Construction and Phenotypes of Double Sporulation Deficient Mutants in *Streptomyces coelicolor* A3(2)

By K. F. CHATER

Genetics Department, John Innes Institute, Colney Lane, Norwich NR4 7UH

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SUMMARY

In *whiA*, *B*, *G* and *H* mutants of *Streptomyces coelicolor* A3(2), aerial hyphae develop but sporulation septa are not formed. Septa are produced by *whiI* mutants but are spaced abnormally far apart. Mutants in each locus have a distinctive aerial mycelium morphology, except for *whiA* and *B* mutants which are closely similar. Seven strains were made with pairwise combinations of *whiA* and *B* mutations with *whiG*, *H* and *I* mutations and with each other. The genotypes of these strains were confirmed by suitable crosses and their aerial mycelium morphology examined. An indirect procedure was used to determine the aerial mycelium morphology of *whiGH*, *GI* and *HI* double mutants. The double mutants always closely resembled one of the single mutant parent strains in morphology and a consistent scheme of epistasis was obtained – *whiG* being epistatic to *whiH*, *A*, *B* and *I*; *whiH* to *whiA*, *B* and *I*; and *whiA* or *B* to *whiI*. These results point to the absence of any complex interactions between gene products, which might have been revealed by the occurrence of novel phenotypes in double mutants or by inconsistencies in the epistasis scheme.

INTRODUCTION

Previous papers have described the isolation, phenotypes and genetic mapping of white colony (*whi*) mutants of *Streptomyces coelicolor* A3(2) defective in the conversion of aerial hyphae into mature spore chains (Hopwood, Wildermuth & Palmer, 1970; Chater, 1972; Chater & Merrick, 1974; McVittie, 1974). Eight *whi* genes (*A* to *E*, *G* to *I*) have been identified; the status of a ninth (*whiF*) is doubtful (Chater & Hopwood, 1973; Chater & Merrick, 1974). Of these, *whiA*, *B*, *G* and *H* mutants were apparently unable to form the special sporulation septa (Wildermuth & Hopwood, 1970) required to subdivide the long aerial hyphal cells into spore-sized compartments; *whiI* mutants produced aberrantly spaced sporulation septa (Hopwood *et al.* 1970); the single *whiC* mutant was oligosporogenous (Chater & Merrick, 1974); and *whiF* mutants were defective in the formation of mature spores from spore compartments (McVittie, 1974). The frequency with which *whiA*, *B*, *G*, *H* and *I* mutants occurred suggested that few if any other genes were specifically involved in the early stages of sporulation of aerial hyphae (Chater & Hopwood, 1973).

Most of these mutants had aerial hyphal morphology resembling intermediate stages in spore formation in the wild type. The sequence in which these stages take place should be determinable by studies of the morphology of strains carrying two different *whi* mutations. In this paper I describe the construction and phenotypes of a set of double mutant strains involving the five genes *whiA*, *B*, *G*, *H* and *I*.

Fig. 1. Linkage map of markers employed. The map and gene symbols are based on those of Hopwood *et al.* (1973). Morphological markers are given inside the circle.

METHODS

Strains. All strains (Table 1) were originally derived from *Streptomyces coelicolor* A3(2) (Hopwood, 1959). The locations on the circular linkage map of all the markers employed are given in Fig. 1.

Media, culture methods, mating techniques and light microscopy. These were all as described by Chater (1972), microscopy being done on impression preparations.

Fertility testing. Fertility tests to distinguish IF and NF types were as described by Vivian & Hopwood (1970), with UF strains 1190 or A200 as testers.

RESULTS

Morphology of constructed double whi mutants containing whiA or whiB mutations

The sections immediately following describe the synthesis of seven strains containing pairwise combinations of the two mutations *whiA*170 and *whiB*218 with *whiG*71, *whiH*250 or *whiI*80, and with each other. The genetic confirmation of the presence of the pairs of *whi* mutations in each strain is also described. To facilitate understanding of some of the genetics, the phenotypes of the double *whi* mutants and the single mutant parent strains are described first.

The mapping and phenotypes of all five mutations were given by Chater (1972) and are summarized in Figs. 1 and 2 and in Table 1. Each is typical of mutations of its locus; *whiA* and *B* mutants usually have type II phenotype (non-fragmenting, often tightly coiled aerial hyphae); *whiG* mutants have type I phenotype (non-fragmenting aerial hyphae showing no coiling); *whiH* mutants have type III phenotype (non-fragmenting, loosely coiled hyphae); *whiI* mutants have type IV phenotype (coiled, much-fragmented aerial hyphae).

Each double *whi* strain was found to resemble closely one of the single *whi* parent strains (Fig. 2, Table 1). These data suggested that *whiG* and *H* were epistatic to *whiA* and *B*, and *whiA* and *B* to *whiI*, while the *whiAB* double mutant was indistinguishable in phenotype from either single mutant parent strain. These conclusions were supported by data, given

Fig. 2. Phase-contrast photomicrographs of impression preparations of the aerial hyphae of single and double *whi* mutants. All bar markers indicate 20 μ m. (a) *whiA170* (phenotype II); (b) *whiB218* (II); (c) *whiA170B218* (II); (d) *whiA170G71* (I); (e) *whiB218G71* (I); (f) *whiG71* (I); (g) *whiA170H250* (III); (h) *whiB218H250* (III); (i) *whiH250* (III); (j) *whiA170I80* (II); (k) *whiB218I80* (II); (l) *whiI80* (IV). Phenotypic designations (Roman numerals) are those of Chater (1972).

Table 1. *List of strains*

Explanations of symbols and fertility type designations are given in Hopwood, Chater, Dowding & Vivian (1973). Morphological phenotypes are those given by Chater (1972) (see also Fig. 2).

Strain no.	Genotype	Fertility type†	Morpho-logical phenotype
602	<i>hisA1 pheA1 strA1 acrA9</i>	NF	w.t.
1107	<i>hisA1 cysD18 leuB5 strA1</i>	NF	w.t.
1190	<i>hisA1 uraA1 strA1</i>	UF	w.t.
1258*	<i>hisC9 proA1 argA1 uraA1 cysD18 strA1</i>	NF	w.t.
A200	<i>adeCv10 pheA1 strA1</i>	UF	w.t.
J354	<i>cysD1</i>	NF	w.t.
Single <i>whi</i> mutants			
J279	<i>cysD18 strA1 whiA170</i>	n.t.	II
J327	<i>proA1 strA1 whiA170</i>	n.t.	II
J171	<i>cysD18 strA1 whiB218</i>	n.t.	II
J961	<i>hisC9 argA1 uraA1 cysD18 strA1 whiB218</i>	IF	II
J90	<i>hisA1 argA1 mthB2 strA1 whiG71</i>	n.t.	I
J118	<i>cysD18 strA1 whiG71</i>	n.t.	I
J119	<i>leuB5 strA1 whiG71</i>	n.t.	I
J116	<i>hisA1 argA1 mthB2 strA1 whiH250</i>	n.t.	III
J198	<i>leuB5 strA1 whiH250</i>	n.t.	III
J409	<i>cysC3 strA1 whiH250</i>	n.t.	III
J159	<i>hisA1 argA1 mthB2 strA1 whiI80</i>	n.t.	IV
J86	<i>leuB5 strA1 whiI17</i>	n.t.	IV
J127	<i>cysD18 strA1 whiI17</i>	n.t.	IV
Double <i>whi</i> mutants			
J1003	<i>uraA1 strA1 whiA170 whiB218</i>	IF	II
J481	<i>mthB2 strA1 whiA170 whiG71</i>	n.t.	I
J518	<i>mthB2 strA1 whiA170 whiH250</i>	n.t.	III
J960	<i>mthB2 strA1 whiA170 whiI80</i>	n.t.	II
J539	<i>mthB2 strA1 whiB218 whiG71</i>	IF	I
J526	<i>mthB2 strA1 whiB218 whiH250</i>	NF	III
J953	<i>mthB2 strA1 whiB218 whiI80</i>	NF	II

* Genotype given incorrectly by Chater (1972).

† n.t., Not tested.

Fig. 3. Synthesis of a *whiAG* double mutant. Strains J90 and J279 were crossed and *hisA*⁺ *argA*⁺ *cysD*⁺ recombinants selected (triangles). These were screened for homoserine-requirement (the *mthB2* phenotype). A sample of *mthB2* recombinants was then tested for inheritance of the two *whi* mutations as shown in Tables 2 and 3. Similar crosses, in which strain J90 was replaced by strains J116 or J159, were used to construct *whiAH* and *AI* double mutants.

Table 2. *Analysis of a strain (J481) carrying whiA and G mutations*

Strain J481 was crossed with 1258 and selection (triangles) made for *proA*⁺*mthB*⁺ recombinants. These were analysed for non-selective markers, and samples of *whi* recombinants re-streaked and examined microscopically. The phenotypic classification is that of Chater (1972) (see also Fig. 2). The frequency of each allele among the progeny is given in the diagram, the frequency of *whi* recombinants being determined by the inheritance of *whiA* (see text).

Genotype	Frequency of recombinants	Phenotype of aerial mycelium in a sample of recombinants	
		Type I	Type II
<i>uraA whi</i>	12	—	—
<i>whi</i>	120	20	2
<i>cysD uraA whi</i>	4	0	1
<i>cysD whi</i>	8	0	4
<i>argA cysD uraA whi</i>	1	0	1
<i>hisC argA cysD uraA</i>	1	—	—
<i>hisC argA cysD</i>	1	—	—
<i>hisC uraA cysD*</i>	1	—	—
Total	148		

* Multiple crossover class.

below, from crosses involving the double mutants, where the segregation of parental *whi* mutant phenotypes among *whi* recombinants was only explicable in terms of the epistatic relationships summarized above.

Synthesis and testing of whiAG, AH and AI double mutants

The diagrams and Tables relating to this section all describe in detail the situation for the *whiAG* double mutant. To save space and preserve clarity, the methods and results for the *whiAH* and *AI* mutants will merely be summarized in the text.

Fig. 3 illustrates the type of cross used to synthesize these strains. The selection of strains requiring only homoserine from these crosses ensured that, unless multiple crossing-over had occurred, the recombinants would inherit both the *whiA* and the *whiG*, *H* or *I* mutation.

In the crosses in which the presence of both *whi* mutations in each double mutant was confirmed (Tables 2, 3) the double *whi* strain was always counterselected at *mthB2*, the counterselection against the tester strains being such that the two *whi* mutations were not separated

Table 3. Further analysis of a strain (J481) carrying *whiA* and *G* mutations

Strain J481 was crossed with 1107 and selection (triangles) made for *mtbB*⁺ *leuB*⁺ recombinants.
For further explanation, see Table 2 and text.

Genotype	Frequency of recombinants	Phenotype of aerial mycelium in a sample of recombinants	
		Type I	Type II
<i>hisA cysD</i>	1	—	—
<i>hisA cysD whi</i>	4	1	0
<i>hisA whi</i>	39	6	0
<i>whi</i>	261	6	0
<i>cysD whi</i> *	2	1	0
<i>hisA</i> *	2	—	—
Total	309		

* Multiple crossover class.

by the selection points. As a result, when the segregation of white and grey colony colour was scored, a *whi* map location would be obtained corresponding to that of the *whi* mutation situated nearer to the counterselection point of the tester strain. In Table 2 this point was at *proA*, so that white colony colour segregated as if determined by *whiA*, while in Table 3 it was at *leuB*, so that the pattern of *whiG* segregation determined the frequency of *whi* recombinants. These data confirmed the genotype of the *whiAG* strain. Very similar data were obtained in crosses with the *whiAH* and *AI* strains, confirming their genotypes.

Assuming negligible multiple crossing-over, all *whi* recombinants should have the *whiA* mutation, and some should also have the *whiG* mutation. The *whiG* mutation, however, should only be found among *cys*⁺ recombinants. Since the phenotype of the parental double mutant was identical to that of a *whiG* mutant, it follows that some *cys*⁺ recombinants (i.e. those carrying *whiA* and *whiG*) should have the *whiG* (type I) phenotype, and the rest the *whiA* (type II) phenotype, while all *cys*⁻ recombinants should have the type II phenotype. This was indeed observed (Table 2), confirming the epistasis of *whiG* over *whiA*. Similarly, with the equivalent *whiAH* double mutant test cross, segregation of aerial mycelium phenotype was observed only among *cys*⁺ recombinants (3 type II, 6 type III) while five *cys*⁻

Fig. 4. Synthesis of a *whiBH* double mutant. (a) Strains J116 and J171 were crossed and *hisA*⁺ *argA*⁺ *cysC*⁺ recombinants selected (triangles). These were screened for homoserine requirement (the *mthB2* phenotype). (b) Patches of a number of *mthB2* recombinants were then plate-crossed with strain 1190 and the plate crosses replica-plated to medium lacking histidine and homoserine. The frequency of recombinants and their aerial mycelium colour (grey or white) was scored, allowing the identification of IF recombinants (which would be expected to possess the *whiB218* mutation) and of those NF recombinants that possessed the *whiB218* mutation. For further explanation, see text. *whiBG* and *BI* double mutants were obtained by the same procedure, but substituting strains J90 or J159 for J116.

recombinants tested were all type II. This confirmed the epistasis of *whiH* over *whiA*. The prediction was different for the equivalent *whiAI* double mutant test cross: since all *whi* segregants should carry the *whiA* mutation and *whiA* appears epistatic to *whiI*, segregation of aerial mycelium type was not expected. This was the case, all of nine *cys*⁺ and two *cys*⁻ recombinants having the type II phenotype.

Similar consideration of the diagram in Table 3 leads to the prediction that, for the *whiAG* and *AH* crosses, all *whi* recombinants should have the *whiG* or *whiH* phenotype. However, in the *whiAI* cross all *cysD hisA whi* and most *cys*⁺ *hisA whi* recombinants should inherit only the *whiI* mutation and hence the type IV phenotype, while a few *cys*⁺ *hisA whi* recombinants, and all *cys*⁺ *his*⁺ recombinants, should also have the *whiA* mutation and hence the epistatic type II phenotype. These predictions were confirmed, no segregants of type II phenotype being observed with the *whiAG* or *AH* crosses, while the expected segregation of type II and IV phenotypes occurred in the *whiAI* cross. In this latter cross all 23 *cys*⁺ *his*⁺ *whi* recombinants tested were type II, while only one of 16 *cys*⁺ *hisA whi* recombinants tested was type II, the rest being type IV.

It is concluded from these results that the proposed genotypes of the prospective double *whi* mutants were correct, that *whiG* and *H* are epistatic to *whiA*, and that *whiA* is epistatic to *whiI*.

Synthesis and testing of whiBG, BH and BI double mutants

As in the previous section, data for only one double mutant are given in detail (Fig. 5), similar data for the other strains being mainly summarized in the text.

Fig. 4a illustrates the cross used to synthesize a *whiBH* double mutant. Ignoring multiple crossing-over, strains inheriting *whiH250* could have inherited *whiB218* as well. To identify those that did, *mthB2* recombinants (arrayed in patches on master plates) were replica-plated to a lawn of spores of UF strain 1190. After a period of growth, these 'plate crosses'

Fig. 5. Analysis of a strain (J526) carrying *whiB* and *H* mutations. Strains J526 and 1258 were crossed and selection (open triangles) made for *hisA*⁺ *mthB*⁺ recombinants. These were analysed for non-selective markers, and samples of *whi* recombinants re-streaked and examined microscopically. (a) The allele frequencies obtained by considering only *cysD* recombinants (additional selection indicated by black triangle) which are all presumed to be *whiH*⁺ but are free to segregate for *whiB218* or *whiB*⁺. All the *cysD whi* recombinants tested had the *whiB* (type II) phenotype. (b) The allele frequencies obtained by considering only *proA uraA* recombinants (black triangles) which are all presumed to be *whiB*⁺ but are free to segregate for *whiH250* or *whiH*⁺. All the *proA uraA whi* recombinants tested had the *whiH* (type III) phenotype.

were replica-plated to selective medium lacking histidine and homoserine (Fig. 4*b*). This allowed the identification of any IF strains among the patches on the master plate, as these gave few if any recombinants with the tester strain. It can be seen (Fig. 4*a, b*) that such IF recombinants should have inherited the *whiB218* mutation. The plate cross with strain 1190 also allowed the identification of NF strains carrying the *whiB218* mutation. Selection for *his*⁺ in the plate cross ensured that the *whiB* region of any NF strain tested should be inherited by most of the recombinants, while the selection for *mthB*⁺, together with the asymmetrical nature of zygote structure in NF × UF crosses (Hopwood, Harold, Vivian & Ferguson, 1969) ensured that the *whiH*⁺ allele of strain 1190 would be inherited by almost all recombinants. Thus the predominant colour, white or grey, of the aerial mycelium of the recombinant patches after 4 days' incubation indicated the presence or absence of the *whiB218* mutation in the appropriate patch on the master plate. The *whiBH* double mutant chosen for study, J526, was NF (Fig. 5), as was the *whiBI* double mutant used, J953 (Table 4). The *whiBG* double mutant studied, however, was IF. (The fertility type of these recombinants had significance only for genetic analysis, and did not affect morphology).

The presence of the expected *whi* mutations in the double mutants was confirmed by crossing with strain 1258 and, for the *whiBI* double mutant, also with strain 1107. In the crosses with strain 1258 (Fig. 5), the selection was different from that described earlier (Table 2) for *whiAG*, *AH* and *AI* double mutants, since the two *whi* mutations were separated by the points of selection and segregated independently to a large extent. To show that *whiB218* was present, only recombinants requiring cysteine were considered (Fig. 5*a*), since the *whiH*⁺ allele of strain 1258 would be present in nearly all these progeny. Among these, a *whi* mutation giving the *whiB218* phenotype was found to segregate in a way consistent

Table 4. Further analysis of a strain (J953) carrying *whiB* and *I* mutations

Strains J953 and 1107 were crossed, and selection (triangles) made for *methB*⁺ *leuB*⁺ recombinants. For further details, see Tables 2 and 3 and text.

Genotype	Frequency of recombinants	Phenotype of aerial mycelium in a sample of recombinants	
		Type II	Type IV
<i>hisA cysD</i>	17	—	—
<i>hisA cysD whi</i>	1	—	—
<i>hisA whi</i>	16	0	15
<i>whi</i>	45	15	5
Total	79		

only with a location between *proA* and *uraA*. Conversely, considering only *proA uraA* recombinants, nearly all of which should be *whiB*⁺ (Fig. 5b), a *whi* mutation having the *whiH250* phenotype segregated in a way consistent only with a map location in the *methB-cysD* interval. Thus the presence of the two *whi* mutations was confirmed in a single cross. In addition, the aerial mycelium phenotype of *proA*⁺ *uraA*⁺ (and hence *whiB218*) recombinants was examined. All those inheriting *cysD* should be *whiH*⁺ and therefore have the type II (*whiB218*) phenotype. However, a proportion of the *cys*⁺ recombinants should inherit the *whiH250* mutation and therefore should have the type III phenotype shown by the parental double mutant (strain J526; Table 1, Fig. 2). These predictions were confirmed; all three *cysD* recombinants scored were type II, as compared with only two out of 32 *cysD*⁺, the rest of which were type III. This substantiated the earlier conclusion that *whiH* was epistatic to *whiB*.

This kind of analysis gave a similar and equally unambiguous result with the *whiBG* mutant, confirming its genotype and the epistasis of *whiG* over *whiB*. With the *whiBI* double mutant, too few recombinants of the appropriate genotype were obtained in the cross with strain 1258 to confirm the presence of *whiI80*, though the presence of *whiB218* was amply confirmed. A further cross, with strain 1107, was therefore used (Table 4). The pattern of white colony segregation was compatible only with the presence in strain J953 of a *whi* mutation between the *leuB* and *cysD* genes, i.e. in the region of the *whiI* gene. The morphology of *whi* recombinants was also examined, in order to test the predicted pattern of

Fig. 6. The synthesis of a *whiA170 whiB218* double mutant. (a) Strains J327 and J961 were crossed and selection (triangles) made for *proA*⁺ *hisA*⁺ *argA*⁺ *cysD*⁺ recombinants, most of which should also have inherited *whiA170* from strain J327. The recombinants were classified for fertility (IF or NF) and uracil requirement, and seven *ura*⁻ IF recombinants picked. Most of these should have inherited *whiB218* from strain J961. The presence of *whiA170* was confirmed by the absence of *whi*⁺ recombinants in a backcross with strain J327 (see text). (b) For confirmation of the presence of *whiB218*, strain J1003 was crossed with strain 602 and selection (triangles) made for *pheA*⁺ *acrA* (acriflavine-resistant) recombinants. After recombinant analysis, the nine *whi* recombinants were re-streaked and examined microscopically. All were of phenotype II, and they included one carrying *hisA1*. Allele frequencies are given on the diagram.

segregation. The *whiBI* parent strain (J953) had type II (*whiB*) morphology (Table 1, Fig. 2), so that the presence or absence of *whiB218* rather than *whiI80* should have been decisive in controlling the phenotypes of recombinants. This led to the prediction that a proportion of the *hisA*⁺ recombinants should have been type II, all other *whi* recombinants being type IV. This was confirmed (Table 4).

Synthesis and testing of a *whiAB* double mutant

The cross used to synthesize a strain carrying *whiA170* and *B218* is illustrated in Fig. 6a. The presence of *whiA170*, in seven presumptive double mutants, was confirmed by backcrossing to the parent *whiA170* strain J327. In the case of strain J1003, finally selected, all 100 re-streaked recombinants were white. Similarly, no grey recombinants were observed with any of the other six strains. The presence of *whiB218* in strain J1003 was confirmed by a cross (Fig. 6b) in which the white/grey colony colour segregated with a frequency indicating the presence of a *whi* mutation located between the *hisA* and *uraA* genes.

Epistasis among *whiG*, *H* and *I*

The *whiG*, *H* and *I* loci are all situated between *leuB* and *cysD* (Fig. 1) (Chater, 1972), and with the currently available methods of gene transfer in *S. coelicolor* it would be exceedingly arduous to make and confirm *whiGH*, *GI* and *HI* double mutants. A different method was therefore used to test epistasis. The frequency of different mutant phenotypes was scored among the progeny of crosses where the double *whi* genotype was expected to occur with a significant and predictable frequency. This situation was provided in a series of crosses made between *cysD18* (or in one case *cysC3*) and *leuB5* mutants carrying *whi*⁺, *whiG71*, *H250* or *I17*, with selection for *leu*⁺ *cys*⁺ recombinants (Table 5).

In crosses homozygous for any *whi* allele, only *whi* (never *whi*⁺) recombinants arose, and

Table 5. Epistasis among *whiG71*, *H250* and *I17*

Nine crosses involving *whiG71*, *H250* and *I17*, both singly and in pairwise combinations, were carried out and *leu⁺ cys⁺* recombinants selected in each case (triangles). Data from crosses 1 to 6 were summed, as indicated by lower case letters, to give recombination frequencies for each interval. Roman numerals refer to the microscopic morphology of *whi* recombinants. The summed data were used to make predictions of the frequency of each *whi* phenotype among prototrophic recombinants in crosses 7 to 9. Observed and expected frequencies were compared by chi-square tests.

Cross		CROSSES 1 to 6				
<i>leuB5</i>	<i>G71</i>	<i>leuB</i>	<i>whiG</i>	<i>whiH</i>	<i>whiI</i>	<i>cysCD</i>
1. <i>leuB5</i> +	<i>G71</i> +	(a)8/50(I)	(b)42/50(+)			
2. <i>leuB5</i> +	<i>H250</i> +	(c)62/102(III)		(d)40/102(+)		
3. <i>leuB5</i> +	<i>I17</i> +		(e)32/49(IV)		(f)17/49(+)	
4. <i>leuB5</i> +	<i>G71</i> +	(g)9/50(I)	(h)11/50(+)		(i)30/50(III)	
5. <i>leuB5</i> +	<i>H250</i> +	(j)11/55(I)	(k)36/55(+)			(l)8/55(IV)
6. <i>leuB5</i> +	<i>I17</i> +	(m)57/111(III)		(m)37/111(+)		(o)17/111(IV)

Summed frequency of crossing-over in each interval

$leuB-whiG = a + g + j = 28/155$
 $leuB-whiH = c + g + h + m = 139/263$
 $leuB-whiI = e + j + k + m + n = 173/152$
 $whiG-cysCD = b + h + i + k + l = 127/155$
 $whiH-whiI = n = 11/50$

$whiG-whiI = k = 36/55$
 $whiH-cysCD = d + i + n + o = 124/263$
 $whiI-whiI = n = 37/111$
 $whiI-cysCD = f + l + o = 42/215$

CROSSES 7 to 9

Cross	Phenotype	Observed frequency	Expected frequency for epistasis of		
			<i>whiG71</i>	<i>whiH250</i>	<i>whiI17</i>
7.* <div><div><div><i>leuB5</i></div><div>+</div><div><i>H250</i></div><div>+</div><div><i>G71</i></div><div>+</div><div><i>cysD18</i></div></div><div><div>+</div><div>+</div><div>+</div><div>+</div><div>+</div><div>+</div><div>+</div></div></div>	I	73	80 $\chi^2_1 = 3.3$ $P = 0.07$	46 $\chi^2_1 = 29$ $P \ll 0.001$	—
	III	25	18	52	
8. <div><div><div><i>leuB5</i></div><div>+</div><div><i>H250</i></div><div>+</div><div><i>G71</i></div><div>+</div><div><i>cysD18</i></div></div><div><div>+</div><div>+</div><div>+</div><div>+</div><div>+</div><div>+</div><div>+</div></div></div>	I	45	47 $\chi^2_1 = 0.49$ $P = 0.5$	—	11 $\chi^2_1 = 130$ $P \ll 0.001$
	IV	12	10	46	
9. <div><div><div><i>leuB5</i></div><div>+</div><div><i>H250</i></div><div>+</div><div><i>I17</i></div><div>+</div><div><i>cysC3</i></div></div><div><div>+</div><div>+</div><div>+</div><div>+</div><div>+</div><div>+</div><div>+</div></div></div>	III	47	—	47 $\chi^2_1 = 0$ $P = 1.0$	20 $\chi^2_1 = 46$ $P \ll 0.001$
	IV	53		53	80

* One *whi*⁺ recombinant was obtained in this cross.

these were all of the expected microscopic phenotype (data not given). This confirmed the validity of the scoring of the phenotypes. In crosses 1 to 3 of Table 5, only the *leuB5* parent was a *whi* mutant. From these crosses were derived estimates of the relative frequencies of crossing-over in the *leuB-whi* and *whi-cysD* intervals. The validity of the scoring of microscopic phenotypes was again upheld.

Crosses 4 to 9 of Table 5 were heterozygous for all three possible pairwise combinations of *whi* mutations, each in both coupling arrangements with the selective markers. For example, crosses 4 and 7 involved *whiG71* and *H250* in alternative coupling arrangements. Ignoring rare multiple crossover classes, in cross 4 frequent *whi*⁺ recombinants were generated by crossing-over between *whiG* and *H*, while crossing-over in the same region in cross 7 generated *whiG whiH* recombinants. Assuming no unknown selective effects, these two classes should be equally frequent. Three possible phenotypes can be envisaged for a given double *whi* mutant: like one *whi* parent, like the other, or different from both. In the present crosses, only the parental phenotypes were observed; thus the double mutants always resembled one of the parent strains. From the relative frequencies of the two *whi* phenotypes segregating in the cross this parent could be determined, with the result that *whiG* was found to be epistatic to *whiH* and *I*, and *whiH* to *whiI*. In each case very good agreement of the observed segregation with that expected from epistasis of one of the *whi* genes was obtained (Table 5).

DISCUSSION

The main conclusions arising from this work are: (i) the aerial mycelium of double *whi* mutants for the *whiA*, *B*, *G*, *H* and *I* genes is always morphologically similar to one of the single mutant parent strains; (ii) a consistent scheme of epistasis emerges, with *whiG* epistatic to *whiA*, *B*, *H* and *I*, *whiH* to *whiA*, *B* and *I*, and *whiA* and *B* to *whiI*. Thus no complex interactions were detected.

It is difficult to make definite interpretations of these results, although we may reasonably state that, directly or indirectly, the *whiG* gene product causes limited coiling, and the *whiH* product further coiling, of aerial hyphal cells, and that the *whiA* and *B* gene products are perhaps more directly involved in sporulation septation. It is certainly not reasonable to assume that the epistatic scheme reflects the temporal sequence of either gene expression or gene product activity in any simple way.

Combining all the available information on these *whi* genes, a very speculative model can be put forward. On this model, the *whiG* and *H* gene products would be involved in cell wall changes required for the initiation and localization of sporulation septation. The coiling observed would be a secondary consequence of these changes. The *whiA* and *B* gene products might be directly involved as structural or catalytic elements in the development of sporulation septa. Since this scheme takes away from the *whiI* gene product the task of regulating the spacing of sporulation septa, it may be that the *whiI* effect upon spacing (Hopwood *et al.* 1970) is indirect and might result from, for example, an effect upon the distribution of chromosomal material to spore compartments that occurs during normal sporulation septation.

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