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Resistance to *p*-fluorophenylalanine in diploid/haploid dikaryons: dominance modifier gene explained as a controller of hybrid multimer formation

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SUMMARY

The gene *pfp*^{r}*-10* in *Coprinus* confers resistance to *p*-fluorophenylalanine. The resistance of the heterozygote *pfp*^{r}*-10*/*pfp*^{s}*-10* is completely recessive, both in diploids and dikaryons. A dominance modifier *Mod*$^+$ makes *pfp*^{r}*-10* dominant in a dikaryon but not in a diploid. It also enhances the degree of resistance in *r* homozygotes. These results are explained on a hypothetical model based upon a hexameric product of the *pfp-10* gene, with resistance to PFP being proportional to the percentage of homo r hexamers. The *Mod*$^+$ is presumed to act by keeping the r and s products separate so that hybrid multimers are reduced to a minimum. Critical threshold concentrations of PFP for diploid/haploid dikaryons in 40 different combinations of *pfp*^{r} and *Mod*$^+$ genes cover a wide range of gene doses and percentage of homo r multimers. The relationship between the critical threshold concentrations and the calculated percentage of homo r multimers supports the model.

1. INTRODUCTION

Extensive selection experiments for resistance to the inhibitory concentration of 1×10^{-4} M *p*-fluorophenylalanine, using haploids of *Coprinus lagopus*, have given only mutants which are recessive in a dikaryon. Selection in diploids has given no resistant mutants;* recessive mutants would not be expressed and presumably dominant mutations are either lethal in the haploid or are not produced. Selection in haploid/haploid dikaryons results in resistant mutant dikaryons. These resistant dikaryons are the result of a matched pair of mutations, a recessive resistance gene and a specific dominance modifier (Senathirajah & Lewis, 1975). The presence of the modifier confers resistance on a dikaryon which is heterozygous for the otherwise recessive resistance gene. The modifier, however, has no effect in a similarly heterozygous diploid. The actions of the modifier and the resistance gene have been explained by assuming that the product of the *pfp-10* gene is a multimer and that resistance results from the presence of homo r multimers and that these are pro-

* *Note added in proof.* By using haploid stocks with *Mod*$^+$ and high concentrations of PFP, mutants dominant in dikaryons have now been obtained in haploids (Lewis & Talmud unpub.). A rare class of mutants dominant in diploids has also been obtained (M. Jehan unpub.).

duced in low amounts when the *r* and *s* alleles are in the same nucleus because of the proximity of the respective messenger RNAs. When *r* and *s* are separated in different nuclei, the messenger RNAs and the translational sites can be kept separate, but only by the presence of *Mod*$^+$. The *Mod*$^+$ localizes the aggregation and probably the translation process. This novel gene action can be readily perceived only by comparison of a diploid and a stable dikaryon in the same organism. *Coprinus lagopus* is, at present, the only organism with these in a readily available and stable form for study. The paired gene action may, therefore, be a feature of other organisms and they may be characteristic of genes affecting membranes or ribosomes which are multimolecular structures.

The present work was designed to substantiate or refute the localization hypothesis and to exclude or suggest alternative explanations based upon gene dosage or on special properties of a diploid nucleus. Dikaryons with a diploid and a haploid nucleus have been tested for resistance in the 40 possible combinations of modifier and resistant genes and their alleles. Tests with a graded series of concentrations of *p*-fluorophenylalanine from 1×10^{-6}–1×10^{-2} M have provided a quantitative estimate of the effect of the modifier and the *pfp*^{r} gene in all the combinations. The additional data support the hypothesis and have given confidence to specify the molecular mechanism more precisely in terms of a hexameric gene product.

2. MATERIALS AND METHODS

Details of culture methods, media, production of diploids and many of the monokaryotic haploid stocks used are given in Senathirajah & Lewis (1975). Details of diploid stocks produced and used in the course of this work are given in Table 1.

(i) *Testing for resistance and sensitivity of cultures*

This was done by measuring the radius of colonies at 24 and 48 h on agar medium incubated in the dark at 37 °C with and without 1×10^{-4} M DL *p*-fluorophenylalanine (PFP). The ratio of the radii on the two media expressed as a percentage was used as a criterion of resistance. At the concentration used the two classes, sensitive and resistant, could be distinguished unambiguously. After the initial classification each culture was tested on a range of concentrations from 1×10^{-6} to 1×10^{-2} M in intervals of 2·5. The highest concentration which gave $< 50\,\%$ growth was denoted as the *critical threshold concentration*.

(ii) *Diploid/haploid dikaryons*

The dominant phase of the life-cycle of *Coprinus* in nature is a stable, long-lived dikaryon with two haploid nuclei in each cell. An artificial dikaryon with one diploid and one haploid nucleus is made by mating a diploid and haploid monokaryon which have compatible alleles of the mating type genes *A and B*. The diploid has complementing, forcing auxotrophic mutations, and the haploid may or may not have one or more auxotrophic markers. These dikaryons are stable and show all the main features of the natural haploid/haploid dikaryon. The only difference is

Table 1. *Stocks used in different diploid combinations*

Combination	Diploid no.	Source of the diploid	Mating type	Markers present	
r+r+	D13	RS96 × RS66	*A5B5 × A5B6*	RS96:	*pab-1 pfp^r-10 ad his-1 Mod$^+$-10*
	D14	,,	,,	RS66:	*ad-8 pfp^r-10 ad-12 Mod$^+$-10*
	D15	,,	,,		
r+r−	D6	RS96 × RS67	*A5B5 × A5B6*	RS67:	*ad-8 pfp^r-10 ad-12 Mod$^-$-10*
	D9	,,	,,		
	D21	RS96 × RS62	*A5B5 × A5B6*	RS62:	*ad-8 pfp^r-10 ad-12 Mod$^-$-10*
r−r−	12a	no. 12 × RS62	*A5B5 × A5B6*	no. 12:	*pab-1 pfp^r-10 ad his-1 Mod$^-$-10*
	12b	,,	,,		
	12c	,,	,,		
r+s+	E	RS96 × RS49	*A5B5 × A5B2*	RS49:	*ad-8 pfp^s-10 ad-12 Mod$^+$-10*
r+s−	D81	RS81 × RS29	*A6B6 × A6B52*	RS81:	*pfp^r-10 Mod$^+$-10*
				RS29:	*pfp^s-10 Mod$^-$-10*
r−s+	DI DII DIII	no. 12 × DT1	*A5B5 × A5B3*	DT1:	*pfp^s-10 Mod$^+$-10*
	DIV DV DVI	no. 12 × RS49			
r−s−	LI	LR4 × no. 12	*A5B6 × A5B5*	LR4:	*pfp^s-10 Mod$^-$-10 met-1*
s+s+	S1 S2 S3	SR68 × DT2	*A5B5 × A5B3*	SR68:	*pab-1 pfp^s-10 ad his-1 Mod$^+$-10*
				DT2:	*pfp^s-10 Mod$^+$-10*
s+s−	L/M 1	LR4 × MR801	*A5B6 × A5B5*	MR801:	*pfp^s-10 Mod$^+$-10 ad-3*
	L/M 4	,,	,,		
	L/M 6	,,	,,		
s−s−	T1	IND3 × TC1	*A2B5 × A2B3*	TC1:	*pfp^s-10 Mod$^-$-10*
	T2	,,	,,	IND3:	*pfp^s-10 Mod$^-$-10 met-1*

that the diploid nucleus has a degree of instability which results in some loss of chromosomes (Casselton & Lewis, 1966). Only three of the many diploid/haploid dikaryons exhibited instability as shown by irregular growth and sectoring. The products of these instabilities were tested by dikaryon resolution into its components from chlamydospores (Lewis, 1961). This was followed by the di-mon mating test for the loss of a mating type gene, by the loss of auxotrophic markers and by repeated subculturing on media with and without PFP to determine whether a permanent or a reversible change had occurred

Most diploid/haploid dikaryons did not show instability. A sample of these and the unstable dikaryons were treated with the haploidizing agent, griseofulvin, at 1 μg per ml of medium (North, 1977). The unstable dikaryons were affected by griseofulvin, but the others showed no breakdown even with griseofulvin. This was important for the interpretation of the effects of the *pfp*^{r} and the *Mod* gene on resistance.

3. RESULTS

Haploid monokaryons, diploids, and haploid/haploid dikaryons with different combinations of *r* and *s* alleles of the resistant gene *pfp-10* and the + and − alleles of the dominance modifier gene *Mod-10* were tested on 1×10^{-4} M DL PFP by Senathirajah & Lewis (1975). Diploid/haploid dikaryons have been made by mating a diploid and a haploid monokaryon. There are 40 different diploid/haploid

Table 2. *Forty different diploid/haploid dikaryons with their growth on 10^{-4} M PFP expressed as a percentage of the growth on MM, and their classification into resistant R and sensitive S.*

$$\frac{\text{Growth on PFP}}{\text{Growth on MM}} \times 100$$

	Diploid									
Haploid	R *rr*++	R *rr*+−	R *rr*−−	S *rs*++	S *rs*+−	S *rs*−+	S *rs*−−	S *ss*++	S *ss*+−	S *ss*−−
R *r*+	95 R	100 R	109 R	80 R	100 R	90 R	100 R	99 R	87 R	92 R
R *r*−	107 R	105 R	84 R	85 R	82 R	93 R	82 R	100 R	7 S	12–18* S
S *s*+	94 R	97 R	87 R	0–11* S	0 S	11 S	0 S	3·6 S	3·3 S	3·0 S
S *s*−	92 R	90 R	77 R	11–20* S	1·3 S	7·5 S	1·7 S	3·4 S	1·8 S	1·5 S

r = *pfp*^{r} + = *Mod*$^+$ R and S = Phenotype
s = *pfp*^{s} − = *Mod*$^-$ * = *Irregular*.

combinations with respect to *r* and *s* of *pfp-10* and of + and − of *Mod-10*. Each dikaryon was tested with two mycelial inocula. Twenty-eight of the 40 dikaryons were replicated using parents with the same combination of *r* and *s* and + and −, but with slightly different genetic backgrounds. These replications varied from

2 to 5 dependent upon the number of stocks available. The results were completely reproducible and the effect of genetic background differences was trivial and insignificant.

The results given in Table 2 were obtained from the first series of tests, the percentage values are based on either a single dikaryon or the mean of the replicates. In a second repeat series of tests, because of the close agreement between different replicates, one dikaryon of each combination was tested. All except three gave such close agreement with the first test that these values have not been included in Table 2. The three exceptional dikaryons, diploid $rs++$ with haploids $s+$ and $s-$ and diploid $ss--$ with haploid $r-$, gave irregular results with sectoring and uneven growth. These exceptions are due to instability of the diploid component and the subsequent loss of the chromosome with r or s. This made it possible to assign the gene *pfp-10* to the *A* chromosome. From Table 2 it will be seen that there are two sharply contrasted reactions; resistant R, in which the % growth on 1×10^{-4} M PFP varies from 107 to 77 % as compared with the growth on non-PFP medium, and sensitive S with 0 to 18 % growth on PFP.

A visual survey of the table reveals that resistance and sensitivity are not related only to dosages of r, s, + and − alleles. For example, the comparison of four different combinations of two s alleles with one r allele, given below, shows that other important variables are whether the sr heterozygosity is *within* a diploid nucleus or *between* nuclei and whether Mod^+ is present or absent.

Diploid	Haploid	Phenotype
$r+s+$	$s+$	S
$r-s-$	$s+$	S
$s-s-$	$r+$	R
$s-s-$	$r-$	S

This finding supports and extends the data obtained from diploids and haploid/haploid dikaryons (Senathirajah & Lewis, 1975). We believe that the extensive new data justify a detailed elaboration of the hypothesis in molecular terms previously outlined to explain the gene action in H/H dikaryons and diploids.

(i) *Hypothesis*

Mod^+ localizes translation and aggregation resulting in homomultimers.

The hypothesis has the following four assumptions:

1. The product of the *pfp* gene is a multimer.
2. The amount of subunit product is proportional to gene dosage.
3. Products of pfp^r and pfp^s form homo- and heteromultimers by aggregation at random when the modifier gene Mod^+ is absent.
4. When Mod^+ is present the gene products from separate nuclei, but not from the same nucleus, are kept separate and do not cross-multimerize.

There is no direct evidence in this system for any of these assumptions. In general terms many examples of the first assumptions are known, although exceptions to each have been recorded. The fourth assumption is novel and, therefore, it is the central focus in the testing and in searching for alternative explanations.

The criterion for accepting or rejecting an assumption is the simple one of consistency with the data. Only contrary data lead to rejection, the choice of alternatives is to prefer the one which gives the maximum difference in terms of the hypothesis between the resistant (R) and the sensitive (S) phenotypes. The expected proportions of homo- and heteromultimers are calculated from the simple law of probability. From an *rs* heterozygote the proportion of r and s homomultimers is $(\frac{1}{2})^n$, where n is the number of subunits in the multimer, and the proportion of rs heteromultimers is $1-2(\frac{1}{2})^n$; with an *rrs* diploid/haploid heterozygote, the homomultimers are r, $(\frac{2}{3})^n$; s, $(\frac{1}{3})^n$. In the calculations the action of *Mod*$^+$ is presumed to prevent the formation of all hybrid multimers between products of different nuclei so that an *rs* dikaryon with *Mod*$^+$ will produce 50 % r and 50 % s homomultimers irrespective of the number of subunits.

A comparison of the expected fractions expressed as percentages, of the types of multimers formed in haploids, diploids and haploid/haploid dikaryons (Table 3), excludes some of the possible assumptions. A dimer is highly improbable because the difference in amounts of r and s homomultimers between R and S phenotypes is only twofold, i.e. between 50 and 25 %. A tetramer appears to be possible with an eightfold difference, and a hexamer gives a better agreement with a 33-fold difference.

Table 3. *The expected percentage of homo- and heteromultimers from haploids, heterozygous diploids and dikaryons for different numbers of subunits*

		Hypothetical gene products r and s (%)			
	Phenotype	Dimer	Tetramer	Hexamer	
Haploids	R	100	100	100	all rr homos
	S	100	100	100	all ss homos
Hap/Hap dikaryons	R	50	50	50	rr
		0	0	0	rs
		50	50	50	ss
	S	25	6·2	1·5	rr
		50	87·6	97·0	rs
		25	6·2	1·5	ss
Diploids	S	25	6·2	1·5	rr
		50	87·6	97·0	rs
		25	6·2	1·5	ss

The expected values given in Table 3 also show some discrimination of the type of action of *r* and *s* products in terms of the resistant/sensitive phenotype. Four possible types of action with expected percentages of active multimers in R and S phenotypes, based upon the expected values from haploid/haploid dikaryons and diploids, given in Table 3, are presented in Table 4.

Two of the four variants, nos. 2 and 3, are excluded because the amount of presumed active product is too low. Similar calculations of *r* and *s* products based upon 40 different diploid/haploid dikaryons are summarized in Table 5. These values also exclude the variants 2 and 3 because of the high value of ss multimers in the

Table 4. *Four possible types of* pfp^r *and* pfp^s *gene action to account for resistance* (*ratios less than one are not acceptable*)

1. ss homos and rs heteros active → sensitivity; rr homos passive

		Dim	Tet	Hex	
Ratio of % of rs + ss	$\frac{S}{R}$	$\frac{75}{50}$	$\frac{93{\cdot}8}{50}$	$\frac{98{\cdot}5}{50}$	Possible but unlikely

2. ss homos active → sensitivity; rr and rs passive

Ratio of % of ss	$\frac{S}{R}$	$\frac{25}{50}$	$\frac{6{\cdot}2}{50}$	$\frac{1{\cdot}5}{50}$	Impossible

3. rr homos and rs heteros active → resistance; ss passive

Ration of % of rr + rs	$\frac{R}{S}$	$\frac{50}{75}$	$\frac{50}{93{\cdot}8}$	$\frac{50}{98{\cdot}5}$	Impossible

4. rr homos active → resistance; ss and rs passive

Ratio of % of rr	$\frac{R}{S}$	$\frac{50}{25}$	$\frac{50}{6{\cdot}2}$	$\frac{50}{1{\cdot}5}$	Possible
			x8	x34	

Table 5. *Hypothetical gene products from diploid/haploid dikaryons expressed as percentages; rr, ss, homomultimers, rs, heteromultimers*

	Dip/Hap dikaryons, hypothetical gene products				
	Dip	Hap	Phenotype	Tet	Hex
Mod+	*rr*	*s*	R	rr 66	66
				rs 0	0
				ss 33	33
Mod−	*rr*	*s*	R	rr 19·8	8·2
	rs	*r*		rs 79	91·7
				ss 1·2	0·1
Mod+	*rs*	*r*	R	rr 37	34
				rs 59	65
				ss 4	1
Mod+	*ss*	*r*	R	rr 33	33
				rs 0	0
				ss 66	66
Mod−	*ss*	*r*	S	rr 1·2	0·1
	rs	*s*		rs 79	91·7
				ss 19·8	8·2
Mod+	*rs*	*s*	S	rr 4	1
				rs 59	65
				ss 37	34

Mod+ *ss r* resistant dikaryon and the high percentage of rr and rs multimers in the sensitive dikaryons. Furthermore, the values decisively exclude variant 1 because of the high proportion of ss and rs multimers in two resistant dikaryons.

For simplicity in presenting the data of the diploid/haploid dikaryon, the dosage of *Mod*+ has not been indicated. In fact, one dose of *Mod*+ is effective except in the

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diploid nucleus where two are necessary. *Mod*$^+$ is recessive in the diploid nucleus but dominant in the haploid. In this respect it resembles the action of the *pfp-10* gene, whose action it modifies.

A comparison between the presumed active rs + ss multimers to give sensitivity as in variant 1 and Table 4, in resistant and sensitive phenotypes, shows only a $\times 1{\cdot}2$ difference with a tetramer and a $\times 1{\cdot}1$ with a hexamer. This variant is excluded. The variant 4, in which rr homomultimers result in active resistance, gives comparable figures of $\times 5$ for a tetramer and $\times 8$ for a hexamer.

The conclusions from this are *that resistance is achieved as an activity of r homomultimers, which is dominant and that the active multimer is at least a tetramer or possibly a hexamer or higher aggregate.*

(ii) *Threshold concentration for inhibition of growth*

Haploid monokaryons, haploid/haploid dikaryons, diploids and diploid/haploid dikaryons have been tested for growth on medium with molar concentrations varying from 10^{-6} to 10^{-2} M. The critical threshold concentration is taken as the concentration which gives 50–80 % growth and the next highest concentration gives complete inhibition. Most genotypes gave a sharp cut off at one concentration but genotypes with the highest threshold concentration had a tail of slight inhibition over several concentrations. Most of the results for the different genotypes are given in Tables 6 and 7, and Figs. 1 and 2.

Table 6. *Values for critical threshold concentrations of PFP* $\times 10^{-5}$ M *for haploids, haploid/haploid dikaryons and diploids; (−) and (+) refer to the haploids*

	Hap	Dik	Dip	Dik	Dip	Hap	Dik	Dip
pfp-10...	*s*	*s/s*	*s/s*	*r/s*	*r/s*	*r*	*r/r*	*r/r*
Mod								
(−) −/−	1	1·0	0·25	1	0·4	25	50	100
(+) +/−	1	1·0	0·50	25	0·7	25–250	500	250
+/+	—	2·5	0·25	50	1·0	—	1000	1000
Average effect of one *Mod*$^+$	$\times 1$	$\times 1{\cdot}5$	$\times 1$	$\times 25$	$\times 1{\cdot}5$	$\times 10$	$\times 10$	$\times 5$

Table 7. *Figures for critical threshold concentrations of PFP* $\times 10^{-5}$ M *for diploid/haploid dikaryons; figures in parentheses are hypothetical percentages of r homomultimers*

pfp-10	*s/ss*	*s/rs*	*r/ss*	*s/rr*	*r/rs*	*r/rr*
Mod						
−/−	1 (0)	1 (0·13)	1 (0·13)	10 (8·7)	10 (8·7)	10 (100)
+/−	1 (0)	2·5 (1·1)	10 (33·3)	10 (8·7)	50 (34·3)	50 (100)
−/+	1 (0)	1 (0·13)	2·5 (0·13)	10 (8·7)	10 (8·7)	50 (100)
+/+	5 (0)	5 (1·1)	25 (33·3)	10 (8·7)	50 (34·3)	250 (100)
−/+ +	2·5 (0)	1 (1·1)	10 (33·3)	100 (66·6)	10 (34·3)	100 (100)
+/+ +	2·5 (0)	2·5 (1·1)	100 (33·3)	100 (66·6)	10 (34·3)	1000 (100)
Effect of one *Mod*$^+$	$\times 1{\cdot}5$	$\times 1$–$1{\cdot}5$	$\times 33$	$\times 3$	$\times 1$	$\times 5$–33

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The results on critical threshold concentrations given in Tables 6 and 7 again show the effect of the *Mod*$^+$ on the *r*/*s* heterozygous haploid/haploid dikaryon where a 25-fold effect of one dose of *Mod*$^+$ is found. In the diploid/haploid dikaryon a 33-fold effect is found where the heterozygosity is between different nuclei. There is no effect of *Mod*$^+$ in the diploid or in the diploid/haploid dikaryon where the heterozygosity is within the diploid nucleus. The *Mod*$^+$ has no effect in *s* homozygotes whether they are haploid, dikaryon or diploid. The *Mod*$^+$ has a 5–10-fold effect on *r* homozygous haploids, haploid/haploid dikaryons and diploids, and a 5–33-fold effect on *r* homozygous diploid/haploid dikaryons.

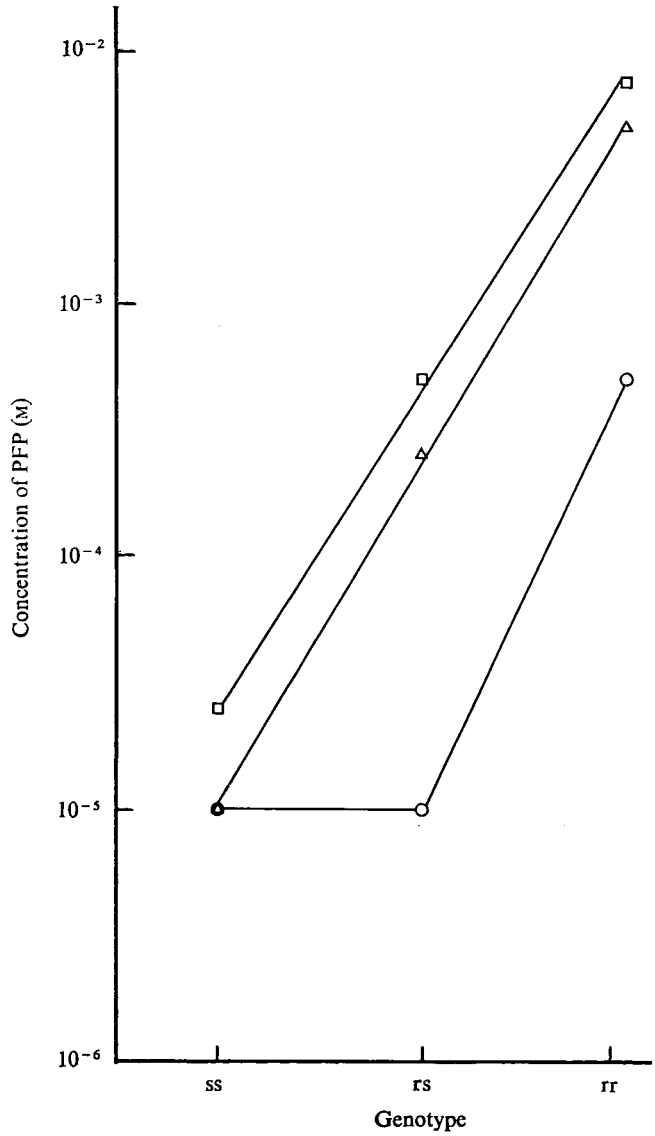

Fig. 1. Critical threshold concentrations of PFP for three genotypes, *ss*, *rs*, and *rr*, of haploid/haploid dikaryons. ○—○, Modifier absent; △—△, one dose of modifier; □—□, two doses of modifier.

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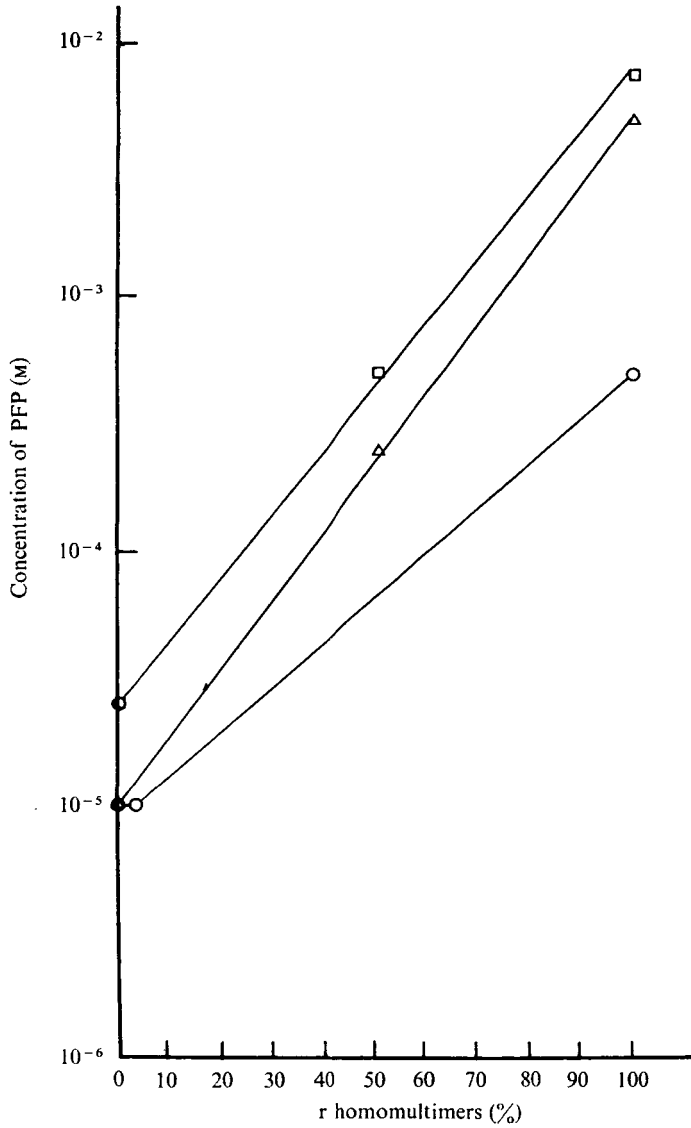

Fig. 2. Critical threshold concentration of PFP plotted against the percentage of r homomultimers in haploid/haploid dikaryons. ○—○, Modifier absent; △—△, one dose of modifier; □—□, two doses of modifier.

An attempt to explain the critical threshold concentrations on a dosage basis of *pfp-10* and *Mod* genes, where *Mod*$^+$ is a tenfold enhancer of *pfp*^{r}, has been made and completely failed. A comparison of the *r/rs*, +/+ + and *r/rr*, +/+ + in Table 7, where the ratio of $r \times +$ genes is only 6:9 between the two genotypes, shows that there is a 100-fold difference in resistance.

A comparison of the critical threshold concentration and the hypothetical % of r homomultimer given in Table 7 and Fig. 2 for the diploid/haploid dikaryons reveals a relationship between them. There appears to be a direct relationship

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except with the *r/rr* genotypes which, for full expression of resistance, require two and three doses of *Mod*$^+$.

4. DISCUSSION

The dominance modifier gene *Mod-10* has its major effect only in the dikaryon, which is the predominant natural form of the Basidiomycetes to which *Coprinus* belongs. Its effect in the diploid, a laboratory creation, is negligible. The novel action of the modifier to reverse dominance in a heterozygote for the *pfp*^{r}*-10* gene is explained on the prevention of the formation of hybrid multimeric products of the *pfp-10* gene. The hybrid multimeric products are presumed to be inactive in a similar way to the i^{-d} of the mutants of the lac operon (Müller-Hill, 1975). The evidence for this rests on the comparison of critical threshold concentrations for a whole range of different genotypes exhibiting a range of gene dosages and of relative positions of the gene between and within nuclei. This is not a standard approach to the problem and it leaves a large gap between this analysis of the gene action and identification of the gene product. However, the internal consistencies with the hypothesis in this wide series of analysis give some confidence in the integrity of the basic hypothesis. Only further work at the molecular level will establish whether the genes are affecting a protein, a ribosome or a membrane.

The novel mode of action of *Mod*$^+$*-10*, which has been postulated to explain the results, has been elaborated only after the exclusion of more conventional explanations. One of these will be discussed. Assume that the *Mod*$^+$*-10* gene blocks the action of the *pfp*^{s}*-10* gene which actively confers sensitivity, and assume that the *Mod*$^+$*-10* gene is induced in the dikaryon but repressed in the haploid monokaryon. The haploid monokaryon and the diploid are similar in morphology and physiology; they do not have clamp-connexions or produce fruiting bodies, they produce asexual oidia. The dikaryon is different, it does produce clamp-connexions and fruiting bodies and it does not produce oidia, it has a higher growth rate and an increased production of certain enzymes. It is, therefore, possible that the uptake and metabolism of amino acids in these two different mycelial phases involve different parts of the system by differential induction or repression. The fact that there are two separate permeases (Lewis & North, 1974) and probably a third (unpublished) for phenylalanine uptake, provides a different route of uptake, and therefore resistance to PFP in monokaryons and diploids. With these assumptions we can postulate that *pfp*^{s}*-10* positively produces a sensitive phenotype, and this is dominant in diploids and dikaryons. The action of the *Mod*$^+$*-10* is confined to the dikaryon. The modifier is presumed to have the conventional action (cf. Hiwatashi & Myohara, 1976) by blocking some stage in the production of the final product of the *pfp*^{s}*-10* gene. Even with the published results with haploid/haploid dikaryons (Senathirajah & Lewis, 1975), there is a difficulty with this explanation because the effect of *Mod*$^+$*-10* is negligible on the *ss* homozygous dikaryon and has its major effect only on the *rs* heterozygote. The present tests with the diploid/haploid dikaryons completely exclude this explanation. The diploid/haploid dikaryon has

all the morphology and physiology of the haploid/haploid dikaryon. In the diploid/haploid dikaryon the *Mod*$^+$*-10* has the expected action for a dikaryon in that *Mod*$^+$*-10* acts as a dominance modifier when *s* and *r* are in different nuclei, but also has the expected absence of effect when *s* and *r* are in the diploid nucleus which is present in the same dikaryon.

One further test to exclude the conventional explanation was to compare common *A* heterokaryons with either $r+s+$ or $r-s-$ genotypes. These heterokaryons are quite different from dikaryons and in most features resemble monokaryons and diploids. The *Mod*$^+$*-10* has its effect in these heterokaryons and, in this respect, they resemble dikaryons. In both the dikaryon and the heterokaryon the *r* and *s* alleles are in separate nuclei.

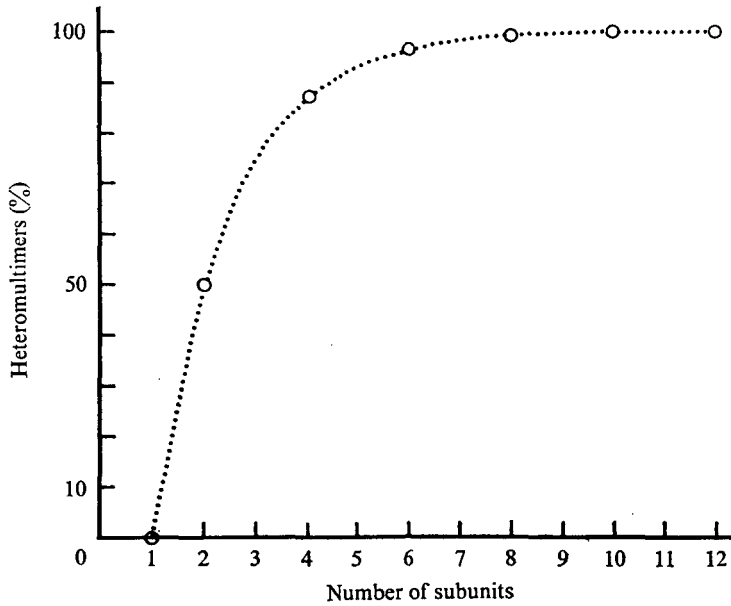

Fig. 3. Theoretical percentages of hybrid multimers plotted against the number of sub-units in the multimer.

The new finding that *Mod*$^+$ is necessary for the full expression of *r/rr* homozygotes may be an expression of an increased production of *pfp*^{r}*-10* products; this could be a pleiotropic effect of *Mod*$^+$ or it might be the primary effect which, by producing a higher concentration of r products near the *pfp*^{r} containing nucleus, might lead to homoaggregation.

The fact that three other similar systems of dominance modifiers in *Coprinus* (unpublished) have been found suggests that they may play an important part in dikaryotic and, possibly in diploid organisms. The theoretical analysis used in terms of multimeric gene products illustrates the power of the relationship between the number of sub-units and hybrid multimer formation by a heterozygote.

With the proportion of hybrid multimers given according to the formula $1-2(\frac{1}{2})^n$, we obtain the asymptotic curve in Fig. 3. From this it is clear that the types of

mutations, surviving natural selection, of a structural gene or its modifier will be quite different for a monomer and a duodecamer. In the monomer a mutation must survive unaided in competition with wild type; in a duodecamer a mutation will survive only if it makes a workable aggregate with the wild type.

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