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Resistance to amino acid analogues in *Coprinus*: Dominance modifier genes and dominance reversal in dikaryons and diploids

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SUMMARY

Wild-type strains of *Coprinus lagopus* are sensitive to para-fluorophenylalanine and ethionine. Resistant mutants to these two analogues are known but all these mutants are recessive in a heterozygous dikaryon except for F7 (*pfp*^{r}*-3*) which is semi-dominant. Resistance to two other analogues, however – canavanine sulphate and azetidine-2-carboxylic acid – were found to be wild-type features. One strain of *C. lagopus* sensitive to canavanine was identified. Selection for canavanine resistance in monokaryons always yielded only dominant resistance, while selection for para-fluorophenylalanine resistance in monokaryons gave only recessive resistance. Canavanine-resistant mutants were due to a single gene mutation which, like the wild-type resistance, were dominant in heterozygous dikaryons. The wild-type resistance was also dominant in a diploid but the mutant resistance was recessive. Selection for resistance to para-fluorophenylalanine in auxotrophically balanced dikaryons resulted in the identification of two new loci (*pfp*^{r}*-10* and *pfp*^{r}*-11*), and two specific dominance modifiers (*mod*$^+$*-10* and *mod*$^+$*-11*). In the absence of the specific modifier, *pfp*^{r}*-10* and *pfp*^{r}*-11* were recessive while, in the presence of even one dose of the specific modifier, resistance was dominant in the dikaryon. The *pfp*^{r}*-10* and *pfp*^{r}*-11* even in the presence of two doses of modifier were fully recessive in the diploid. The action of the modifier genes and the reversal of dominance in dikaryon and diploid is discussed in terms of negative complementation in an oligomeric product of the *pfp*^{r} gene and localized translation of the relevant mRNA in the cell with the modifier acting as a reinforcer of localization.

1. INTRODUCTION

Genes which modify the dominance of other genes as postulated by Fisher (1931) in his theory of the evolution of dominance, have been amply demonstrated in wild populations of several species by Clarke & Sheppard (1960). A more recent trend in the conception of dominance is not evolutionary but mechanistic. This originated with the success of the *lac* operon concept, which was at first largely based upon the trans-dominance of i^+ on the phenotype of the i^-/i^+ heterogenote and the cis dominance of the o^c mutant (Jacob & Monod, 1961). It was assumed

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that the dominant i^+ produces a product which is not produced by the recessive allele. This concept has been repeatedly used with varied success in regulatory systems (Englesberg *et al.* 1969). The dominance of suppressor genes has also been used in defining their action (Whitfield, 1972).

In a rigorous selection for dominant resistance to amino acid analogues in *Coprinus lagopus*, only recessive resistant mutants have been found but these mutants are accompanied by a mutation of a separate gene that specifically changes the phenotype of the heterozygote from full sensitivity to full resistance. The modifier mutant gene is polymorphic in several wild populations which offers in this system an opportunity to study dominance both from the aspects of evolution and gene action (cf. Goldschmidt, 1938).

A comparison of the effects of the resistance and modifier genes in diploids and dikaryons has revealed the first difference to be reported between these two comparable genomes.

Table 1. *Wild type and marker strains*

Strain	Mating type and markers	Strain	Mating type and markers
RF50	*A51 B52*	MR201	*can^R A5 pab-1 B5*
MP101	*A6 pab-1 B6 met-1*	RS2	*can^s A6 pab-1 B6*
WMR198/66a	*A6 B6 ad-12 nic-4 pab-2*	RS3	*met-1 can^s A51 B52*
MAE192	*ad-8 A5 met-2 B2*	RS7	*ad-12 nic-4 can^s A51 B6*
H_2	*A6 B5*	RS10	*can^s ad-8 A5 B2*
MAE110	*A6 B5*	RS11	*can^s ad-8 A5 B52*
SR68	*A5 pab-1 B5 ad his-1*	RS36	*ad-12 nic-4 can^R A6 B52*
MAE213	*A6 pfp^r-1 B5*	RS37	*ad-12 can^R ad-8 A5 B52*
54	*A1 pfp^r-2 B2*	RS38	*ad-12 nic-4 can^R A51 B2*
F111	*A6 B5 pfp^r-4*	RS46	*ad-12 can^R ad-8 A5 B6*
MAE141	*A6 pfp^r-5 B5*	RS47	*ad-12 nic-4 can^R A6 B2*
MAE277	*A6 pfp^r-6 B5*	RS49	*ad-12 can^R ad-8 A5 B2*
MAE140	*A6 B5 pfp^r-8*	RS61	*ad-12 nic-4 pfp^r-10 mod$^-$-10 A51 B2*
G1905	*A6 met-5 B6*	RS69	*ad-12 nic-4 pfp^r-10 mod$^+$-10 ad-8 A5 B6*
TC1	*A2 B3*	RS72	*ad-12 nic-4 pfp^r-11 mod$^-$-11 A51 B6*
PR28-183	*A2 B1 ad his-1*	RS74	*ad-12 nic-4 pfp^r-11 mod$^-$-11 ad-8 A5 B6*

Symbols and nomenclature

Character	Wild type	Mutant
Reaction to canavanine	*can^R* (resistant)	*can^r* (resistant) *can^s* (sensitive)
p-Fluorophenylalanine	*pfp^s* (sensitive)	*pfp^r* (resistant)
Dominance modification	*mod$^+$* (dominant resistance) *mod$^-$* (recessive resistance)	*mod$^+$* (dominant resistance)

p-Fluorophenylalanine PFP

2. MATERIALS

The life-cycle of *C. lagopus* has been described by Lewis (1961). Wild-type haploid and marker strains are listed in Table 1. Most wild-type strains are sensi-

tive to ethionine and *p*-fluorophenylalanine and resistant to canavanine. Sensitivity to canavanine has been found in only two basidiospore isolates of the wild-type stock, RF, collected at Cluny, France. One of these sensitive isolates, RF50, was the source of all other canavanine-sensitive strains.

3. METHODS

(i) *Culture media*

Details of the composition of minimal, complete and fruiting media used for the culture of *Coprinus* were similar to those described by Lewis (1961). The supplementation of minimal medium for the growth of auxotrophs was similar to that described by Casselton (1965).

The analogues used in this investigation were added to minimal medium at the following final concentrations: DL-ethionine ($6{\cdot}12 \times 10^{-4}$ M); DL-para-fluorophenylalanine (1×10^{-4} M); L-canavanine sulphate (1×10^{-4} M); L-azetidine-2-carboxylic acid (1×10^{-3} M).

Initially, when testing for canavanine resistance/sensitivity there was often inconsistency in the results. This was attributed to sterilization of the medium along with glucose. It was subsequently discovered that if the medium was made up without glucose, sterilized at a pressure of 10–12 lb/in.^{2} for 20 min, and then supplemented with filter-sterile glucose and 1×10^{-4} M canavanine, consistent results were obtained.

(ii) *Culture, UV irradiation, selection, techniques*

The culture techniques used for storage, fruiting and comparison of growth rates and the treatment by ultraviolet light followed those of Lewis (1961) and Casselton (1965). To select for resistance in oidia and chlamydospores they were plated on the appropriately supplemented medium and incubated at 37 °C for 48 h. All plates were overlayered with a layer of agar medium with the appropriate analogue. Fully resistant mutants grow through the overpoured layer after 48 h of incubation.

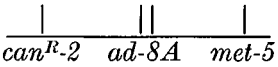
(iii) *Genetical analysis, test for dominance and production of diploid*

Resistant mutant dikaryons were subjected to chlamydospore and basidiospore analysis. After germination the tips of the hyphae from the chlamydospores were excised and isolated. These gave rise to one or both of the component monokaryons. The test for dominance was after Lewis (1961) and Casselton (1965). Resistant haploid isolates were mated to compatible sensitive strains on solid complete medium and incubated at 37 °C. Rectangular strips, cut in the direction of mycelial growth of the dikaryon, were transferred to minimal medium and growth measurements recorded after 24 and 48 h. Diploids were produced and analysed genetically by chlamydospores, basidiospores and by di-mon matings (Casselton, 1965; Casselton & Lewis, 1966).

4. DOMINANCE MODIFIER GENES

(i) *The genetics of wild-type resistance to canavanine*

The canavanine-sensitive strain RF 50 was crossed to six different wild-type canavanine-resistant strains with different auxotrophic markers. The resistant/sensitive colonies segregated as a single gene which was linked with 26 % recombination to *ad-8* on the A mating-type chromosome (Lewis & North, 1974). The position of the *can*^{R}*-2* gene is



The canavanine-sensitive strain RF 50 was originally obtained as one of the two sensitive basidiospore colonies among 35 resistant colonies from a single fruiting body in the wild. Fruiting bodies in the wild, as in the laboratory, are the result of a mating between two, and only two, monokaryons. Therefore if the two parents are different at only one *can*^{R} gene then 50 % of the basidiospore colonies should be sensitive. The two sensitive colonies in 37 implies 2 *can*^{R} genes that are linked (10·8 %) or 3 *can*^{R} genes with loose linkage. Sensitivity would then require mutant alleles at all the 2 or 3 *can*^{R} genes.

Crosses between resistant RF cultures segregated two other *can*^{R} genes which were linked with 14 % recombination, were not linked to the A mating type but were linked to *eth*^{r}*-1* at the right-hand end of the *A* chromosome. The explanation consistent with all the segregating families is that there are at least three genes for resistance to canavanine: the wild-type allele at any one of these will give a resistant phenotype, and all three must be mutant to produce a sensitive phenotype. The RF 50 stock, on this assumption, would have the genotype *can*^{s}*-2 can*^{s}*-3 can*^{s}*-1*. The parents of the wild-type fruiting body which gave rise to RF50 would be

Parent I	*can*^{s}*-2*	*can*^{R}*-3*	*can*^{s}*-1*
Parent II	*can*^{s}*-2*	*can*^{s}*-3*	*can*^{R}*-1*

With 14 % recombination between *can*^{R}*-3* and *can*^{R}*-1* the expected frequency of sensitive progeny from the wild fruiting body would be 7 %. One fully sensitive and one leaky sensitive progeny out of 37 is in good agreement.

(ii) *Selection and analysis of canavanine-resistant mutants*

Chlamydospores from three different auxotrophically balanced dikaryons were treated with UV and selected for growth on minimal medium supplemented with canavanine. Five UV-induced and one spontaneous-resistant dikaryons were obtained. Four of these were subjected to analysis by chlamydospores and basidiospores and resistant segregates were tested for dominance. In all four dikaryons a single dominant resistant gene segregated. All the resistant progeny from sexual crosses were dominant in a heterozygous dikaryon. In one dikaryon one of the components was lethal.

Selection of canavanine resistance in haploid monokaryons resulted in five spontaneous and five UV-induced resistant mutants. All ten when tested in dikaryons were dominant.

(iii) *Mutant PFP resistant dikaryons*

An auxotrophically balanced dikaryon of the following genotype was produced:

RS 10 + + *pfp*^{s} *ad-8 A5 B2*
RS 6 *ad-12 nic-4 pfp*^{s} + *A51 B6*

Chlamydospores from this dikaryon were treated with UV light and sown on MM + 10^{-4} M PFP to select for PFP resistance. The chlamydospores, if left to germinate freely, reproduce the dikaryon. Four resistant dikaryons were obtained but only two of these were successfully analysed. A control batch of spores without UV treatment produced no resistant dikaryons. The two resistant dikaryons are named *pfp*^{r} *dikaryon I UV* and *pfp*^{r} *dikaryon II UV*. A similar selection in another balanced dikaryon gave four resistant dikaryons but all were sterile and could not be analysed.

Resolution of the chlamydospores of *pfp*^{r} *dikaryon I* (*UV*) gave only the RS10 component which was sensitive to PFP like the original, but had slow dense restricted growth. The RS 6 component could not be recovered. The minute hyphal tip which developed from one end of the chlamydospore failed to grow. Since the dikaryon I was vigorous and completely resistant to PFP, it was presumed that the unrecovered RS6 component must carry a PFP-resistant mutant. Either the *pfp*^{r} mutant is also a recessive lethal or a second lethal mutation had occurred in RS6.

The chlamydospores of dikaryon II germinated to give both components. The RS10 component was sensitive to PFP but with slow sparse growth. The RS6 component was resistant but had extremely slow growth.

Both dikaryons I UV and II UV segregated *pfp*-resistant and *pfp*-sensitive basidiospore colonies. The germination was low and segregations of resistance and marker genes were disturbed. This is probably a consequence of the recessive lethality and slow growth shown by the chlamydospore analysis. The resistant segregates of both dikaryons, when tested in a dikaryon with sensitive strains, were found to be of two types: dominant and recessive resistance. The radial growth of a dikaryon on minimal medium is 22–26 mm in 48 hr; a dikaryon heterozygous for dominant resistance has a radial growth of 20 mm on PFP; a dikaryon heterozygous for recessive resistance has a radial growth of 4 mm. Dikaryon I produced eight dominant and ten recessive resistant colonies (Plate 1*a*, *b*). From a cross of a basidiospore segregate of dikaryon II 5 were dominant and 16 recessive. A detailed analysis of these and other crosses showed that two genes were segregating in each dikaryon. In dikaryon I the two genes were a recessive resistance gene, *pfp*^{r}*-10* and a dominance modifier *mod*$^{+}$*-10*. These were not linked and also not linked to *ad-8*, A or *met-5* on chromosome I or to *nic-4* on chromosome IV and more importantly not linked to B on chromosome II.

In dikaryon II UV the recessive resistance gene *pfp^r-11* and modifier gene *mod$^+$-11* were linked with $20 \pm 5{\cdot}8\,\%$ recombination. The *pfp^r-11* gene was linked to B on chromosome II with $33{\cdot}7 \pm 3{\cdot}3\,\%$ recombination. The main conclusion from the genetic and linkage tests is that both dikaryons have a recessive resistance gene and a dominance modifier, and that these *pfp^r* genes are different in the two dikaryons, the modifiers may or may not be different.

(iv) *Complementation and specificity tests*

The two recessive resistant genes were tested for complementation in a dikaryon which was heterozygous both for *pfp^r-10* and *pfp^r-11* in the absence of the two modifiers. The dikaryon was fully sensitive to PFP, thus showing that they were functionally two different genes. Dikaryons which were heterozygous for one of the *pfp^r* (*pfp^r-10*) genes and with the other modifier (*mod$^+$-11*) were sensitive, thus showing complete specificity of the modifier for its *pfp^r* gene.

Dikaryons containing either the *mod$^+$-10* or *mod$^+$-11* and which were heterozygous for the known recessive resistance genes *pfp^r 1*, *2*, *4*, *5*, *6* and *8* were all sensitive to the analogue, thus showing that the modifiers have no effect on these *pfp^r* genes.

(v) *Origin of the modifiers*

Wild-type stocks were mated to resistant *pfp^r-10* and *pfp^r-11* isolates and the dikaryons tested for resistance. The results of this test revealed that the modifier genes are a regular and common feature of wild populations.

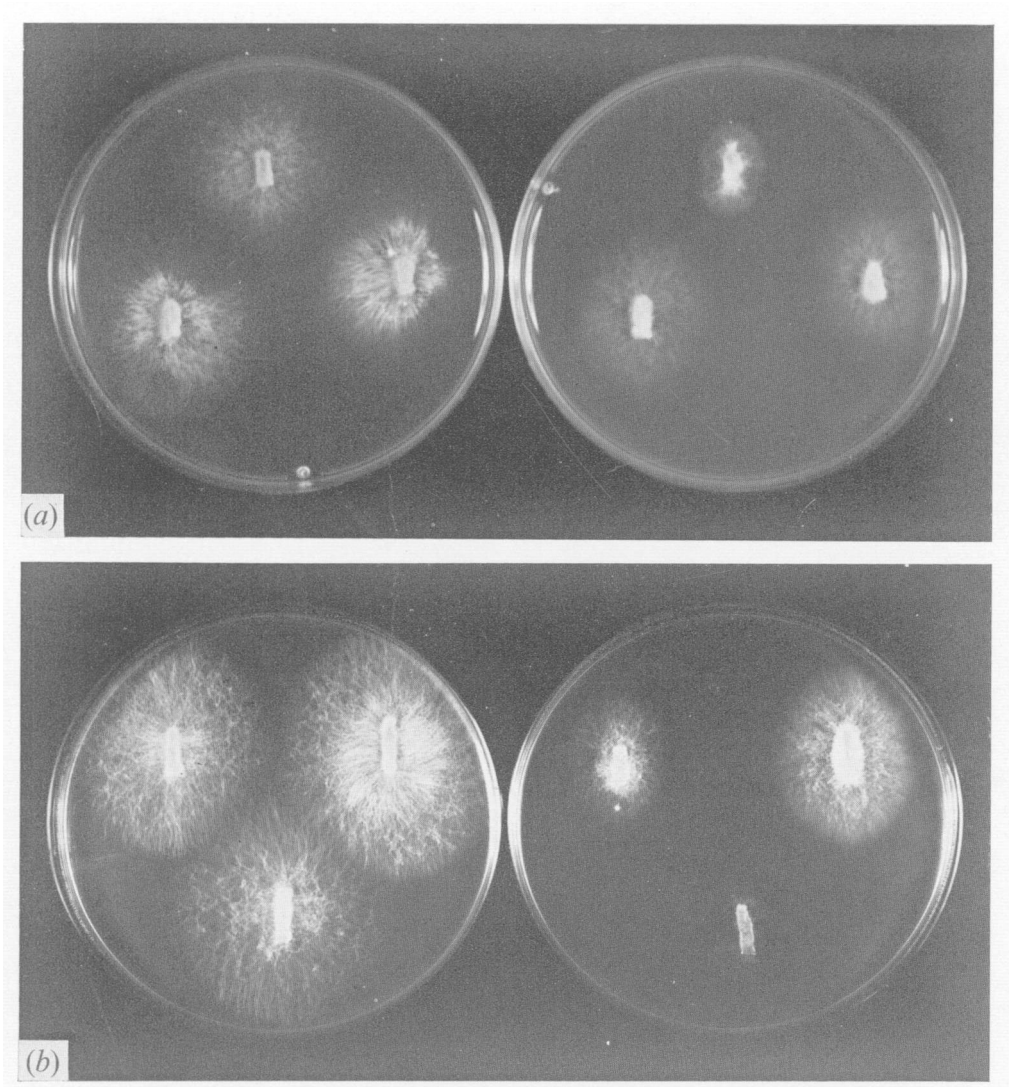
The stocks tested could be divided into four categories:

	No.
(1) Lacking *mod$^+$-10* and *mod$^+$-11*	16
(2) Carrying only *mod$^+$-10*	1
(3) Carrying only *mod$^+$-11*	9
(4) Carrying both *mod$^+$-10* and *mod$^+$-11*	17

Because the modifiers were common in wild-type stocks the original components of the dikaryons I UV and II UV were tested for modifiers, component RS10 lacked both *mod$^+$-10* and *mod$^+$-11* the other component lacked *mod$^+$-10* but no suitable stocks were available to test *mod$^+$-11*. It can be concluded that in the mutation experiment *mod$^+$-10* arose by mutation in dikaryon I but that *mod$^+$-11* arose either by mutation or was originally present in dikaryon II.

(vi) *Selection of dominant resistance in monokaryons and diploids*

More than 200 PFP-resistant mutants have been selected in monokaryons over the last few years and all that were tested for dominance have been recessive except one which was semi-dominant (Barker & Lewis, 1974). With the knowledge of the modifier gene and the slow growth of the resistant component in a dikaryon a search was made for dominant resistant mutants in a monokaryon with the active modifier, *mod$^+$-11*. Oidia from RS^3 *met-1* A_{51} *pfp^s mod$^+$-11* B_2 were selected for resistance to PFP. Both UV-induced and spontaneous mutants were tested.



(*a*) Left, control plate. Right, PFP plate. Minimal medium, 1×10^{-4} M. *pfp*^{r} *dikaryon I* (*UV*), culture: top, RS67 × RF50; bottom left, RS67 × MAE110; bottom right, RS67 × H2. All dikaryons heterozygous (*pfp*^{r}-*10*) × (*pfp*^{s}), 48 h growth.
(*b*) Left, control plate. Right, PFP plate. Minimal medium, 1×10^{-4} M. *pfp*^{r} *dikaryon I* (*UV*), culture: top left, RS82 × RF50; top right, RS81 × RF50; bottom, RS82 × RF50. All dikaryons heterozygous (*pfp*^{r}-*10*) × (*pfp*^{s}), 48 h growth.

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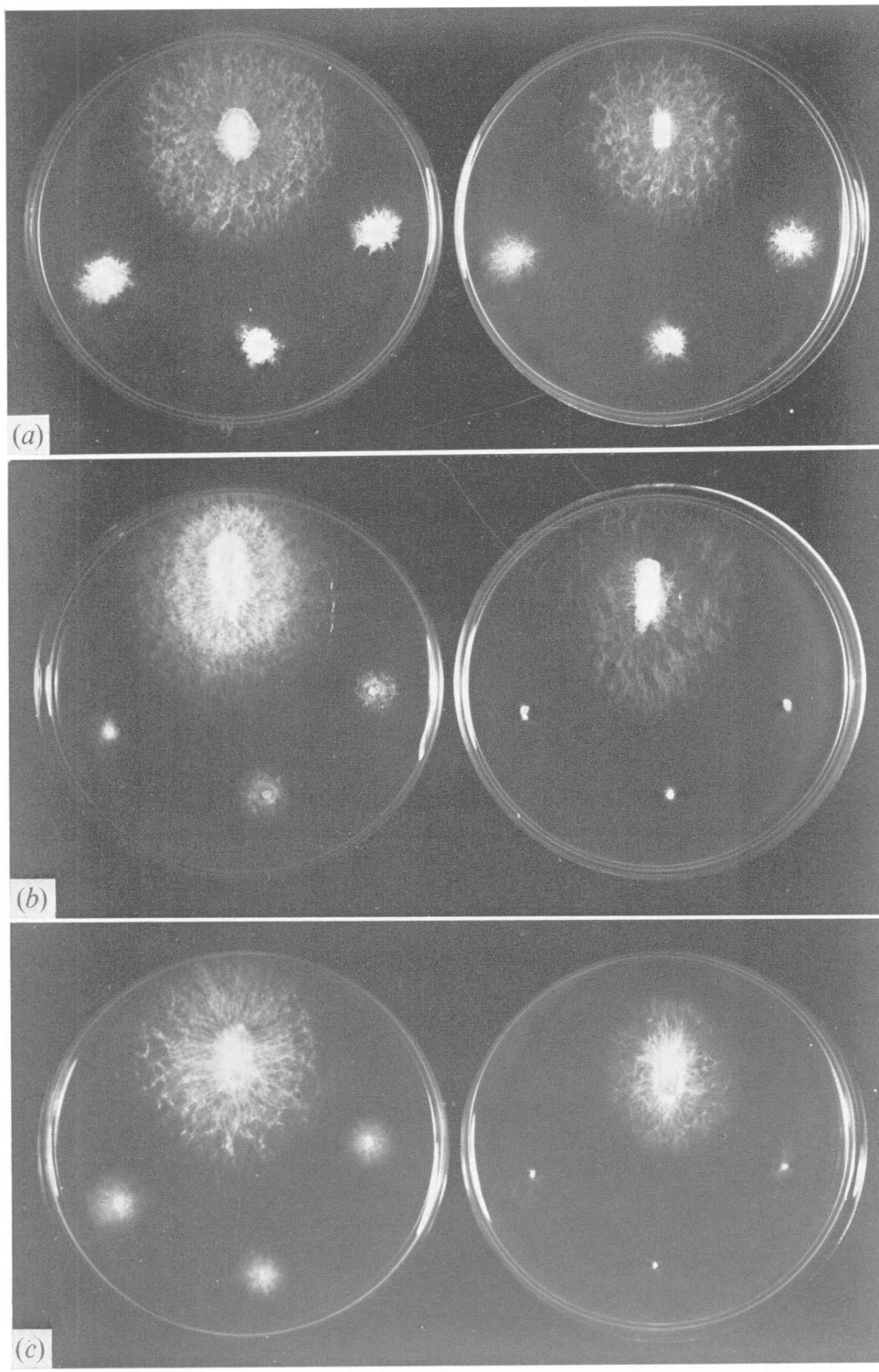

PLATE 2. For legend see facing page.

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All were recessive including seven resistant colonies which had restricted slow growth.

Table 2. *Growth rates of strains with* can^R, pfp^r-10, pfp^r-11, mod^+-10 *and* mod^+-11 *genotypes*

Strain	Genotype	Radius in 24 h (mm)
MAE10	Wild type, *mod⁺-10 mod⁺-11*	10·3
H1	Wild type, *mod⁺-10 mod⁺-11*	8·5
TC1	Wild type, *mod⁺-10 mod⁺-11*	7·4
RS52	*can^r* mutant *can^r* mutant	8·8
RS72	*pfp^r-11*	7·3
RS78	*pfp^r-11 mod⁺-11*	6·3
RS71	*pfp^r-11*	6·0
RS61	*pfp^r-10*	3·0
RS81	*pfp^r-10 mod⁺-10*	2·2
RS69	*pfp^r-10 mod⁺-10*	0·7

(All wild types are canavanine-resistant and para-fluorophenylalanine-sensitive.)

Oidia of analogue-sensitive diploids were selected for dominant resistance. From repeated and extensive tests only one semi-dominant canavanine-resistant and one semi-dominant PFP-resistant mutant were obtained. Genetical analysis by mating dikaryons with the diploids by the methods of Casselton (1965) and Casselton & Lewis (1966) were not successful because of instability of the diploid when put into a dikaryon. The significance of this failure to produce resistant diploids will be apparent after the results of *pfp^r-10* and *pfp^r-11* and their modifiers in diploids have been described in the next section.

Because PFP resistance is not a wild-type feature and canavanine resistance is, a comparison of growth rates is of interest. Canavanine-resistant mutants obtained in the experiments did not differ from wild type in their growth rate (Table 2). The growth rate of the dominant PFP-resistant strains was slower than wild-type-sensitive strains. This difference was most marked in the *pfp^r-10 mod⁺-10*. Outcrossing improved the growth rate of resistant strains. Recessive resistant isolates without the modifier had faster growth rates than the comparable resistant strains with the modifier. PFP-sensitive strains with the modifier had normal growth rate.

LEGEND TO PLATE 2

The petri dishes on the left contain minimal medium; the ones on the right contain minimal medium with PFP. In each dish there is one dikaryon at the top and three diploids below.

(*a*) Cultures are heterozygous for wild-type canavanine resistance, *Can^R/can^s*.

(*b*) Cultures are heterozygous for mutant canavanine resistance, *can^r/can^s*.

(*c*) Cultures are heterozygous for PFP resistance *pfp^r-11/pfp^s* in the presence of one dose of the modifier, *mod^+-11*.

5. DOMINANCE REVERSAL IN DIKARYONS AND DIPLOIDS

A wild-type canavanine-resistant strain, MR201, which, as shown earlier, exhibits dominant resistance in a dikaryon, was forced into a diploid with the canavanine-sensitive strain, RS11. Again resistance was dominant in the diploid (see Plate 2*a*).

To study the behaviour of the mutant genes for canavanine and PFP resistance in heterozygous dikaryons and diploids, two resistant strains and two sensitive strains were arranged to form a foursome group as shown in Table 3. The ideal would have been to use the same components to make the dikaryon and the diploid. This is not possible, however, since for dikaryon formation compatibility at both the A and B loci of the component monokaryons is necessary. For diploid formation, a common A allele and compatibility of the B allele is necessary. In some cases it was not possible to complete the foursome because of the difficulty in producing the right stocks.

Table 3. *The foursome arrangement of matings used to make compatible heterozygous dikaryons and diploids*

	Dikaryon	Diploid
1. Resistant mutant Ax Bx 2. Resistant mutant Ay Bx	1 × 3	1 × 4
3. Sensitive strain Ay By 4. Sensitive strain Ax By	2 × 4	2 × 3

Table 4. *The resistance/sensitivity to canavanine of the foursome test for three canavanine-resistant mutants*

	can^r *dikaryon II* (*UV*)	*can*^{r} *dikaryon III* (*spon*)	*can*^{r} *dikaryon IV* (*UV*)	Dikaryon	Diploid
1	RS 36	RS 38	RS 47	1 × 3	1 × 4
2	RS 37	RS 46	RS 49	resistant	sensitive
3	RS 10	RS 11	RS 11	2 × 4	2 × 3
4	RS 2	RS 3	RS 2	resistant	unobtainable

The reactions of isolates from three canavanine-resistant mutant dikaryons *II UV*, *III spon* and *IV UV* are given in Table 4 (see also Plate 2*a*). Diploid 2 × 3 could not be constructed. All three mutant resistant isolates differ from wild-type resistant by being dominant in a heterozygous dikaryon but recessive in a heterozygous diploid (Plate 2*b*).

The PFP-resistant isolates with the genes *pfp*^{r}*-10* or *pfp*^{r}*-11* with their respective modifiers are compared in dikaryons and diploids in Table 5 (see plate 2*c*). A summary of the different combinations of *pfp*^{r}*-10* and *pfp*^{r}*-11* with their modifiers is given in Fig. 1. All the dikaryons heterozygous for the *pfp*^{r} allele and for the modifiers are resistant; that is, *pfp*^{r} is dominant: this is true both when the active

modifier allele is in the nucleus with the *pfp*^{r} allele or in the other nucleus with the *pfp*^{s} allele. All diploids are sensitive; that is, *pfp*^{r} is recessive, even with two doses of the modifier allele.

Table 5. *Genotypes of dikaryons and diploids and their resistance/sensitivity to* p-*fluorophenylalanine*

Dikaryons	Reaction to PFP
pfp^r *dikaryon I (UV)*	
1 × 3 (*pfp*^{r}-*10 mod-10*) × (*pfp*^{s}-*10 mod*$^+$-*10*)	Resistant
2 × 4 (*pfp*^{r}-*10 mod*$^+$-*10*) × (*pfp*^{s}-*10 mod*$^-$-*10*)	Resistant
pfp^r *dikaryon II (UV)*	
1 × 3 (*pfp*^{r}-*11 mod*$^-$-*11*) × (*pfp*^{s}-*11 mod*$^+$-*11*)	Resistant
2 × 4 (*pfp*^{r}-*11 mod*$^-$-*11*) × (*pfp*^{s}-*11 mod*$^+$-*11*)	Resistant
Diploids	
pfp^r *dikaryon I (UV)*	
1 × 4 (*pfp*^{r}-*10 mod*$^-$-*10 pfp*^{s}-*10 mod*$^-$-*10*	Sensitive with slight peripheral growth
2 × 3 *pfp*^{r}-*10 mod*$^+$-*10 pfp*^{s}-*10 mod*$^+$-*10*	Sensitive with slight peripheral growth
pfp^r *dikaryon II (UV)*	
1 × 4 = *pfp*^{r}-*11 mod*$^-$-*11 pfp*^{s}-*11 mod*$^+$-*11*	Sensitive with slight peripheral growth
2 × 3 = *pfp*^{r}-*11 mod*$^-$-*11 pfp*^{s}-*11 mod*$^+$-*11*	Sensitive with slight peripheral growth

6. DISCUSSION

(i) *Wild type and mutant resistance*

Coprinus in nature exists for the main part as a dikaryon. Its genetic variation depends mainly upon sexual reproduction by the dikaryotic fruiting body and the resulting haploid monokaryotic basidiospores. These germinate to give monokaryons, which under natural conditions rapidly fuse to give dikaryons. Any character of great selective value would have to be expressed in both the dikaryon and monokaryon. The diploid condition is a laboratory artifact which has no significance in the wild and in the evolution of the organism because it is rare, has to be forced and is unstable in the dikaryon (Casselton, 1965; Casselton & Lewis, 1966).

Wild-type characters are usually dominant to mutant characters (Fisher, 1931) and this has been found with resistance to canavanine which is dominant and the wild-type character. A rare sensitive culture was found to be mutant at three loci; all three have to be mutant to produce the sensitive phenotype. This implies that there are three reiterated and parallel pathways to resistance any one of which, if complete, will give the resistant phenotype. Selection both in monokaryons and dikaryons readily produced single gene mutants which gave a dominant resistant phenotype in the dikaryon. The genetic background in the species was homogeneous for the expression of dominance of these genes. All these

features are to be expected from the assumption that resistance to canavanine, and some other phenotypic characters with greater selective importance which might accompany it, have been a long established part of the wild-type monokaryon and dikaryon.

In contrast, sensitivity to PFP is the wild character. Resistant mutants when selected in monokaryons are recessive and represent nine separate gene loci. When rigorous selection in a dikaryon is practised dominant resistance is obtained, but this is the result of four gene mutations:

(1) *pfp^r-10* or *pfp^r-11*	recessive resistance,
(2) *mod$^+$-10* or *mod$^+$-11*	specific dominant dominance modifiers,
(3) *let.*	recessive lethal in Dik I, semi-lethal in Dik II,
(4) *slo.*	recessive slow growth in Dik I and Dik II.

The *pfp^r* allele is not found in wild populations but the modifier is found in about half the wild-type stocks tested. No other effect of the modifier has yet been found. This polymorphism of the modifier implies that it has some other effect which is important to the organism. It also supports Haldane's (1930) prediction that a dominance modifier to operate in the Fisherian sense would have to possess wild type fitness, and 'will be fairly common in the population'.

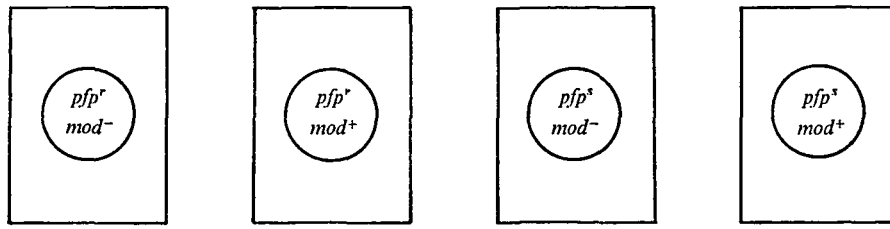
The four main biochemical ways in which resistance to an amino acid analogue may be achieved (Fowden, Lewis & Tristram, 1967) are: (1) a lack of a specific permease; such mutants are usually recessive; (2) a changed amino-acyel tRNA synthetase; such mutants are usually recessive; (3) a new metabolic pathway which will detoxify the analogue by breaking it down; such mutants could be recessive or dominant; (4) a breakdown in the regulatory system so that there is an overproduction of the particular normal amino acid; such mutants could be (*a*) a defective feedback inhibition (these would be recessive or at most semi-dominant) or (*b*) a non-repressible enzyme synthesis; these could be recessive or dominant.

Of these four methods listed, (1), (2) and (3) are more applicable to resistance which is the wild-type character such as canavanine resistance in *C. lagopus*. Examples of the three methods have been found in wild-type resistance (Dunnill & Fowden, 1965; Peterson & Fowden, 1963). The over-production of an amino acid as in method (4) is unlikely to be a wild-type character because it would be wasteful to cellular economy.

(ii) *The behaviour of the modifier genes* mod$^+$-10 *and* mod$^+$-11

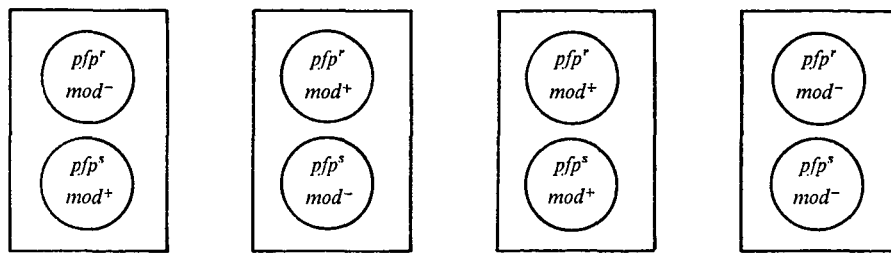
A comparison of the sensitive and resistant phenotypes obtained in monokaryons, dikaryons and diploids is helpful in limiting the possible explanations for the action of the modifier genes. Since both *mod$^+$-10* and *mod$^+$-11* react with their respective *pfp^r* in exactly the same way, only *mod$^+$-10* will be discussed. The reactions of the *pfp^r-10* mutant and its modifier in monokaryons are summarized in Fig. 1(*a*) which gives a diagrammatic representation of the four possible combinations between the *pfp^r* gene and the modifier. It is concluded that resistance

(*a*) Monokaryons



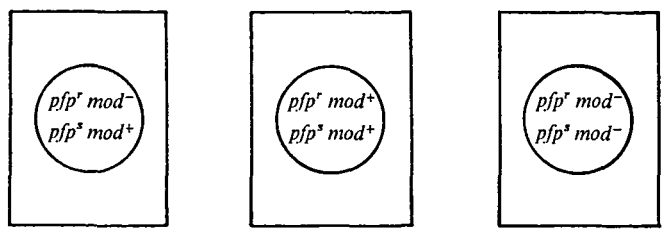
1 Resistant | 2 Resistant | 3 Sensitive | 4 Sensitive

(*b*) Dikaryons



1 Resistant | 2 Resistant | 3 Resistant | 4 Sensitive

(*c*) Diploids



1 Sensitive | 2 Sensitive | 3 Sensitive

Fig. 1. The comparison of the resistance/sensitivity to PFP in different genotypes in monokaryons, dikaryons and diploids.

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is caused by either one of (*a*) the presence of an active *pfp*^{r} allele or (*b*) the absence of an active *pfp*^{s} allele or (*c*) the *pfp*^{r} allele gives resistance but only in the absence of an active *pfp*^{s} allele. It must also be concluded that the presence or absence of the *mod*$^+$ or its allele do not affect the degree of resistance in the monokaryon. From the dikaryons shown in Fig. 1(*b*) and the result obtained in dikaryon 4, the alternative action (*a*) is ruled out, but (*b*) and (*c*) are equally possible. From dikaryons 1, 2 and 3 the explanations (*b*) and (*c*) in its simplest form are also excluded. A modified form of (*c*) is possible. The modified form of (*c*) is that the *pfp*^{s} allele can be present for the expression of a resistant phenotype but its active product must not interact or combine with the product of the *pfp*^{r} allele. From the results of the dikaryons we can conclude that the modifier, *mod*$^+$, prevents the interaction of *pfp*^{r} and *pfp*^{s} products. This could be caused by keeping the *pfp* products separate in the cell, either by localized synthesis or localized transport. The modifier allele has its full action in the dikaryon whether it is in single or double dose and whether it is in the nucleus containing the *pfp*^{r} allele or in the one containing the *pfp*$^+$ allele.

If the action of the modifier is to localize the synthesis of the *pfp* product close to the nuclear membrane the modifier should have no effect in a diploid where both *pfp*^{r} and *pfp*^{s} products would be synthesized side by side near the same nucleus. The results of the diploids, Fig. 1(*c*), fully confirm this. Even with two doses of *mod*$^+$ the diploid is sensitive.

Without biochemical studies it is premature to speculate on the nature of the gene products, but suffice it to say that negative complementation by the production of a hybrid oligomer (Fincham, 1966; Muller Hill, Craps & Gilbert, 1968; Lieberman, Buchanan & Markovitz, 1970) would be consistent with the results. The oligomer might be an enzyme or a ribosomal component. The nearest parallel may be the dominance in a heterozygous diploid yeast caused by hybrid enzyme formation (Zimmerman & Gundelach, 1969).

Pontecorvo (1952, 1963), Luig (1962), Roberts (1964) and Ayling (1969) have observed differences in dominance and complementation between heterozygous allele pairs in diploids and heterokaryons of *Aspergillus nidulans*. Casselton & Lewis (1967) found similar differences in *C. lagopus* between heterokaryons and dikaryons and diploids. But the dikaryon and diploid were identical. This was confirmed for the dikaryon and diploid by Day & Roberts (1969). The similarity in behaviour of diploids and dikaryons in contrast to that of heterokaryons has been attributed by Casselton & Lewis (1967) to the extreme dilution of cytoplasmic gene products in heterokaryons. The present interpretation on the modifiers is an application of this dilution concept to the immediate vicinity of the nucleus.

One of the puzzling points about the *pfp* system is that there are two separate and non complementary *pfp* genes each with its own specific modifier.

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