

## A Morphological and Genetic Mapping Study of White Colony Mutants of *Streptomyces coelicolor*

By K. F. CHATER

John Innes Institute, Colney Lane, Norwich, NOR 70F

(Accepted for publication 9 March 1972)

### SUMMARY

Fifty *whi* mutants of *Streptomyces coelicolor*, having white instead of the wild-type grey colonies, were examined microscopically and genetically. The aerial mycelium structure of the mutants was broadly classified into six types, ranging from the complete absence of any stage of sporulation to the presence of apparently normal spores. Eight map locations were discovered for *whi* genes, all in previously well-marked regions of the map. Closely linked mutations possessed similar aerial mycelium structure, with few exceptions.

### INTRODUCTION

Hopwood, Wildermuth & Palmer (1970) described a simple visual selection for sporulation mutants of *Streptomyces coelicolor*: because the wild-type colony turns from white to grey as its spores mature, it was reasoned that colonies remaining white on prolonged incubation might be unable to make mature spores. In that study about one hundred white (*whi*) mutants were isolated, of which three were chosen for further study because they were apparently defective in the formation or spacing of sporulation septa. The present report describes genetic mapping studies with many of the remaining mutants, and their phenotypic classification by phase-contrast microscopy.

### METHODS

*Organisms.* All the strains used in this work were ultimately derived from the wild-type *Streptomyces coelicolor* (*S. violaceoruber sensu* Kutzner & Waksman, 1959) strain A3(2) (Hopwood, 1959). All *whi* mutants were isolated from this strain by Professor D. A. Hopwood, Mrs Helen Palmer and Mrs Helen Wright, either as spontaneous mutants or following ultraviolet irradiation (Hopwood & Sermonti, 1962) or treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Delić, Hopwood & Friend, 1970). The morphologically wild-type, genetically marked strains used for mapping *whi* mutants are listed in Table 1, and the locations on the circular linkage map of all the markers employed are shown in Fig. 5 (inner circle). Genetic symbols, which follow the recommendations of Demerec, Adelberg, Clark & Hartman (1966), are as listed by Hopwood (1967) and Harold & Hopwood (1970a). All recombinant derivatives of *whi* mutants possessed *strA1*, because this was the only marker available for selection in primary crosses with the prototrophic *whi* mutants.

*Media and general methods of culture and genetic analysis.* These were as described by Hopwood (1967) and Hopwood *et al.* (1970). Scoring of ultraviolet sensitivity of recombinant progeny on replica plates was as described by Harold & Hopwood (1970b).

Table 1. *Characteristics of morphologically wild-type strains used for mapping*

All strains were of the NF fertility type except A3(2) which is IF (Vivian & Hopwood, 1970).

Strain	Genetic markers
A3(2)	Wild-type
9	<i>hisD3</i>
35	<i>pheA1 uraA1</i>
417	<i>hisC9</i>
461	<i>metB4 pheA1</i>
782	<i>argA1 cysC3 hisA1 mthB2 strA1</i>
876	<i>argA1 cysC3 hisC9 pheA1 proA1 strA1</i>
955	<i>cysA15 hisA1 nicA1 pheA1 strA1 tps-30</i>
1076	<i>argA1 serB2 strA1 uraA1</i>
1107	<i>cysD18 hisA1 leuB5 strA1</i>
1258	<i>argA1 cysC3 hisC9 proA1 strA1 uraA1</i>
A321	<i>uraA1</i>
J192	<i>cysD18 leuB5 strA1</i>
J240	<i>cysD18 hisD3 strA1</i>
V31	<i>mthB2 proA1 uraA1 strA1 uvsB6</i>
V115	<i>hisC9 proA1 strA1 uvsD18</i>
V151	<i>argA1 uvsD18 strA1</i>

*Microscopy.* Impression preparations of aerial mycelium were made by touching a glass coverslip on a 5- to 7-day-old colony growing on defined medium. Alternatively, if the aerial mycelium was to be examined *in situ*, coverslip preparations were made. A sterile coverslip was inserted obliquely into the agar growth medium, and the strain was inoculated in the acute angle so formed. This permitted aerial mycelium to grow against the coverslip, so that when the latter was carefully removed after 5 to 7 days very little disturbance occurred (Williams & Davies, 1967). Preparations of both kinds were mounted in water and examined and photographed using a Zeiss Photomicroscope II and Ilford Microneg film.

## RESULTS

### *Phenotypic classification of whi mutants*

All the *whi* mutants were examined by phase-contrast microscopy, using impression preparations presumably composed largely of aerial mycelium. This process allowed their rough classification into six classes (I–VI) on the basis of aerial mycelium structure, the numerical sequence not necessarily reflecting a temporal sequence of morphogenetic stages. Representative mutants of the various classes were also examined by the coverslip method, which produced less disruption of the fragile aerial parts of the colony.

**Class I.** Impression preparations of these mutants contained only long, non-helical fibrous hyphae (Fig. 1*a, b*). At high magnification (Fig. 1*b*) sporulation septa (Wildermuth & Hopwood, 1970) were never seen though, as in wild-type, occasional cross-walls were observed in the substrate mycelium in coverslip preparations, suggesting that, had sporulation septa been present, they too would have been visible.

**Class II.** In impression preparations of class II mutants the aerial hyphae were often curled into long, tight helices (Fig. 1*c*), which never showed fragmentation, or any sign at high magnification of sporulation septa (Fig. 1*d, e, f*). The frequency of helical pieces of mycelium varied considerably among mutants and preparations.

**Class III.** These mutants resembled the mutant *whi-46* described by Hopwood *et al.* (1970), who observed that impression preparations contained 'unfragmented helical hyphae'.

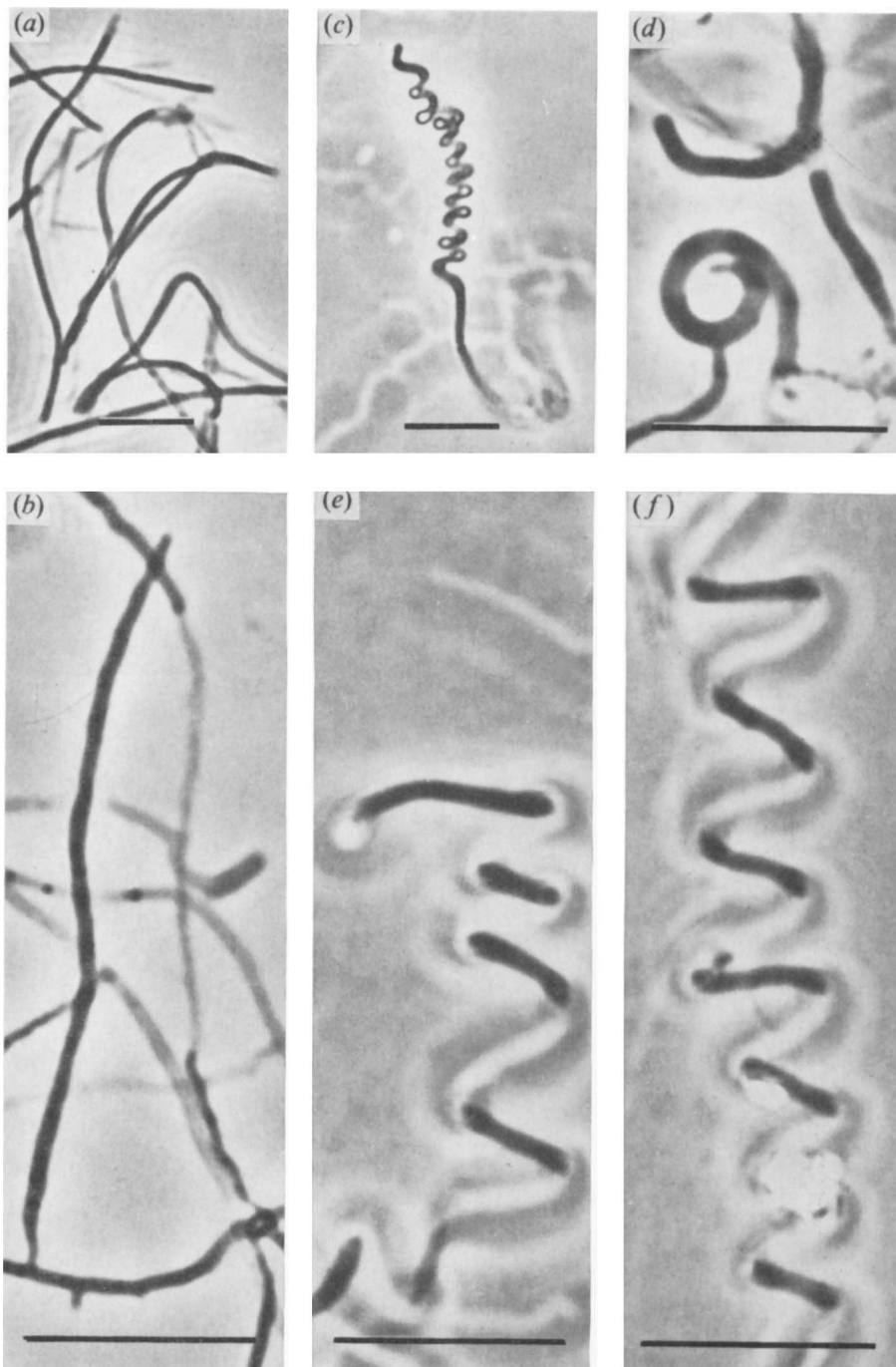


Fig. 1. Aerial mycelium of *whi* mutants (phase contrast). Scale marks represent 10  $\mu$ m. (a) and (b) Class I mutants: (a) *whi-103*; (b) *whi-75*. Note absence of helical coiling, fragmentation and sporulation septa. (c) and (d) Class II mutant (*whi-70*). Note tight helical coiling but absence of fragmentation and sporulation septa. (e) and (f) Class II mutant (*whi-72*). High magnification photomicrographs showing absence of sporulation septa in helical regions.

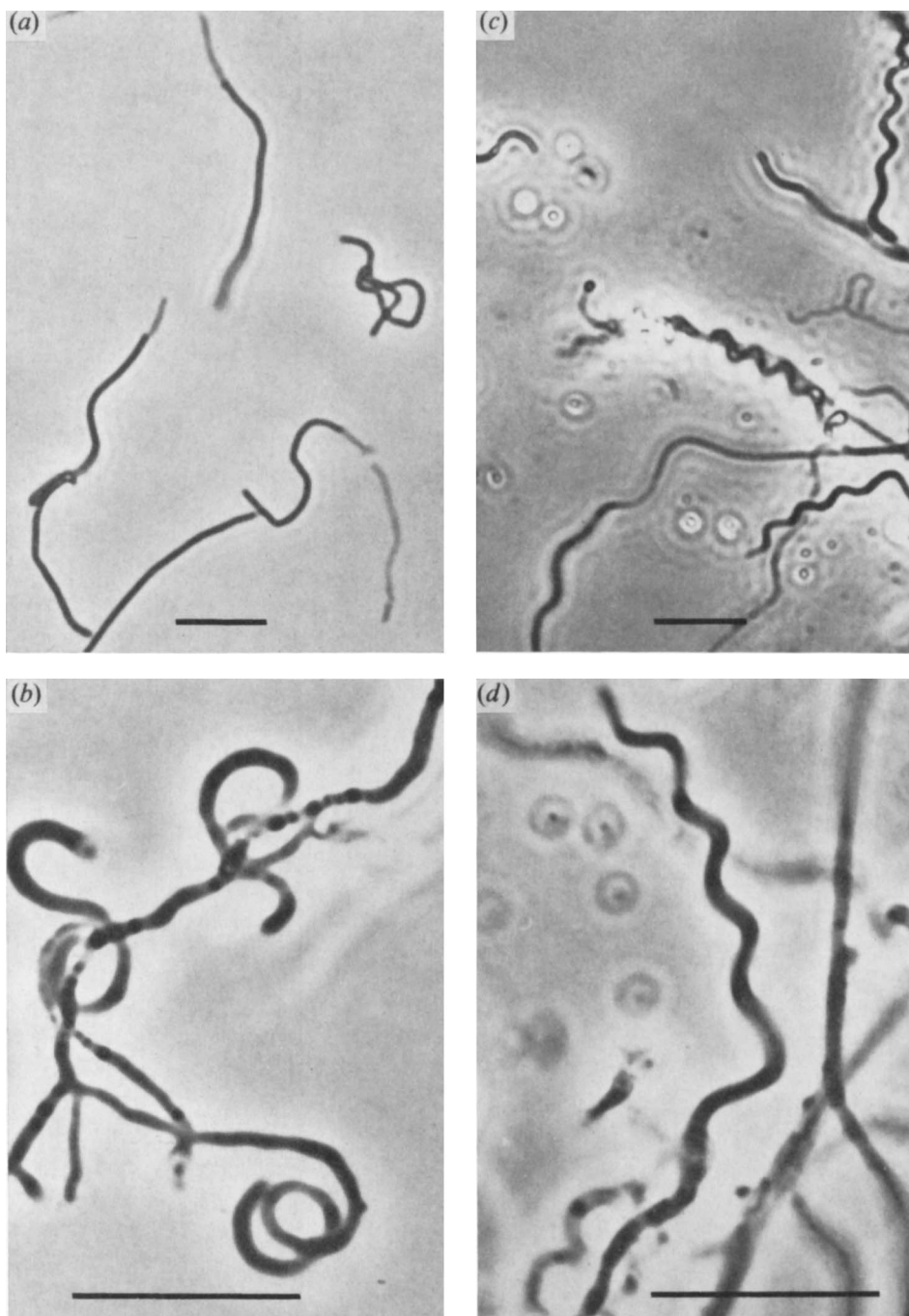


Fig. 2. Aerial mycelium of *whi* mutants (phase contrast). Scale marks represent 10  $\mu\text{m}$ . (a) and (b) *whi-193*. Note irregular coiling but absence of fragmentation and sporulation septa. (c) and (d) Class III mutant (*whi-119*). Hyphae are wavy and do not fragment or make sporulation septa.

The pitch of the helices was longer than in class II mutants, giving the aerial mycelium a wavy rather than spring-like appearance (Fig. 2*c, d*). Sporulation septa were not seen at high magnification (Fig. 2*d*).

One mutant, *whi-193*, was difficult to classify, having features of each of groups I and III. Its aerial hyphae never fragmented, and occasionally formed irregular helices (Fig. 2*a, b*) apparently having no sporulation septa. Small numbers of spores were sometimes encountered, their frequency increasing with colony age.

Class IV. The mutants *whi-6* and *-13* of Hopwood *et al.* (1970) were both members of this class, which comprised those mutants producing many short, often tightly coiled, fragments in impression preparations, occasionally with some spores (Fig. 3*b*). From cover-slip preparations (Fig. 3*a, c*) it appeared that *in situ* the aerial hyphae consisted of a short stem of straight mycelium, in which widely spaced cross-walls could occasionally be seen, surmounted by a tightly coiled terminal knot within which closely spaced sporulation septa were often discernible. It was presumably at these septa that fragmentation, observed in impression preparations, took place. In the particular case of *whi-13*, although fragmentation occurred, there was also some resemblance to class II mutants in that long tightly coiled helical hyphae apparently lacking sporulation septa were often seen.

Class V. Only one class V mutant (*whi-99*) has been identified. It produced regular chains of spore-like bodies (Fig. 4*a, b*) which differed from mature wild-type spores (Fig. 4*d*) in being rod-shaped rather than oval. Measurements of the dimensions of these units, made on chains in photomicrographs, gave average dimensions of  $0.765 \times 1.64 \mu\text{m}$  (sample size 36) for *whi-99* and  $0.865 \times 1.26 \mu\text{m}$  (sample size 64) for the wild-type. The calculated cytoplasmic volumes of such objects, assuming a spore wall thickness of  $0.05 \mu\text{m}$ , are  $0.39$  and  $0.32 \mu\text{m}^3$  respectively. Thus the difference between the two types is one of shape rather than size. This rod-shape also occurs in immature wild-type spore chains (Fig. 4*d*); perhaps *whi-99* is unable to perform a 'rounding-up' of spore units.

Class VI. Impression preparations of these mutants contained many spores and spore-chains closely resembling those of the wild-type; indeed, those of *whi-107* and *-124* were indistinguishable from wild-type spores. These two mutants may be unable to make the grey pigment of wild-type spores (if the grey colouration is in fact due to a pigment). The spores of *whi-16* (Fig. 4*c*) were more distinctive, being more nearly spherical and slightly larger than wild-type spores and forming somewhat irregular chains. Particles showing Brownian movement were often seen within *whi-16* spores, suggesting that the spores had lost their structural integrity, and that the spore wall might be more fragile in this mutant than in the wild-type.

#### *Genetic analysis of whi mutants*

Because the *whi* mutants were all isolated directly from the wild-type strain A3(2), they were all prototrophic, streptomycin-sensitive, and of the 'IF' fertility type (Vivian & Hopwood, 1970).

In preliminary mapping experiments, each mutant was crossed with one of the multiply marked, streptomycin-resistant strains 876 and 1258, which were both NF in fertility type (Hopwood, Harold, Vivian & Ferguson, 1969; Vivian & Hopwood, 1970). Spore progeny from the cross were plated on a medium containing streptomycin and lacking histidine (or arginine). Only those recombinants that had inherited the *hisC*<sup>+</sup> (or *argA*<sup>+</sup>) allele of the *whi* parent strain, and the *strA1* allele of 876 or 1258, were able to grow on this medium; the remaining growth requirements of 876 or 1258 were present in the medium, permitting the segregation of the other markers present in the cross.

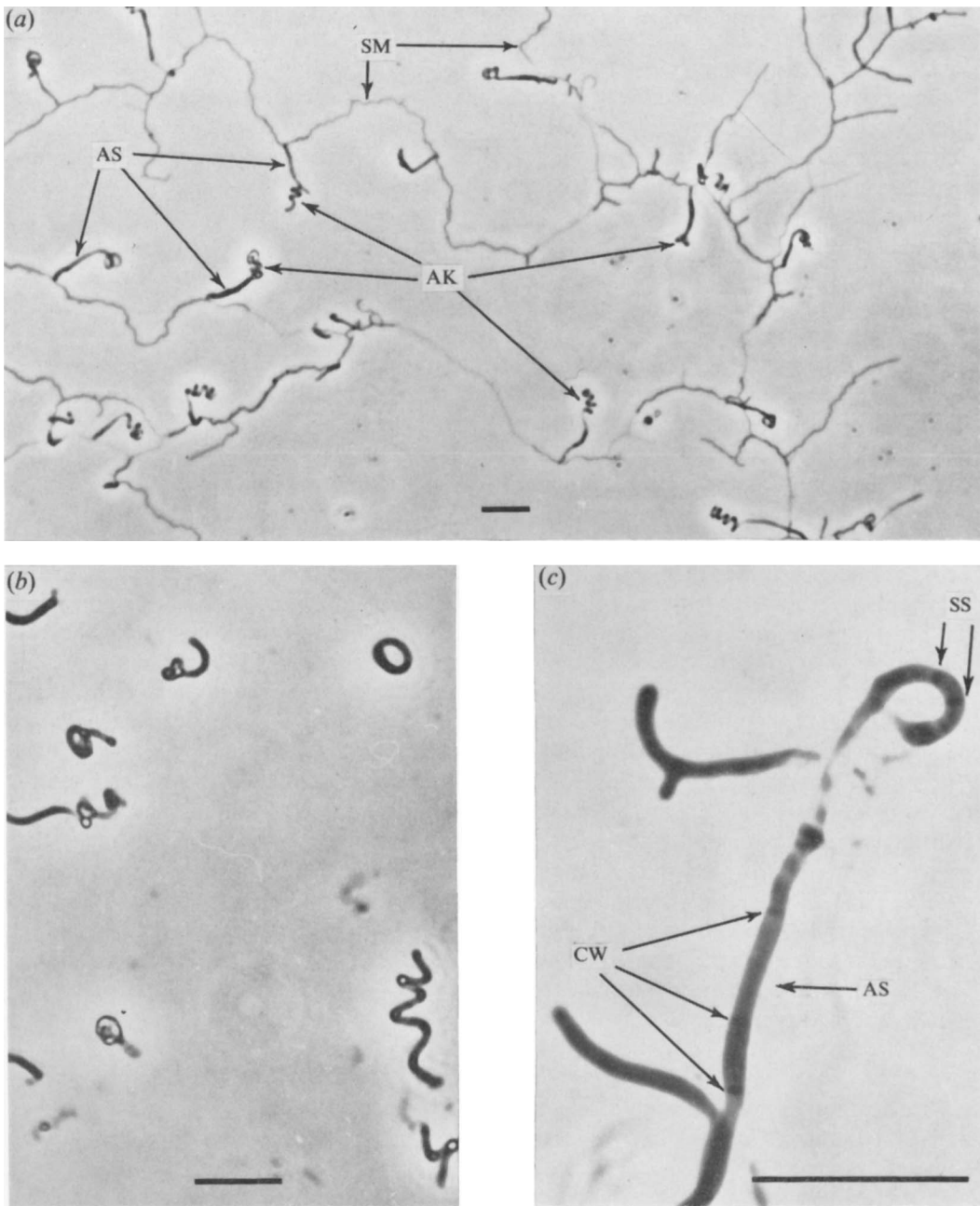


Fig. 3. Aerial mycelium of *whi* mutants (phase contrast). Scale marks represent 10 μm. (a) *whi-17*, coverslip preparation; (b) *whi-229*, impression preparation; (c) *whi-40*, coverslip preparation. All three mutants are examples of the class IV phenotype. Aerial hyphae growing on the substrate mycelium (SM) have straight stems (AS) containing widely spaced cross-walls (CW); the stems are surmounted by tightly coiled knots (AK) often containing relatively closely spaced sporulation septa (SS). The fragments seen in impression preparations are presumably derived from the knots.

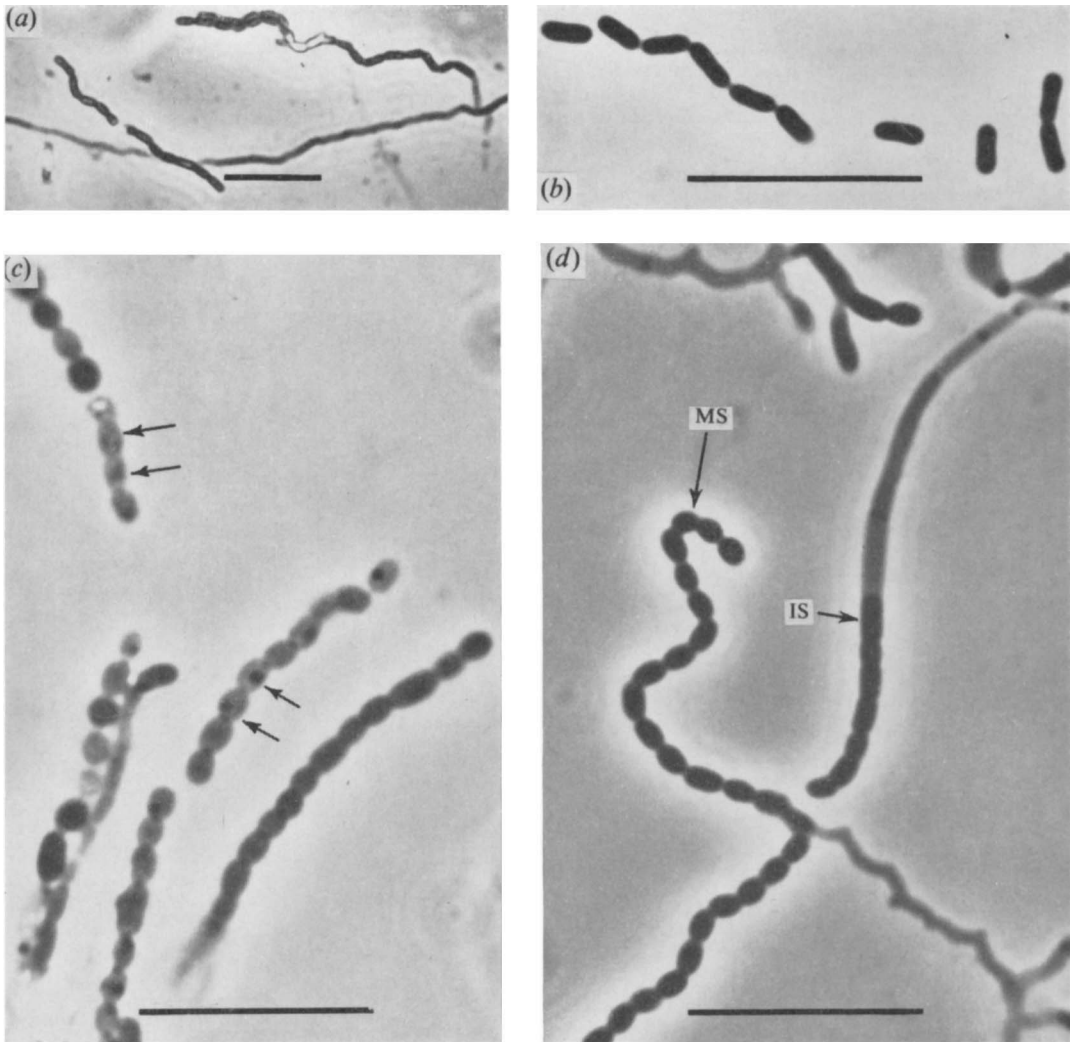
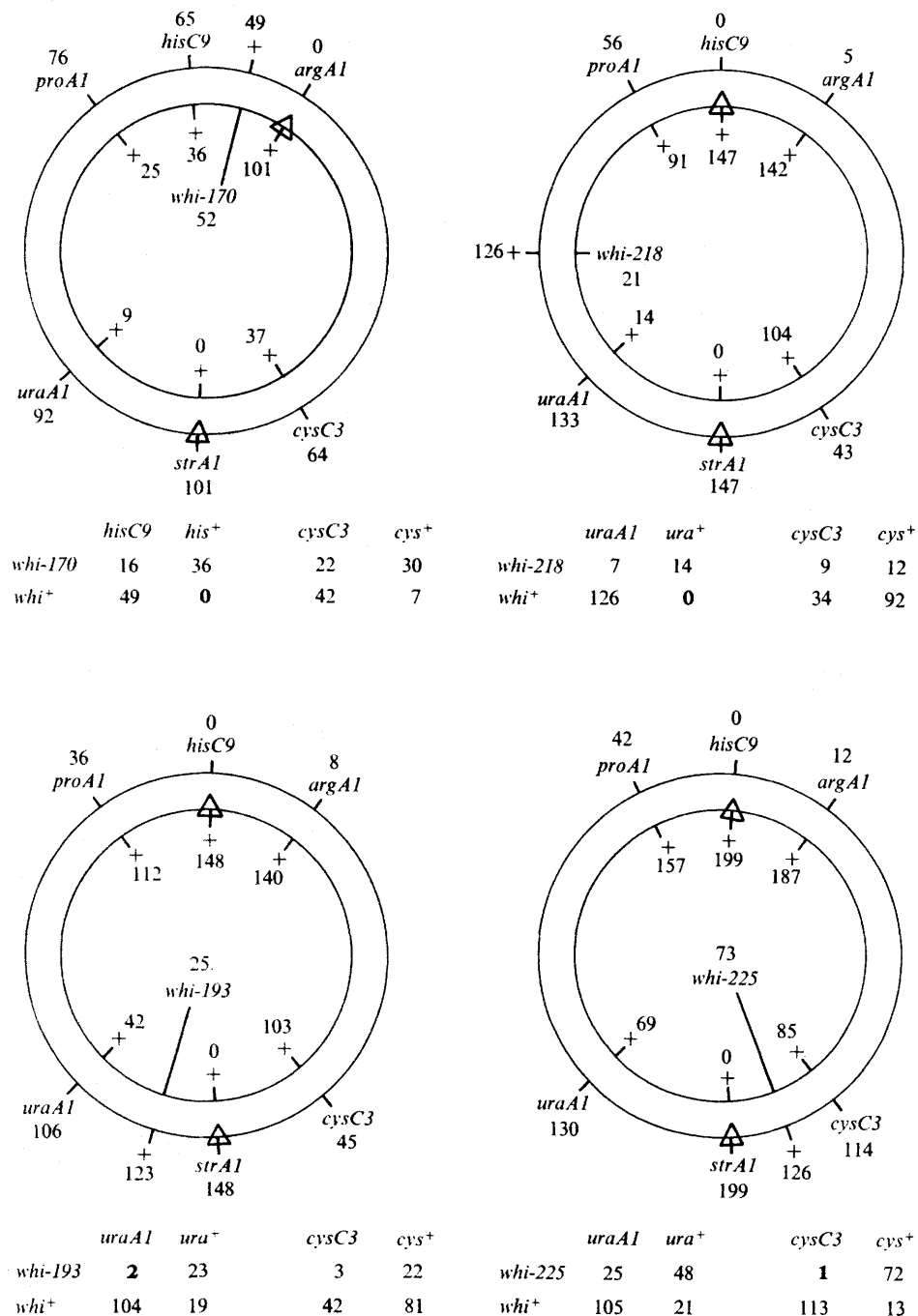


Fig. 4. Spores of *whi* mutants and the wild-type (phase-contrast). Scale marks represent 10  $\mu$ m. (a) and (b) Class V mutant (*whi-99*). This mutant produces chains of rod-shaped spores. Double chains (a) are also found in the wild-type and in other *whi* mutants. (c) Class VI mutant (*whi-16*). The spore chains are irregular and the spores rounder than in the wild-type. Some spores (arrow) are transparent and contain particles showing Brownian movement. Most other class VI mutants possess spores that are indistinguishable from those of the wild-type. (d) Wild-type. Note rod-like appearance of immature spores (IS) and ellipsoidal shape of mature ones (MS).

After scoring of recombinant phenotypes, the frequency of each segregating allele among the recombinants was calculated, and the values inserted in a diagrammatic representation of the cross (Table 2). The frequency of an allele is related to the closeness of its linkage to the selected marker with which it was coupled in the parent strain, so that the frequency of the *whi* mutation in each cross indicated the closeness of its linkage to *hisC*<sup>+</sup> (or *argA*<sup>+</sup>) compared with that of other markers in the cross. The *whi* mutation was thus given two alternative locations, one in each of the arcs separating the selected markers. To decide

Table 2. *Preliminary mapping of whi mutations*

Examples are given of crosses with four genetically distinct *whi* mutants. Each *whi* mutant (inner circle in diagrams) was crossed with strain 1258 (outer circle) and *hisA*<sup>+</sup> (or *argA*<sup>+</sup>) and *strA*1 (indicated by triangles) were selected. The numbers in the diagrams are the frequencies of alleles among the recombinant progeny. The segregation of each *whi* mutation with respect to a potentially closely linked marker from each of the arcs separating the selected markers is tabulated below each diagram, and shown to be more dependent upon one marker than on the other. In each cross, one recombinant class out of the eight tabulated should have arisen from multiple crossing over, and therefore be rarer than the other seven. This class in each cross is given in bold-face type.





between these locations the segregation of the *whi* mutation with respect to that of a potentially closely linked marker from each arc was tabulated. In each case, segregation of the *whi* mutation was far more dependent on the segregation of one marker, to which it was therefore linked, than on that of the other. In the examples given in Table 2, lack of independent segregation was shown by the following pairs of mutations: *whi-170* and *hisC9*; *whi-218* and *uraA1*; *whi-193* and *uraA1*; and *whi-225* and *cysC3*. Among 50 mutations mapped in this way, six were located in the *hisC-argA* interval, two in the *proA-uraA* interval, one in the *uraA-strA* interval, and 41 between *strA* and *cysC*. With this information, it was possible to devise further crosses that gave more precise map locations, and allowed the subdivision of some groups of mutants. These crosses will be described under separate headings.

*Mutations located between hisC and argA.* Six mutations (*whi-13*, -72, -73, -85, -170 and -213) were located in the *hisC-argA* interval. Of these, five had the class II phenotype and the sixth, *whi-13*, the class IV phenotype. Recombinant derivatives were constructed carrying each mutation coupled with *hisC9* (or the closely linked mutation *hisA1*) and *argA1* respectively (with the exception that no *argA1 whi-85* or *his whi-170* recombinants were obtained). Two types of crosses were then made using these strains.

In the first series of crosses, designed to measure recombination between pairs of *whi* mutations, each *whi hisC9* (or *hisA1*) strain was crossed with all the *whi argA1* strains, and selection was made for *his<sup>+</sup> arg<sup>+</sup>* recombinants, arising from crossing-over in the *his-argA* interval. The colonies arising were scored as *whi* or *whi<sup>+</sup>* by visual examination. The prediction was that *whi<sup>+</sup>* recombinants should arise with much higher frequency in one coupling arrangement than in the other for any pair of *whi* mutations, allowing the sequence of the mutations on the map to be determined. In practice, the frequency of *whi<sup>+</sup>* recombinants was so low (less than 1%) that it was difficult to eliminate the possibility that they had arisen from reversion of one of the parent strains to *whi<sup>+</sup>*. Thus an unambiguous sequence of mutations could not be obtained: but it was clear that all six mutations were very closely linked.

In the second series of crosses, designed to study recombination between the *whi* mutations and the *uvrD18* marker also located in the *hisC-argA* interval, similar selection was applied (for *his<sup>+</sup>* and *arg<sup>+</sup>*), but each cross was between either a *whi hisC9* (or *whi hisA1*) strain and strain V151 (*argA1 uvrD18*), or a *whi argA1* strain and strain V115 (*hisC9 uvrD18*). Because it turned out that the *whi* mutations were somewhat less closely linked to *uvrD* than they had been shown to be to each other, the results for all the mutations tested were summed, giving the following totals:

*argA1 uvrD18* × *whi his*: *uvrD18*, 600; *uvrD18 whi*, 22; *whi*, 126; *uvr<sup>+</sup> whi<sup>+</sup>*, 8;  
*hisC9 uvrD18* × *whi arg*: *uvrD18*, 309; *uvrD18 whi*, 6; *whi*, 249; *uvr<sup>+</sup> whi<sup>+</sup>*, 17.

These data strongly suggested a location for the *whi* mutations close to, and clockwise of, the *uvrD* locus.

It had previously been observed (D. A. Hopwood, personal communication) that the mutant *whi-73* was highly u.v.-sensitive, and it was shown by Harold & Hopwood (1970b) that it possessed a mutation in the *uvrA* gene, designated *uvrA24*, closely linked to *uvrD*. It was thus important to establish whether the *uvrA24* mutation was also responsible for the *whi-73* phenotype. That this was not the case was shown by the phenotype of the recombinant strain *hisC9 whi-73*, which had wild-type u.v. sensitivity and mutant morphology. On the other hand, the *argA1 whi-73* recombinant strain used in some crosses was highly u.v.-sensitive, and could not be used in the crosses involving *uvrD18*. Presumably it had retained

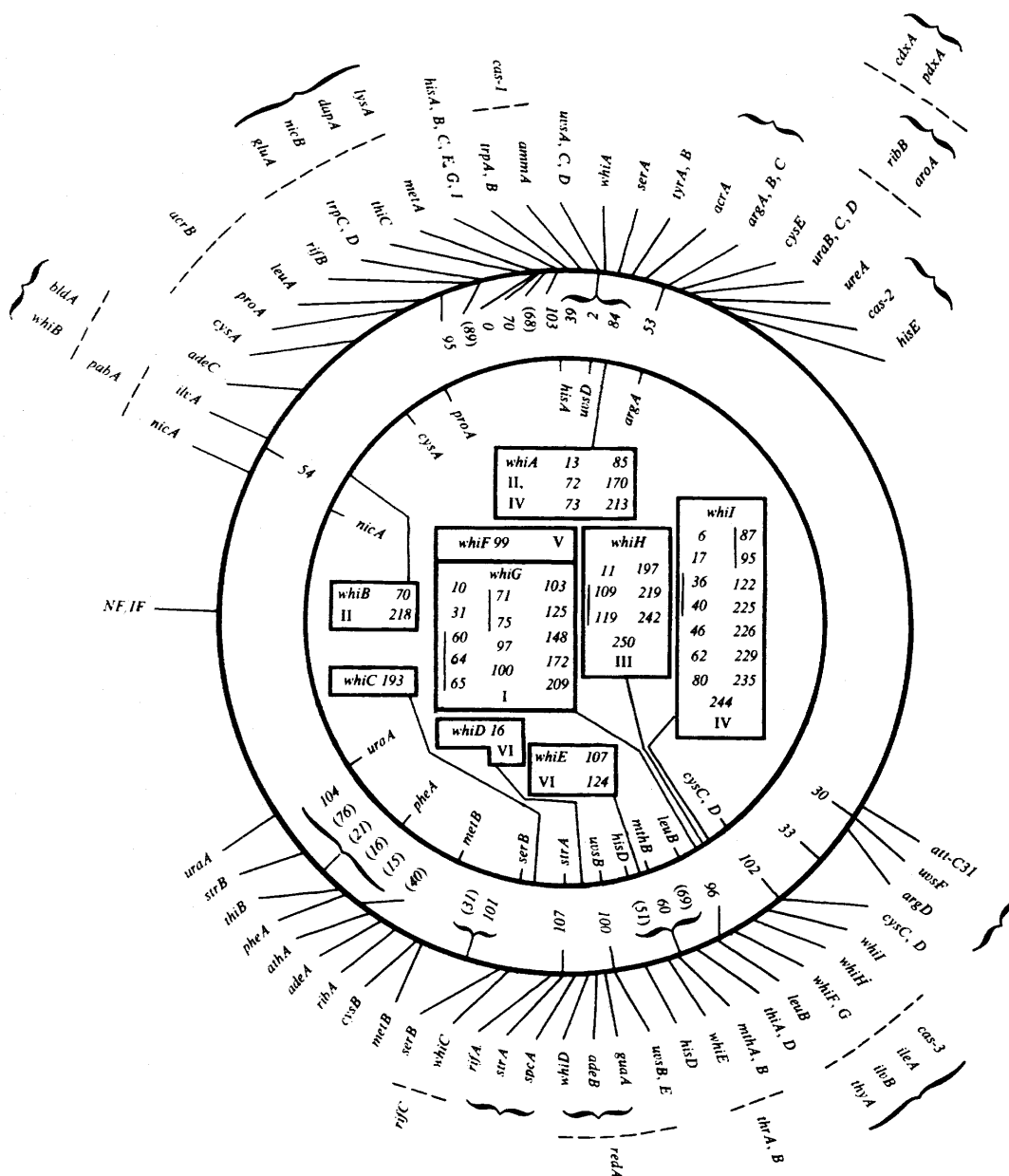


Fig. 5. Linkage map of *Streptomyces coelicolor* and summary of results in this paper. Outer circle: the outer circle is an up-to-date linkage map of *Streptomyces coelicolor* redrawn from that of Hopwood (1970) with later additions. The locus designations are as given by Hopwood (1967, 1970) with the following additions: *att-C31*, attachment site of prophage  $\phi$ C31 (Lomovskaya *et al.* 1971); *bldA*, apparent absence of aerial mycelium (Hopwood, 1967); *NF/IF*, donor versus recipient fertility (Vivian & Hopwood, 1971); *rifA,B,C*, resistance to rifampicin (K. F. Chater, unpublished); *trpA,B,C,D*, requirement for tryptophan (replaces *try*) (P. Engel, personal communication). Numbers on the inside of the outer circle represent indispensable temperature-sensitive mutations (Hopwood, 1966), parentheses indicating mutations which have now been lost. The orders within groups of bracketed loci are unknown. Certain loci have not been ordered relative to groups of loci covered by dotted lines. Inner circle: the inner circle gives the locations of markers referred to in this paper, and contains a summary of the locations and phenotypes of the *whi* mutations examined. Appended to each *whi* locus is a list of the allele numbers of mutations located there; alleles covered by lines are possibly of clonal origin. The predominant phenotypic classes of *whi* loci are given in Roman numerals, the meaning of which is described in the text (Results).

Table 3. More precise location of *whi-70* and *-218*

The *whi* mutants were crossed with strain 955, and *hisA*<sup>+</sup> and *strA1* (indicated by triangles) selected. The number against each allele in the diagrams gives the percentage frequency of that allele among the recombinant progeny. The tabulations of *whi* against *cysA* and *nicA* segregation show the numbers of informative recombinants upon which the presumed location of the *whi* mutations is based; the rarest class in each tabulation, given in bold-face type, is presumed to have arisen from multiple crossing-over.

	0	15	18	24	49	100		
	+	+	+	<i>whi-70</i>	+	+		
	<i>strA1</i>	<i>pheA1</i>	<i>nicA1</i>	+	<i>cysA15</i>	<i>hisA1</i>		
	100	85	82	76	51	0		
	<i>cysA15</i>	<i>cysA</i> <sup>+</sup>	Total		<i>nicA1</i>	<i>nicA</i> <sup>+</sup>	Total	
<i>whi-70</i>	0	13	13		<i>whi-70</i>	3	10	13
<i>whi</i> <sup>+</sup>	28	14	42		<i>whi</i> <sup>+</sup>	42	0	42
Total	28	27	55		Total	45	10	55

	0	13	15	17	35	100		
	+	+	+	<i>whi-218</i>	+	+		
	<i>strA1</i>	<i>pheA1</i>	<i>nicA1</i>	+	<i>cysA15</i>	<i>hisA1</i>		
	100	87	85	83	65	0		
	<i>cysA15</i>	<i>cysA</i> <sup>+</sup>	Total		<i>nicA1</i>	<i>nicA</i> <sup>+</sup>	Total	
<i>whi-218</i>	1	41	42		<i>whi-218</i>	13	29	42
<i>whi</i> <sup>+</sup>	156	45	201		<i>whi</i> <sup>+</sup>	194	7	201
Total	157	86	243		Total	207	36	243

the *uvsA24* mutation. The numbers of crossovers required for the formation of the *hisC9 whi-73* and *argA1 uvsA24 whi-73* strains are minimized if the map sequence of the *uvs* and *whi* genes is as suggested above; on the alternative sequence multiple crossing-over would be required for the formation of the former strain.

The observed frequency of crossing-over between *uvsD* and the *whi* mutations was about 3% in both coupling arrangements. With the same selection, Harold & Hopwood (1970*b*) obtained about 15% recombination between *uvsCAD* mutations and *serA*, and up to 5.6% between mutations within the *uvsCAD* cluster. The *whi* mutations are thus located anti-clockwise of *serA*, and, assuming that the *uvsCAD* cluster comprises three contiguous genes, the number of genes separating *uvsD* from the *whi* mutations is unlikely to be more than one or two.

On the strength of these data, the six *whi* mutations were assigned to the *whiA* locus (Fig. 5). *whi-70* and *-218*. The preliminary crosses (Table 2) indicated a location between *uraA* and *proA* for the mutations *whi-70* and *-218*, both of which had class II phenotypes. Further crosses were made with strain 955, which is well marked in this region, with the results shown in Table 3. Both mutations mapped in the interval between *cysA* and *nicA* at about 10 o'clock on the linkage map; this was the first location of *whi* mutations in this region of the map and the locus so defined was termed *whiB* (Fig. 5).

Table 4. *More precise location of whi-193*

The recombinant strain *mthB2 strA1 whi-193* was crossed with strains 35, 461 and 1076, selection being applied as indicated by triangles. The number against each allele in the diagrams gives the percentage frequency of that allele among the recombinant progeny. Segregation of *strA1* was not scored in crosses with strains 35 or 1076, and the colour of six *serB2* colonies in the latter cross was indeterminate.

Diagram of cross					Genotype	Crossovers in intervals	Frequency
	100	95	78	0			
	+	+	<i>whi-193</i>	<i>mthB2</i>	<i>pheA1</i>	a	5
Strain 35	△	a	b	c	Wild-type	b	20
	<i>uraA1</i>	<i>pheA1</i>	+	+	<i>whi-193</i>	c	86
	0	5	22	100	<i>pheA1 whi-193</i>	a, b, c	1
					Total		112

	100	88	75	58	0			
	+	+	<i>whi-193</i>	<i>strA1</i>	<i>mthB2</i>	<i>metB4</i>	a	13
Strain 461	△	a	b	c	d	Wild-type	b	13
	<i>pheA1</i>	<i>metB4</i>	+	+	+	<i>whi-193</i>	c	18
	0	12	25	42	100	<i>strA1 whi-193</i>	d	61
					Others	a, b, c; a, b, d; b, c, d	0	
					Total		105	

	100	89	≤ 72	0				
	+	+	<i>whi-193</i>	<i>mthB2</i>	<i>serB2</i>	a	12	
Strain 1076	△	a	b	c	Wild-type	b	33	
	<i>uraA1</i>	<i>serB2</i>	+	+	<i>whi-193</i>	c	109	
	0	11	≥ 28	100	<i>serB2</i> (colour uncertain)	a?	6	
					Total		160	

*whi-193*. Preliminary data located *whi-193* (phenotypic class I or III) between *uraA* and *strA* (Table 2). To refine this mapping, a *mthB2 strA1 whi-193* recombinant strain was crossed with strains carrying various marker mutations known to be located in the *uraA-strA* interval (Table 4).

In the first cross, with strain 35, selection was made for recombinants arising by crossing-over between *uraA* and *mthB*, and the segregation of the included marker *pheA1* was examined in relation to that of *whi-193*. *whi*<sup>+</sup> *pheA*<sup>+</sup> recombinants were common, whereas

Table 5. The location of *whi-16*

The mutant *whi-16* was crossed with strains 782 and v31, with selection as indicated by triangles, and the allele frequencies (percentage) obtained are shown in the diagrams.

Diagram of cross				Genotype	Crossovers in intervals	Frequency
0	4	91	100			
+	<i>whi-16</i>	+	+	<i>whi-16</i>	a	4
---	a	b	c	Wild-type	b	81
---	<i>strA1</i>	+	<i>methB2</i>	<i>methB2</i>	c	9
100	96	9	0	<i>methB2 whi-16</i>	a, b, c	0
Total						94
0	55	87	100			
+	<i>whi-16</i>	+	+	<i>whi-16</i>	a	56
---	a	b	c	Wild-type	b	35
---	<i>strA1</i>	+	<i>uvrB6</i>	<i>uvrB6</i>	c	12
100	45	13	0	<i>uvrB6 whi-16</i>	a, b, c	2
Total						105

*whi-193 pheA1* recombinants were rare, showing that the location of *whi-193* was anti-clockwise of *pheA*.

In a second cross, with strain 461, the selection was for recombinants arising from crossing-over between *pheA* and *methB*, and the segregation of the included markers *methB4* and *strA1* was examined in relation to that of *whi-193*. Since *whi-193 strA*<sup>+</sup> recombinants were common, and *whi*<sup>+</sup> *strA1* recombinants undetected, the location of *whi-193* clockwise of *strA* was confirmed: moreover, the detection of 12% of *whi*<sup>+</sup> *metB*<sup>+</sup>, but no *whi-193 metB*, recombinants showed that *whi-193* was situated anti-clockwise of *metB4*.

Finally, a cross was made with strain 1076, which carried the *serB2* marker located between *metB* and *strA*. Selection here was for recombinants arising from crossing-over between *uraA* and *strA*. A high proportion (21%) of recombinants were *whi*<sup>+</sup> *serB*<sup>+</sup>, but the difficulty of scoring the somewhat poorly sporulating *serB2* colonies for colour made it impossible to assess the number of *whi-193 serB2* recombinants. However, examination of the gradient of allele frequencies given in Table 4 reveals that *whi-193* is nearer than *serB2* to *methB*. The location of *whi-193* between *strA* and *serB* unambiguously defines a new *whi* locus, termed *whiC*.

*Mutations located in the strA-cysC region.* Hopwood *et al.* (1970) noted that the majority of the *whi* mutations they examined were located in the *strA-cysC* interval. Of the 50 *whi* mutations whose mapping is described in the present paper I found that 41 mapped in this region in preliminary crosses (Table 2). Differences in the linkage of some of these mutations with *cysC* suggested that several loci were represented, a possibility confirmed in crosses described below. For clarity the mutations will be considered in three separate sections.

Diagram of cross				Genotype	Crossovers in interval	Frequency in crosses with		
						<i>whi-107</i>	<i>whi-124</i>	
Strain J192	0	50	71	100				
	+	<i>whi</i>	+	+	<i>whi</i>	a	50	48
	-----							
	△	a	b	c	Wild-type	b	22	18
	-----							
	<i>strA1</i>	+	<i>leuB5</i>	<i>cysD18</i>	<i>leuB5</i>	c	24	33
	100	50	29	0	<i>leuB5 whi</i>	a, b, c	0	0
					Totals	96	99	
Strain J240	0	47	53	100				
	+	+	<i>whi</i>	+	<i>whi</i>	a	22	70
	-----							
	△	a	b	c	<i>hisD3 whi</i>	b	5	10
	-----							
	<i>strA1</i>	<i>hisD3</i>	+	<i>cysD18</i>	<i>hisD3</i>	c	23	69
	100	53	47	0	Wild-type	a, b, c	1	2
					Totals	51	151	
Strain 9	0	35	46	100				
	+	<i>hisD3</i>	+	+	<i>hisD3</i>	a	33	—
	-----							
	△	a	b	c	Wild-type	b	11	—
	-----							
	<i>strA1</i>	+	<i>whi</i>	<i>cysD18</i>	<i>whi</i>	c	51	—
	100	65	54	0	<i>hisD3 whi</i>	a, b, c	0	—
					Totals	95	—	
Strain 782	0	55	59	100				
	+	<i>whi</i>	+	+	<i>whi</i>	a	154	246
	-----							
	△	a	b	c	Wild-type	b	17	15
	-----							
	<i>strA1</i>	+	<i>mthB2</i>	<i>cysC3</i>	<i>mthB2</i>	c	123	175
	100	45	41	0	<i>mthB2 whi</i>	a, b, c	1	1
					Totals	295	437	

*whi-16*. Preliminary mapping indicated that *whi-16* (phenotypic class VI) was located closer to *strA* than to *cysC*. This was confirmed in a cross of *whi-16* with strain 782, in which selection was made for recombinants resulting from crossing-over in the interval between *strA* and *cysC*, which contained in addition to *whi-16* the marker *mthB2* (Table 5). Wild-type recombinants were very common, and *whi-16 mthB2* recombinants very rare, so it was concluded that *whi-16* was located between *strA* and *mthB*. In a further cross of *whi-16* against strain v31 selection was made for recombinants arising from crossing-over in the *strA-mthB* interval, which included the marker *uvsB6* (Table 5). Wild-type recombinants were again common and the reciprocal *uvsB whi-16* class rare, giving the gene sequence *strA-whi-16-uvsB*. This identified another previously unknown *whi* locus, termed *whiD* (Fig. 5).

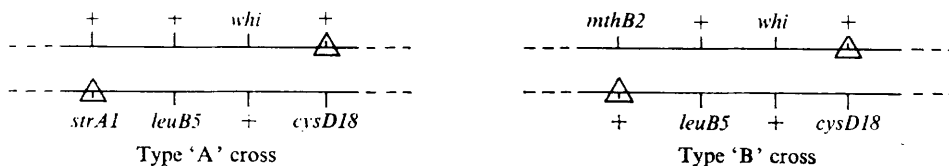
*whi-107 and -124*. A series of crosses was made with the strains *whi-107* and *-124* (phenotypic class VI) in all of which selection was made for recombinants resulting from crossing-over in the region between *strA* and *cysC* or *D*. The segregation of *leuB*, *hisD* or *mthB* (depending on the strain used) was then examined in relation to that of the *whi* mutations (Table 6). Where *leuB* was segregating the abundance of *whi<sup>+</sup> leuB<sup>+</sup>* and the absence of *whi leuB6* recombinants indicated that both *whi* mutations were located between *strA* and *leuB*. In contrast, in crosses involving the *hisD* gene, *whi<sup>+</sup> hisD<sup>+</sup>* recombinants were rare, and the reciprocal *whi hisD3* class quite common, giving the sequence *strA-hisD-whi*. This was confirmed for *whi-107* in a cross in which the same selection was applied, but the coupling arrangement was different, so that the more frequently occurring informative class was *whi<sup>+</sup> hisD<sup>+</sup>*. Finally, the segregation of *whi-107* and *-124* with respect to *mthB2* was examined, again in the same selective conditions. The critical recombinant classes were rare, indicating close linkage between *mthB2* and the *whi* mutations; among the critical recombinants, *mthB<sup>+</sup> whi<sup>+</sup>* greatly outnumbered *mthB2 whi*, giving the sequence *strA1-whi-mthB2*. It was thus concluded that *whi-107* and *-124* identified a new *whi* locus, *whiE* (Fig. 5), mapping between the closely linked loci *hisD* and *mthB*.

*Mutations in the leuB-cysC interval*. Crosses with strain 1107 or J192 (Table 7) served to locate the remaining 38 *whi* mutations between *leuB* and *cysC*, on the criterion that selection for recombinants arising from crossing-over between *strA* and *cysD* gave an excess of *leuB whi* over *leuB<sup>+</sup> whi<sup>+</sup>* recombinants. However, the extent of this excess varied widely, its significance being particularly difficult to assess where informative recombinants were rare. In some cases (cross-type B, Table 7) their relative numbers were increased by selecting for recombinants arising from crossing-over in the shorter interval *mthB* to *cysD*, utilizing *mthB2 whi* recombinants obtained in other crosses. In Table 7 the crosses have been roughly separated into two groups, the first comprising those in which *whi* recombinants occurred relatively frequently among the *leuB* progeny, and the second those in which *leuB whi<sup>+</sup>* were three or more times more frequent than *leuB whi* recombinants. This was done because, taking the ratio of *whi:whi<sup>+</sup>* among *leuB* recombinants to be related to the ratio of the distances *leuB-whi:whi-cysC*, these two groups probably represented at least two distinct *whi* loci, one of which was closely linked to *leuB*.

In order to clarify the linkage relationships of *whi* mutations in the *leuB-cysCD* interval, a series of recombinants was isolated in which *whi* mutations were coupled with either *leuB2* or *cysD18*. Table 8 contains data obtained from a permutation of crosses of such strains involving three representative *whi* mutations: *whi-71*, *-119*, and *-17* (phenotypic classes I, III and IV respectively). Selection was made for recombinants arising by crossing-over in the *leuB-cysC* interval. For each of the three heterologous pairs of mutations *whi<sup>+</sup>* recombinants were found in appreciable numbers in one coupling arrangement only, in which they could

Table 7. *The location of whi mutations between leuB and cysD*

Each *whi* mutant or its *mtbB2* derivative was crossed with strains 1107 or 1192 and *cysD*<sup>+</sup> and *strA1* (cross-type A) or *mtbB*<sup>+</sup> (cross-type B) were selected, with the exception of the cross involving *whi-244*, in which a *hisA1 whi-244 strA1* strain was used, and selection was made for *cysD*<sup>+</sup> and *hisA*<sup>+</sup>. The results have been divided into those giving comparable numbers of *leuB5 whi* and *leuB5 whi*<sup>+</sup> recombinants, and those in which *leuB5 whi*<sup>+</sup> recombinants were three or more times more frequent than *leuB5 whi* ones.



<i>whi</i> mutation	Frequency of recombinant classes				Total number of recombinants scored	Type of cross
	<i>leuB2</i>	<i>leuB2 whi</i>	<i>whi</i>	wild type		
*6	11	13	175	1	200	A
17	3	11	173	1	188	A
36	11	28	61	0	100	A
40	17	32	151	0	200	A
*46	28	27	43	0	101	B
62	38	20	134	6	198	A
80	1	13	234	1	249	A
87	81	45	103	6	235	B
95	4	4	17	0	25	A
103	28	31	22	0	81	B
109	46	23	21	0	90	B
119	30	18	97	1	146	A
122	45	41	64	0	150	A
180	8	25	66	1	100	A
197	70	29	2	0	101	B
219	23	41	83	0	147	A
225	46	21	13	0	80	B
226	20	38	20	0	78	B
229	32	29	27	0	88	B
235	35	31	22	0	88	B
244	30	57	111	2	200	A
10	5	11	71	0	87	See legend
11	73	5	24	0	102	B
31	83	5	10	1	99	A
60	31	2	15	0	48	A
64	70	2	23	2	97	A
65	44	3	3	0	50	B
71	73	6	24	0	103	B
75	88	2	11	0	101	A
97	148	5	51	0	204	B
99	66	2	32	0	100	A
100	332	7	29	3	371	B
125	49	7	28	2	86	A
148	86	9	14	0	109	B
172	25	7	67	0	99	A
209	256	11	33	1	301	B
242	39	6	35	0	80	B
250	77	1	8	0	86	B
	158	12	35	0	205	B
	39	3	48	0	90	A
	42	7	34	0	83	A
	79	15	11	0	105	B
	106	20	15	1	142	B

\* Data of Hopwood *et al.* (1970).



Table 8. Identification and ordering of three *whi* loci mapping between *leuB5* and *cysD18*

A complete permutation of crosses was carried out between recombinant derivatives of *whi-17*, *-71* and *-119* containing *leuB5* or *cysD18*, with selection for *leuB*<sup>+</sup> and *cysD*<sup>+</sup>. The recombinants were classified as *whi* or *whi*<sup>+</sup> either on the primary selective plates or, where leaky parental growth obscured colony colour, after restreaking. The crosses were set up from spore suspensions which were also tested for reversion of auxotrophic and morphological markers. In no case were the observed reversion rates higher than 10<sup>-4</sup> for *leuB5* or *cysD18*, or 10<sup>-2</sup> for *whi* mutations, per plating unit. (These limits represented practical limitations. The actual reversion rates were probably of a much lower order.)

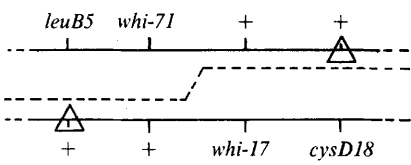
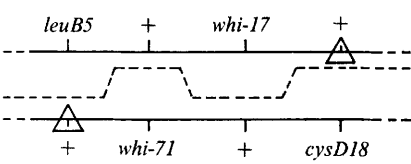
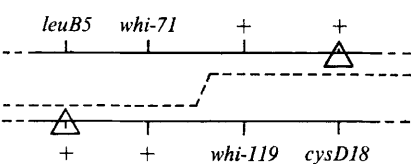
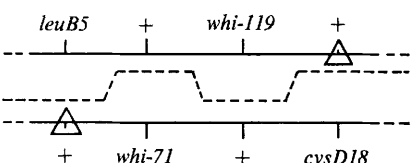
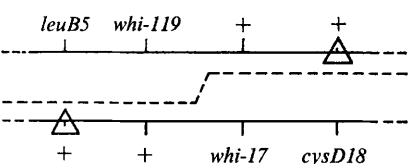
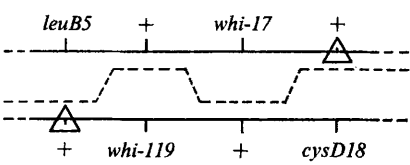
Crossovers required to generate <i>whi</i> <sup>+</sup> recombinants	Number of <i>whi</i> <sup>+</sup> recombinants (%)	Number of recombinants scored	Favoured gene sequence
	89	1314	<i>leuB</i> – <i>whi-71</i> – <i>whi-17</i> – <i>cysD</i>
	0	100	
	38	118	<i>leuB</i> – <i>whi-71</i> – <i>whi-119</i> – <i>cysD</i>
	0	70	
	35	86	<i>leuB</i> – <i>whi-119</i> – <i>whi-17</i> – <i>cysD</i>
	0	99	

Table 9. *Whi* mutations located between *leuB* and *cysD*: their mapping with respect to *whi-71*, *-119* and *-17*

All crosses were made as described in Table 8. The results are arranged in three groups, the members of which were located close to *whi-71*, *-119* and *-17* respectively. Classification of phenotypes was as described earlier in the text.

<i>whi</i> mutation coupled with <i>leuB5</i> (or * <i>mthB2</i> )	Number (%) of <i>whi</i> <sup>+</sup> recombinants with			Phenotype
	<i>cysD18 whi-71</i>	<i>cysD18 whi-119</i>	<i>cysD18 whi-17</i>	
10	0	52	82	I
31	0	37	77	I
60	0	34	47	I
64	0	40	90	I
71	0	38	89	I
75	0	46	79	I
97	0	17	81	I
99	2	40	75	V
100	1	55	86	I/III
*103	0	32	34	I
125	0	32	83	I
172	0	50	79	I
209	0	42	86	III
11	0	0	42	III
109	—	0	42	III
119	0	0	35	III
197	0	0	31	III
219	0	0	51	III
242	—	0	27	III
250	0	0	44	III
6	0	0	0	IV
17	0	0	0	IV
36	0	0	0	IV
40	1	0	0	IV
46	0	0	0	III
62	0	0	0	IV
80	0	0	1	IV
87	0	0	0	IV
95	0	0	0	IV
122	—	0	0	IV
225	0	—	0	IV
226	0	0	0	IV
229	0	0	0	IV
235	—	0	0	IV
244	0	1	0	III/IV

presumably be generated by a single crossover in the *leuB-cysD* interval. This permitted an unambiguous ordering of the three mutations, *whi-71* being located close to *leuB*, *whi-17* close to *cysD*, and *whi-119* approximately midway between the *leu* and *cys* genes.

Having established this basic map, the *cysD18* strains containing *whi-71*, *-119* and *-17* were crossed with *leuB5* strains containing most of the other *whi* mutations mapping in the *leuB-cysD* interval, again with selection for *leuB*<sup>+</sup> and *cysD*<sup>+</sup>. The frequencies of *whi*<sup>+</sup> recombinants are given in Table 9. Each *leuB5 whi* strain showed the same recombination pattern as one of the three *leuB whi* strains listed in Table 8 had done. Those listed in the first part of Table 9 resembled *leuB5 whi-71* in giving no *whi*<sup>+</sup> recombinants with *cysD18 whi-71*, about 30 to 50% with *cysD18 whi-119*, and about 80 to 90% with *cysD18 whi-17*; those in the second part of Table 9 resembled *leuB5 whi-119* in giving no *whi*<sup>+</sup> recombinants with *cysD18 whi-71* or *-119*, but about 30 to 50% with *cysD18 whi-17*; and those in the third

part of the Table resembled *leuB5 whi-17* in giving no *whi*<sup>+</sup> recombinants with any of the tester strains.

A few *whi* mutations were also obtained in combination with *cysD18* and crossed with *leuB5* tester strains carrying *whi-71*, *-119* and *-17* respectively (results not tabulated). The *cysD18* strains carrying *whi-65*, *-99*, *-125*, *-148* and *-180* all failed to give *whi*<sup>+</sup> recombinants with any of the testers. These *whi* mutations were thus located close to *whi-71*, confirming the evidence in Table 9 for *whi-99* and *-125*, and giving locations for *whi-65*, *-148* and *-180* of which *leuB5* derivatives were not available. The location of *whi-80* close to *whi-17* was also confirmed in crosses of *cysD18 whi-80* with the *leuB5 whi* tester strains: 88% *whi*<sup>+</sup> recombinants were obtained with *leuB5 whi-71*, 47% with *leuB5 whi-119* and none with *leuB5 whi-17*.

Excluding *whi-99*, we may conclude that of the 37 mutations examined 15 were located in a cluster close to *leuB*, 15 in a cluster close to *cysD* and seven in a cluster midway between *leuB* and *cysD*. With the exception of *whi-209* (class III), all mutations in the first group had class I phenotype and were assigned to the *whiG* locus; mutations in the group closely linked to *cysD* had class III or IV phenotype and were assigned to the *whiI* locus; and all mutations in the intervening group had class III phenotype and defined the *whiH* locus.

On the strength of its unique phenotype (class V) *whi-99* was taken to represent a distinct locus, *whiF*, very closely linked to *whiG*.

#### DISCUSSION

To permit correlation of the two classifications – morphological and genetic – of white colony mutants, the data have been summarized in Fig. 5. Eight distinct map locations, designated *whiA*, *B*, *C*, *D*, *E*, *G*, *H* and *I*, have been identified, and with a few exceptions mutations in any one location give rise to similar aerial mycelium structure. The exceptions may arise partially from the roughness of the classification; however, Hopwood *et al.* (1970) found that the mutations *whi-6* (class IV phenotype) and *whi-46* (class III phenotype), both of which map at the *whiI* locus, were ultrastructurally distinct in that *whi-6* had abnormally widely spaced sporulation septa while *whi-46* had none. This emphasizes the possibility that some of the clustered mutations found in this study may prove to be non-allelic if genetic functional tests can be applied. Such tests have so far been difficult to carry out either by the use of heteroclones (Hopwood, 1967) or by a technique exploiting the different filterability of wild-type spores and the aerial growth of *whi* mutants of phenotypic classes I–IV (K. F. Chater, unpublished). In the case of *whi-99*, the great difference between its phenotype (class V) and that of all the other mutations closely linked to it at the *whiG* locus (class I) seems sufficient to justify the assumption that *whi-99* is a mutation in a distinct gene, *whiF*.

Since several of the *whi* loci are represented by only one or two mutations, it is reasonable to assume that more loci await discovery, and that the system is genetically more complex than the previous study by Hopwood *et al.* (1970) had indicated. A more rational approach to the search for further loci and to the selection of representative mutants for fine structure and biochemical analysis should be facilitated by this study.

The distribution of *whi* loci on the linkage map (Fig. 5) is similar to that of auxotrophic, resistance and temperature-sensitive mutations (Hopwood, 1966, 1967) in that no *whi* mutations lie in either of the two 'silent' regions: in *Bacillus subtilis*, one previously genetically silent region has recently been found to contain many of the genes involved in sporulation (Ionesco, Michel, Carmi & Schaeffer, 1970). Presumably the silent regions of *Streptomyces coelicolor* are either composed of integrated extraneous genetic material such

as prophages (e.g.  $\phi$ C31; Lomovskaya, Emeljanova & Alikhanian, 1971) and episomes, or they represent recombinational 'hot spots' as discussed by Hopwood (1967).

I am grateful to Judith Humphries and Frances Dixon for their excellent technical assistance and to Professor D. A. Hopwood for his suggestions and criticisms and for allowing me free access to his strains and unpublished results.

#### REFERENCES

- DELIĆ, V., HOPWOOD, D. A. & FRIEND, E. J. (1970). Mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) in *Streptomyces coelicolor*. *Mutation Research* **9**, 167-182.
- DEMEREK, M., ADELBERG, E. A., CLARK, A. J. & HARTMAN, P. E. (1966). A proposal for a uniform nomenclature in bacterial genetics. *Genetics* **54**, 61-76.
- HAROLD, R. J. & HOPWOOD, D. A. (1970a). Ultraviolet sensitive mutants of *Streptomyces coelicolor*. I. Phenotypic characterisation. *Mutation Research* **10**, 427-438.
- HAROLD, R. J. & HOPWOOD, D. A. (1970b). Ultraviolet sensitive mutants of *Streptomyces coelicolor*. II. Genetics. *Mutation Research* **10**, 439-448.
- HOPWOOD, D. A. (1959). Linkage and the mechanism of recombination in *Streptomyces coelicolor*. *Annals of the New York Academy of Sciences* **81**, 887-898.
- HOPWOOD, D. A. (1966). Non-random arrangement of temperature-sensitive mutants on the linkage map of *Streptomyces coelicolor*. *Genetics* **54**, 1169-1176.
- HOPWOOD, D. A. (1967). Genetic analysis and genome structure in *Streptomyces coelicolor*. *Bacteriological Reviews* **31**, 373-403.
- HOPWOOD, D. A. (1970). Linkage map and list of markers of *Streptomyces coelicolor*. In *Handbook of Biochemistry, Selected Data for Molecular Biology*, 2nd edn. Edited by H. A. Sober. Ohio: Chemical Rubber Company.
- HOPWOOD, D. A., HAROLD, R. J., VIVIAN, A. & FERGUSON, H. M. (1969). A new kind of fertility variant in *Streptomyces coelicolor*. *Genetics* **62**, 461-477.
- HOPWOOD, D. A. & SERMONTI, G. (1962). The genetics of *Streptomyces coelicolor*. *Advances in Genetics* **11**, 273-342.
- HOPWOOD, D. A., WILDERMUTH, H. & PALMER, H. M. (1970). Mutants of *Streptomyces coelicolor* defective in sporulation. *Journal of General Microbiology* **61**, 397-408.
- IONESCO, H., MICHEL, J., CAMI, B. & SCHAEFFER, P. (1970). Genetics of sporulation in *Bacillus subtilis* Marburg. *Journal of Applied Microbiology* **33**, 13-24.
- KUTZNER, H. J. & WAKSMAN, S. A. (1959). *Streptomyces coelicolor* Müller and *Streptomyces violaceoruber* Waksman and Curtis, two distinctly different organisms. *Journal of Bacteriology* **78**, 528-538.
- LOMOVSKAYA, N. D., EMEJANOVA, L. K. & ALIKHANIAN, S. I. (1971). The genetic location of prophage on the chromosome of *Streptomyces coelicolor*. *Genetics* **68**, 341-347.
- VIVIAN, A. & HOPWOOD, D. A. (1970). Genetic control of fertility in *Streptomyces coelicolor* A3(2): the IF fertility type. *Journal of General Microbiology* **64**, 101-117.
- WILDERMUTH, H. & HOPWOOD, D. A. (1970). Septation during sporulation in *Streptomyces coelicolor*. *Journal of General Microbiology* **60**, 51-59.
- WILLIAMS, S. T. & DAVIES, F. L. (1967). Use of a scanning electron microscope for the examination of Actinomycetes. *Journal of General Microbiology* **48**, 171-177.