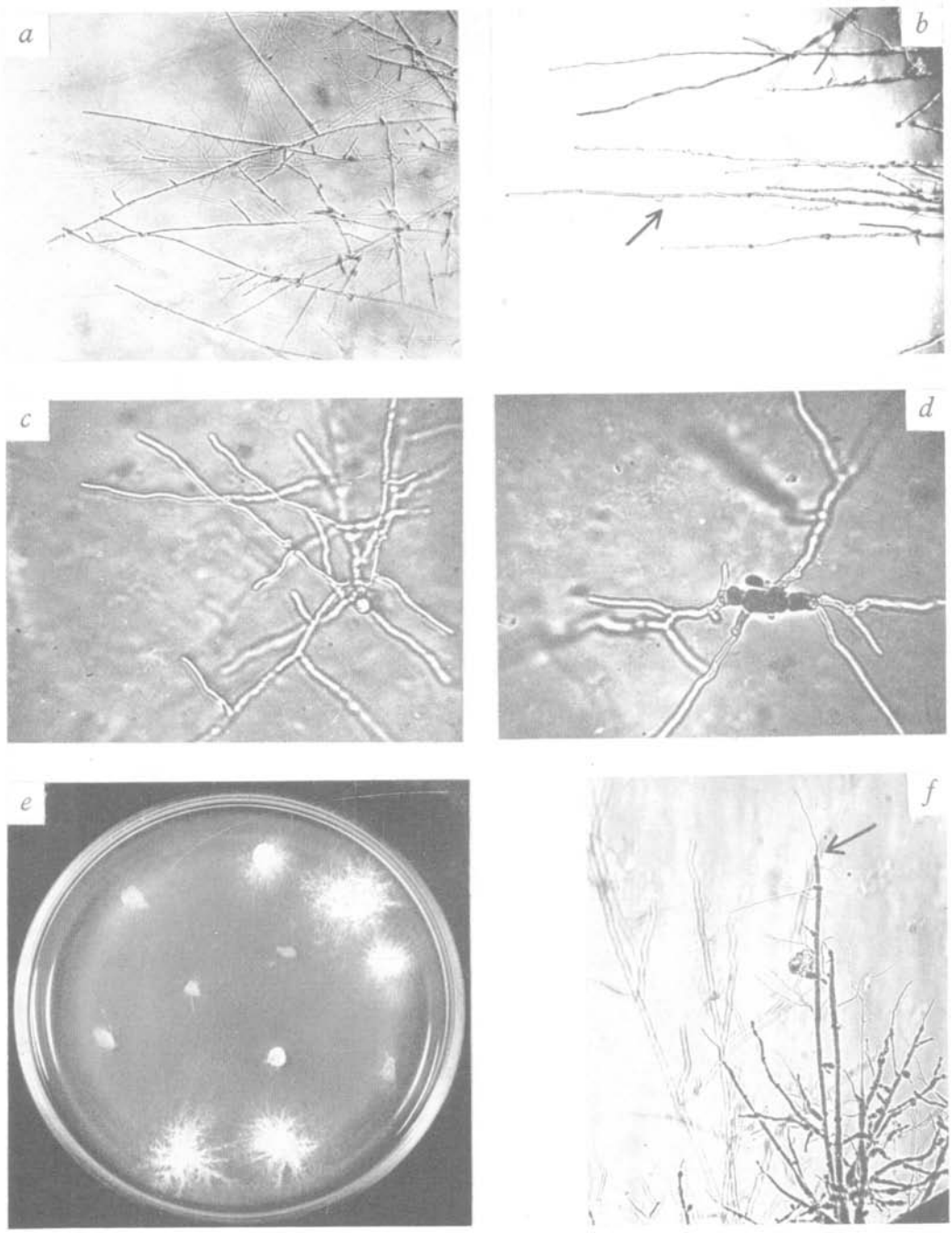

(*a*) Monokaryotic mycelium. × 100
(*b*) Dikaryotic mycelium; arrow points to clamp connexion. × 100
(*c*) Germinating round chlamydospore from dikaryon producing dikaryotic mycelium. × 200
(*d*) Germinating long chlamydospore from dikaryon producing monokaryotic mycelium. × 200
(*e*) Petri dish of minimal medium with eleven transplants of dikaryons—six of the dikaryons are able to grow, five are unable to grow
(*f*) Mycelial growth on minimal medium of dikaryon which will not grow on minimal medium. Left-hand bottom corner, agar block of transplant, arrow points to the point of resolution into the monokaryon—below arrow is dikaryon, above is monokaryon. × 150

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or from a mutation to a dominant suppressor. No growth on minimal medium can result from a mutation to a recessive suppressor of the methionine gene or, if such a possibility exists, to a back-mutation of the methionine gene which has restored wild-type phenotype, but is recessive to the mutant allele which requires methionine (see Table 2).

Treatment of oidia with ultra-violet light

Suspensions of oidia in water were irradiated with a Phillips T.U.V. 6-watt mercury vapour lamp mounted in a glass-lined Perspex housing so that the radiation source was 13 cm. from the oidia. The average energy falling on 1 sq. cm. per second at this distance was 64/65 microwatts. The times of irradiation varied from 4 to 6 minutes. Samples of irradiated oidia were plated on complete medium in order to obtain the percentage kill and on minimal medium in order to obtain prototrophs. The percentage kill varied from 96 to 99%.

RESULTS

Characterization of methionine mutants

The seven methionine mutants are all mutants of different loci, which recombine and which mutually complement in dikaryons. Mutants were tested on complete medium, on minimal medium with methionine, and on medium with known precursors in the synthesis of methionine. The results are given in Table 3.

Table 3. *Growth of methionine mutants in mm. in 4 days on minimal, complete and supplemented media*

	Minimal medium	Complete	Methionine	Homo-cysteine	Cystathio-nine	Cysteine	Homo-serine
me-1	—	15	19	8	0	0	2
me-2	—	15	16	0	0	0	0
me-3	—	17	12	8	0	9	0
me-4	—	19	12	0	0	0	0
me-5	—	25	27	3	0	0	0
me-7	—	19	18	9	3	11	0

Spontaneous and induced mutation rate

The detailed analysis has been made with stock PR2301 *me*-1. Using a total of 28×10^6 oidia in two separate experiments prototrophs of two types appeared spontaneously on minimal medium. There were large diffuse colonies and small compact colonies. The germination of the oidia on complete media was 20%. The total number of prototrophs obtained was 64 large diffuse and 96 small compact colonies. These figures represent frequencies of 2·3 per 10^6 plated oidia for the large colonies and 3·4 per 10^6 for the compact small colonies. If allowance is made for the figure of 20% germination, the spontaneous rate is as follows:

Large diffuse colonies—11·5 per 10^6 viable oidia.
Small compact colonies—17·0 per 10^6 viable oidia.

The other six methionine mutants were tested in a similar way for prototrophs. Mutants *me*-2 and *me*-7 gave prototrophs; *me*-3 gave prototrophs after the oidia had developed at 26° C., but not after the oidia had developed at 37° C. The mutant *me*-6 was too leaky to be able to carry out the test, and mutants *me*-4 and *me*-5 did not give prototrophs under any conditions. Ultra-violet light treatment increased the frequency of prototrophs. A dose of u.v. given to PR2301 *me*-1, which resulted in a 98% kill, produced 260 large diffuse colonies and 460 small compact colonies per 10^6 viable oidia. A dose which resulted in a 40% kill produced 6·5 large diffuse and 4·0 small compact colonies per 10^6 plated oidia. Thus even if the killing of 40% of the spores is not taken into account, there is still nearly a threefold absolute increase in the frequency of large diffuse prototrophs due to the u.v. treatment.

Test for recessiveness and nature of the prototrophs

Sixty-six prototrophs obtained on minimal medium from the oidia of the stock PR2301 *me*-1 mating type A_2B_3 were mated to LR4 *me*-1 A_5B_6. The resulting dikaryons were tested for their ability to grow on minimal medium. Eleven of these prototrophs had been obtained after ultra-violet light treatment, but these appeared to be similar to the spontaneous prototrophs and are not considered separately. All of these dikaryons failed to grow on minimal medium, and all, after variable periods of time, resolved into a monokaryon, which would grow on minimal medium. This monokaryon was the prototrophic component. This clear-cut result showed that the *me*-1 prototrophs are not back-mutations to wild-type at the *me*-1 locus and that they are not dominant suppressors. They are either (1) partial mutations at the *me* locus which are wild in phenotype, but are recessive to the full *me*-1 mutant, or (2) are recessive suppressor mutations at another locus (cf. Table 2).

To test further the prototrophs for the types of mutations a sample of twelve of them were mated to a wild-type stock, H_5. The basidiospores were sown on complete medium and the resulting colonies tested on minimal medium. In all cases auxotrophs appeared in varying percentages from a maximum of 25%. This proved that the change was not at the *me*-1 gene, but at some other gene which suppressed the requirement for methionine. The fact that all the prototrophs analysed are recessive suppressors may represent the full spectrum of prototrophic mutations for the *me*-1 locus. The oidia used in the experiments are uninucleate, therefore recessives as well as dominant mutations, if they occurred, would be selected. During the course of the experiments many hundreds of monokaryotic mycelial transplants containing large numbers of nuclei have been placed on minimal medium and none have sectored to produce a prototroph. The cells of the mycelium are uninucleate, and the fact that no prototrophic sectors appear and that recessive suppressor mutations are common in oidia must mean that a multicellular length of the hyphae acts as a biochemical unit as far as the methionine synthesis and its suppressor system are concerned. Only dominant mutations would be expressed and selected in such a multinucleate hyphal transplant, and the fact that they are not found indicates that dominant suppressors of the system are extremely rare or non-existent.

Complementation

From the crosses of the suppressor mutants to wild-type or to LR4 *me*-1, both of which have $A_5 B_6$ mating type, recombinants of the type $A_5 B_6$ *me*-1 suppressor were selected. These were then crossed to all the other sixty-five *me*-1 prototrophic mutants which have $A_2 B_3$ mating type. The resulting dikaryons were tested on minimal medium. Growth of the dikaryon indicates that the suppressors in the two parents do not complement, failure to grow indicates that the suppressors in the two parents complement one another. The effect of complementation or the lack of it on the growth of the dikaryon and the genetical interpretation are given in Table 4.

Table 4. *Genetic interpretation of dikaryons containing two suppressor mutants*

Components of Dikaryon	Growth on minimal medium	Complementation of suppressor
me-1 *sup*-1 + *me*-1 *sup*-1 +	+	Do not
me-1 + *sup*-2 *me*-1 + *sup*-2	+	Do not
me-1 *sup*-1 + *me*-1 + *sup*-2	−	Do

On the basis of these complementation tests, the sixty-six prototrophs could be readily classified into three groups. Dikaryons formed between components within each group would, as a rule, grow without methionine and therefore did not show complementation. Dikaryons with components of different groups would, in all cases, not grow without methionine and therefore showed complementation. All pairwise combinatorial tests were not made because of the labour of obtaining the necessary recombination stocks with the right mating type.

The results obtained with group 1 prototrophs are given in Table 5. All but seven of the 392 dikaryons which were tested are able to grow without methionine. There are twenty-eight prototrophs in this group and fourteen of them have been tested with all the others. The seven exceptional dikaryons which do not grow on minimal medium and which show complementation are distributed between five of these thirteen which have been fully tested and involve seven of the remaining fourteen. These exceptions have stood up to repetition and appear to be genuine. Sexual recombination data—referred to later—proves this group of prototrophs to be suppressor mutants of the same locus, *sup*-1.

Group 2 mutants have been tested less extensively than group 1. There are nineteen prototrophs in this group, of which five have been tested with all others. All these pairwise dikaryons are able to grow without methionine except two. All the 296 dikaryons containing a component from each of group 1 and group 2 were unable to grow without methionine and therefore showed complementation. From recombination tests this group of suppressors also appears to belong to a single locus, *sup*-2.

The third group of prototrophs contains fourteen different mutants. Owing to difficulties in this group of obtaining suitable recombinants with the mating-type

Table 5. *Tests for complementation between suppressor mutants within group 1 prototrophs. Dikaryons are made between the original prototrophs and their recombinants with suitable mating type.* + = *growth of dikaryon on minimal medium = no complementation between suppressors;* − = *no growth on minimal medium = complementation*

Original prototrophs A_2B_3 *me*-1 *sup*	Recombinant prototroph A_5B_6 *me*-1 *sup*																								
	0	1	3	4	10	11	16	19	23	32	34	36	37	39	40	41	42	43	44	45	47	49	54	59	60	61	65
0	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
1	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
2	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
3	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
4	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
10	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
11	+	+	+	+						+				+			+	+	+		+	−		+	+	+	
16	+	+	+	+						+				+			+	+	+		−	+		+	+	+	
19	+	+	+	+						+				+			+	+	+		+	+		+	−	+	
23	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
32	−	+	+	+						+				+			+	+	+		+	+		+	+	+	
34	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
36	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
37	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
39	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
40	+	+	+	+						+				+			+	+	+		+	−		+	+	+	
41	+	+	+	+						+				+			+	+	+		−	+		+	+	+	
42	+	+	+	−						+				+			+	+	+		+	+		+	+	+	
43	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
44	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
45	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
47	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
49	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
54	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
59	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
60	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
61	+	+	+	+						+				+			+	+	+		+	+		+	+	+	
65	+	+	+	+						+				+			+	+	+		+	+		+	+	+	

loci, only three have been tested with all the others in the group. In general these dikaryons have grown without methionine. There were only three examples of full complementation between the suppressors in which the dikaryon would not grow

Table 6. *Complementation tests within group 3 prototrophs which contains* sup-*3,* sup-*4 and* sup-*5 loci.* + = *between 6–10 mm. growth on minimal medium in 2 days;* +− = *4–6 mm. growth on minimal medium in 2 days;* − = *no growth*

	Original *me*-1 prototrophs													
	sup-3			*sup*-4			*sup*-5				?			
Recombinant	29	30	35	14	25	28	8	13	15	33	17	26	27	31
Sup-3 29	+	+	+	+−	+	+−	+	+	+	−	+	+	+	+
Sup-4 25	+	+	+	+−	+	+−	+−	+−	+−	−	+−	+	+	+
Sup-4 28	+−	+	+	+−	+	+	+	+	+	+	−		+	+

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on minimal medium. But there were also ten dikaryons which grew rather slower than the normal rate for dikaryons which contain the *me*-1 locus and two non-complementary suppressor loci. The results in this group are given in Table 6.

Sexual recombination tests show that this group is by no means homogeneous. The suppressors represent at least two and probably three loci. All the 326 dikaryons formed between this third group and the other two groups of suppressors do not grow on minimal medium and therefore show complementation.

Recombination tests

Tests of recombination between the methionine suppressor genes have been made on a small scale within the groups and between the groups. Within group 1, five different suppressor mutants have been tested for recombination in three crosses. A total of 468 basidiospores were picked from complete medium and tested on minimal. All grew on minimal medium, showing that no recombinant *me*-1+ type had been produced in this number of spores. It is reasonable to assume from this that the group represents mutants at a single locus, and that a selective technique for picking recombinants, if one could be devised in this system, would reveal low frequencies of recombination as has been found within loci in other organisms.

Tests of recombination between group 1 and group 2 suppressors have given, in all three different crosses tested, approximately 25% of basidiospores which did not grow without methionine. Thus suppressor 1 and suppressor 2 show free recombination and are situated on different chromosomes or at least 50 map units apart. Within group 2 prototrophs no recombinants out of 250 tested have been found.

Other information about the location of these two suppressor loci, suppressor 1 and suppressor 2, has been obtained from crosses of these suppressors to the wild-type stock H_5. In all cases with *me*-1, *sup*-1 and *me*-1, *sup*-2 mutants when crossed to H_5, one-quarter of the basidiospore of the progeny required methionine for growth. The expected genotypes are as follows:

Recombinant	$\begin{bmatrix} me\text{-}1 & + \\ + & sup\text{-}1 \end{bmatrix}$	− on minimal medium
Parental	$\begin{bmatrix} me\text{-}1 & sup\text{-}1 \\ + & + \end{bmatrix}$	all + on minimal medium

With free recombination between *me*-1 and the suppressor locus, a quarter auxotrophs are to be expected. Suppressor 1 and suppressor 2 loci are therefore independent of the *me*-1 locus. By testing the three prototrophic genotypes for their mating-type allele an estimate of recombination between the suppressor locus and the *A* and *B* mating-type loci has been obtained. Suppressor 1 shows 33% ± 4% recombination with locus *A*, and suppressor 2 shows free recombination with both *A* and *B* loci.

Recombination tests within the third group of suppressors have given varied results (see Table 7).

From the table it seems probable that three different suppressor loci are included in this group. These may be tentatively designated suppressor 3, which comprises

Table 7. *Crosses between suppressor mutants in the third group; both parents have* me-*1 and a suppressor gene. The percentage recombination between the suppressor mutants is obtained by doubling the percentage of auxotrophs*

Parent of cross	Basidiospores on minimal medium		% recombination
	Total	−	
LR17 (Ms 29 recombinant) × Ms 25	286	41	28
× Ms 28	61	7	23
× Ms 14	86	9	21
× Ms 33	73	20	Free
× Ms 30	97	0	—
× Ms 35	84	0	—
LR57 (Ms 28 recombinant) × Ms 33	30	5	Free

mutants Ms 29, Ms 30 and Ms 35, suppressor 4, which comprises Ms 25, Ms 28 and Ms 14, and suppressor 5, which has Ms 33.

Tests of recombination of these suppressor loci in this third group and the methionine *me*-1 locus are given from crosses to wild-type in Table 8.

Table 8. *Recombination percentage between* me-*1 and the suppressor loci from crosses between Ms stock* me-*1*, sup *and* H_5 *wild-type* + +

	Total	− on minimal medium	% recombination
Ms 29 × H_5	55	8	29·2
Ms 35 × H_5	100	16	32
Ms 25 × H_5	200	2	2
Ms 33 × H_5	96	28	Free
Ms 15 × H_5	96	26	Free
Ms 8 × H_5	100	29	Free
Ms 13 × H_5	96	27	Free

Again three loci are distinguished: suppressor 3 with about 30% recombination with *me*-1, suppressor 4 with about 2% recombination, and suppressor 5 with free recombination with *me*-1. The two methods of distinguishing the suppressor loci agree completely (see Table 9).

Table 9. *Comparison of the grouping of the Ms prototrophs into three suppressor loci on two different recombination tests*

On the basis of recombination between suppressors			On the basis of *me*-1 *sup* recombination		
sup-3	*sup*-4	*sup*-5	*sup*-3	*sup*-4	*sup*-5
Ms 29	Ms 25	Ms 33	Ms 29	Ms 25	Ms 33
Ms 30	Ms 28		Ms 35		Ms 15
Ms 35	Ms 14				Ms 8
					Ms 13

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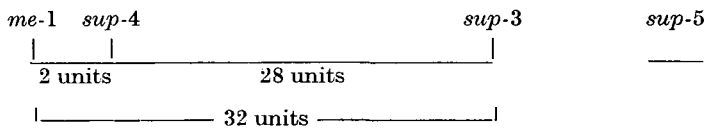
The linkage between *me*-1 and suppressors 3 and 4 is approximately additive and the location of these three suppressor genes can be tentatively summarized as follows:



The question arises that the different recombination values between the different suppressor mutants and between the suppressors and *me*-1 may be due to chromosomal inversions involving one of the suppressor loci. The pairwise complementation tests within this third group show, in all but three exceptional cases, no complementation (see Table 6). This group on the recombinational data contains three well-separated suppressor loci. If the recombinational data are interpreted correctly, then these suppressors show the hitherto unique property of being independent or at most 28 units apart and yet the double heterozygote does not exhibit complementation. Because of this unique situation, and because the recombination data are based on the identification of one of the recombinant classes, detailed tests were concentrated upon one of the dikaryons, Ms 25 × LR17 (Ms 29 recombinant). On the two-suppressor-loci interpretation these components have the genotypes A_2B_3 *me*-1 *sup*-4 + and A_5B_6 *me*-1 + *sup*-3. Two of the auxotrophic progeny obtained from this cross were crossed to a stock which was *me*-1 *sup*-1 and a wild-type stock + + +. In both cases a 1:1 ratio of prototrophs to auxotrophs was obtained in the progeny. This confirmed completely the identification of the auxotroph as *me*-1 + +. The other three presumed classes, two parental *me*-1 *sup*-4 +, *me*-1 + *sup*-3 and the recombinant class *me*-1 *sup*-4 *sup*-3, were identified by backcrossing the prototrophic progeny to the two parents. The progeny were then tested on minimal for auxotrophs. The two parental types should give no auxotrophs with one parent and the 14% auxotrophs with the other parent. The recombinant with both suppressor loci should give no auxotrophs with both parents. Out of seven tested, the following were obtained:

1 recombinant	*me*-1 *sup*-3 *sup*-4
2 parental	*me*-1 + *sup*-3
4 parental	*me*-1 *sup*-4 +

As we expect equal proportions of the parental types and 14% of the recombinant, this limited but conclusive result proves the separation of these two loci with normal recombination between them. The 2% of recombinants between *me*-1 and *sup*-4 estimated on the production of auxotrophs (see Table 8) was also tested further. The prototrophs in the family, wild-type + + × *me*-1 *sup*-4, were again crossed onto *me*-1 + *sup*-1, and the dikaryons tested on minimal medium. If the 2% of recombinants is accurate, then the prototrophs should have contained 49% of + + genotype and 49% of *me*-1 suppressor genotype, instead of 25% of each and 25%

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of + *sup*-4. The reactions on minimal medium of these genotypes in the dikaryon with *me*-1 *sup*-1 is given in Table 10.

Table 10. *The reaction on minimal medium of three prototrophic genotypes in a dikaryon with* me-1 sup-1

Prototrophs	Tester	Expected on independence	Expected on 2% recombination	Growth on minimal medium	Numbers observed
+ + +	*me*-1 + *sup*-1	25%	49·5%	+	50
+ *sup*-4 +	*me*-1 + *sup*-1	25%	1%	+	
me-1 *sup*-4 +	*me*-1 + *sup*-1	25%	49·5%	−	41

The results are in good agreement with the expected on 2% recombination, but differ significantly from the expected on free recombination, $\chi^2 = 5{\cdot}54$, $p = > 0{\cdot}02$. The complete agreement of recombination values rules out the possibility of a chromosome rearrangement being involved.

Suppressor genes and fertility

Coprinus lagopus is similar to *Neurospora crassa* and *Aspergillus nidulans* in being unable to produce fruit-bodies when the dikaryon is homokaryotic for most biochemically deficient mutants despite the fact that adequate supplies of the metabolite are present in the medium. This is true in *Coprinus* for adenine, methionine, choline and nicotinamide mutants. In fact it is true for all the biochemical mutants that have so far been tested on *Coprinus*, with the exception of those that are homokaryotic for para-aminobenzoic acid mutants. Of all the seven methionine loci in *Coprinus* no fruiting has been obtained in homokaryons. If, however, a methionine suppressor gene is present either in a double or a single dose, then they are able to fruit. The effect of these suppressor genes on the ability to produce fruiting bodies is shown below:

Components of dikaryon	Growth on minimal medium	Fruiting
me-1 + + *me*-1 + +	−	No fruiting
me-1 *sup*-1 + *me*-1 *sup*-1 +	+	Fruiting
me-1 + + *me*-1 + *sup*-2	−	Fruiting
me-1 *sup*-1 + *me*-1 + *sup*-2	−	Fruiting

It is perhaps not surprising that the dikaryons which carry suppressor genes which grow on minimal-medium fruit, but the fact that a dikaryon containing one recessive suppressor or complementary suppressors (in which the dikaryon is unable to grow on minimal) also fruits is somewhat surprising. Clearly the suppressor loci are all completely recessive with regard to their effect on the synthesis of methionine and growth on minimal medium, but this is not so in regard to their effect in allowing fruiting to occur. In this respect there is some dominance over the wild-type allele.

DISCUSSION

The present genetic analysis in *Coprinus* shows that at least four and probably five loci, which are widely spaced on the chromosomes, can have recessive alleles that suppress the effects of the methionine mutant *me*-1. How these different suppressor loci and their alleles interact genetically to affect the expression of *me*-1 mutant is known in detail, but what these suppressors are doing biochemically is entirely unknown. There may be a parallel with *Neurospora* in which a suppressor gene acts upon an *me* mutant blocking the cleavage of cystathionine to homocysteine and also on another *me* mutant which blocks an earlier step in the synthesis, the coupling of cysteine and homoserine to form cystathionine (Giles, 1951). The fact that the *sup*-1 in *Coprinus* suppress *me*-1 blocked between cystathionine and homocysteine but not *me*-7 blocked before cystathionine suggests a difference. In *Neurospora* the two blocks in methionine synthesis by the mutants are known to lack the respective enzymes cystathionase 1 and cystathionase 2 (Fischer, 1957). The suppressor restores the production of both enzymes in reduced amount. In general little is known biochemically about the mode of action of suppressor genes (cf. Demerec & Hartman, 1959). There have been suggested two basically different mechanisms: (1) a direct effect on the enzyme restoring its production or a direct repair of some deficiency in the co-enzyme, or (2) an indirect effect removing an inhibition, opening up an alternative pathway. The best-known example of restoration of the enzyme by a suppressor is in tryptophane synthetase in *Neurospora* in which the suppressors are highly specific to certain mutant alleles of the *ts*-locus. But even here the biochemical action appears to be the indirect one of the removal of excess zinc to which the 'mutant' enzyme is excessively sensitive (Suskind & Kurek, 1959). The difficulty of inferring that suppressors which show specificity to certain alleles of the restored gene are restoring the enzyme directly is also brought out by the present study. In the methionine suppressor system in *Coprinus* there is a whole range of action, from non-specific action as shown by the five different suppressor loci which all act upon the same enzyme block, to the highly specific interaction of the suppressor loci and alleles as shown by complementation patterns.

These patterns of complementation between the suppressors have all the usual features well known in other analyses of complementation between pairs of alleles of the same locus (Catcheside & Overton, 1958; Case & Giles, 1960). The complementation pattern found within *sup*-1 locus (see Table 5), as pointed out by my colleague Mr D. H. Morgan, does not, as the data stand, lend itself to a linear arrangement as found in several loci in *Neurospora*. The data in the table on this point, however, are so incomplete that this does not warrant comment without the support of much more detailed analysis of *sup*-1 mutants.

The unique feature about the complementation patterns in the methionine suppressors are the examples of non-complementation between loci that are, in one case, 28 units apart, and in another case, independent. The full explanation of this lack of complementation between distant loci will have to await the biochemical analysis of the system, but certain features about it will be discussed.

The complementation tests are made in a dikaryon so that the lack of complementation between *sup*-3 and *sup*-4 in the double heterozygote is an effect between nuclei and in the cytoplasm. In a haploid nucleus in a monokaryon with wild-type alleles at both suppressor loci there is complementation in the sense that the suppressor system expresses the wild-type phenotype. Of great interest would be the analysis of a diploid or disomic nucleus of the double heterozygote. Would there be complementation under these conditions? Such an analysis has not been feasible in *Coprinus*, but it could be done if a similar case of non-complementation were found in *Aspergillus* or *Neurospora*.

Such a comparison of intra-nuclear and inter-nuclear complementation is relevant from two points of view. Firstly it may help in determining the level of action of the complementation, whether at the RNA template, protein synthesis or at some higher level of dynamic interaction within the cell. Secondly, it may help to explain why such non-complementation between distant loci has not been found before. In other fungi where complementation tests have been made on a large scale in heterokaryons the analysis is based upon a test for complementation between nuclei rather than within a diploid nucleus, except in *Aspergillus* where both intra- and inter-nuclear tests can be made. In *Neurospora* the pseudowild test of Case & Giles (1960) is essentially an intra-nuclear test. In *Aspergillus* and *Neurospora* the intra- and inter-nuclear tests agree, although Case & Giles have reported slight discrepancies between them in the *pan*-2 system.

But so far these analyses in *Neurospora* and *Aspergillus* have been on mutants blocking enzyme action and have not dealt with suppressor genes. It may be that suppressor genes are acting at a different level in synthesis and that such long-distance effects found in *Coprinus* are confined to such genes. As Pontecorvo (1958) suggests, there may be genes which are not directly concerned with protein synthesis and these suppressors may be of this type. The extensive analysis of gene interaction in *Drosophila* has undoubtedly included all types of gene action, and it is tempting to think that the lack of long-distance non-complementation in *Drosophila* is due to the strictly intra-nuclear analysis. But of course a special search may reveal examples. Clearly these suggestions can only be refuted or supported when more examples of long distance non-complementation have been found, and when a close comparison between intra- and inter-nuclear effects are analysed. Perhaps the most likely place to look is in other suppressor systems.

SUMMARY

Seven different genes, *me*-1 to *me*-7, controlling the steps in the synthesis of methionine in the Basidiomycete *Coprinus lagopus* have been tested for the production of prototrophs on minimal medium. Cultures carrying *me*-1 and *me*-7 produced prototrophs spontaneously at a rate of $2{\cdot}6 \times 10^{-5}$. These prototrophs were the result of mutations of suppressor genes and not due to back-mutation of the *me*-gene.

An intensive study of sixty-four mutants of an independent origin suppressing *me*-1 has revealed five different suppressor loci.

Tests for complementation between suppressor mutants and for their recessiveness to the wild allele were made in dikaryons.

All suppressor mutants were recessive to the wild allele. The five suppressor loci were all separated from one another by recombination, twenty-eight map units being the smallest distance between any two pairs. Mutants of the same locus did not complement one another, with few exceptions. Mutants of different loci, as tested in the trans-position in a dikaryon, complemented one another with the exception of pairs between *sup*-3, *sup*-4 and *sup*-5. *Sup*-3 and *sup*-4 are 28 units apart and are independent of *sup*-5 and yet they did not complement. This unique example of long-distance non-complementation is discussed in terms of gene action.

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