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New data on the linkage map of *Streptomyces coelicolor*

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1. INTRODUCTION

The main interest of a formal genetic study of *Streptomyces coelicolor* lies in the fact that this organism is not closely related to any of the other bacteria that have been subjected to genetic analysis; it is also one of the very few bacteria in which it has so far been possible to investigate the topology of long sections, and possibly the whole, of the genetic map. In most other bacterial species, in particular those in which gene transfer occurs by transformation or transduction and is therefore fragmentary, only short sections of the linkage map have been investigated, albeit with a high degree of precision. The notable exceptions to this state of affairs are provided by the two related bacteria *Escherichia coli* and *Salmonella typhimurium*, in which a picture of the whole linkage map has been obtained. The linkage maps of these two bacteria resemble one another closely (Zinder, 1960; Falcow, Rownd & Baron, 1962; Sanderson & Demerec, 1964), but differ in several important respects from those of organisms other than bacteria, notably in consisting of a single linkage group, in their circularity, and in the arrangement of loci controlling related functions. It would be interesting to know whether all these features are characteristic of bacteria as a whole, or are specializations of these Eubacteria. To answer this question we would need a knowledge of the linkage maps of a wide range of bacteria, and study of the Actinomycete *Str. coelicolor* is relevant as an approach to this problem.

Genetic recombination in species of the genus *Streptomyces* was discovered independently by several people (reviews: Hopwood & Sermonti, 1962; Sermonti & Hopwood, 1964). Linkage was first demonstrated by Hopwood (1957) in a strain of *Str. coelicolor* known as A3(2). A linear arrangement of loci in two linkage groups was found by means of recombination tests involving a selective analysis of the haploid recombinants arising from crosses (Hopwood, 1959). Later, with the discovery of colonies originating from unstable heterozygotes (heteroclones: Sermonti, Mancinelli & Spada-Sermonti, 1960; Hopwood, Sermonti & Spada-Sermonti, 1963), a non-selective analysis of linkage became possible. Present knowledge of the linkage map is based on a combined analysis of haploid recombinants and of heteroclones.

2. MATERIALS AND METHODS

Media

Glucose-asparagine minimal medium (Hopwood & Sermonti, 1962) has been modified by adding 0·02% $MgSO_4.7H_2O$. The complete medium is 'reproductive medium 2' of Hopwood & Sermonti (1962).

Strains of the organism

All strains have been derived, by successive mutation and recombination, from a single prototrophic wild-type culture, A3(2) (Hopwood, 1959).

Isolation and characterization of mutants

For the isolation of auxotrophic and streptomycin-resistant mutants, see Hopwood & Sermonti (1962). Acriflavine-resistant mutants were selected by plating spores on minimal medium containing 0·0015% acriflavine (I. Spada-Sermonti, personal communication). Urease-negative mutants were recognized by overlaying colonies (about 300 per dish) with 1·5% agar in M/100 phosphate buffer at pH 6, containing 1% urea and enough bromo-thymol blue to give a strong orange colour (Hopwood, 1964). Colonies lacking urease activity failed to produce the blue colour which, in the wild-type colonies, results from the liberation of ammonia by the action of urease.

Crossing procedure and analysis of haploid recombinants and heteroclones

Full practical details can be found in the review by Hopwood & Sermonti (1962).

In the selection of heteroclones, two closely linked (5 units or less) mutants are required, one in each parent. Previously, and in some of the present work, the pairs *metA-2* and *hisA-1* in linkage group I and *adeA-3* and *pheA-1* in group II were used; the members of each pair are some 3–5 units apart, and in crosses involving either pair, heteroclones are some 5–10% of the total colonies arising on selective media, the remainder being haploid recombinants. In much of the present work, use of the more tightly linked pairs consisting of *hisA-1* and *hisC-9* in group I and *cysC-3* and *cysD-18* in group II has resulted in the proportion of heteroclones exceeding 50%. In the case of the *his* markers, the double mutant amongst the segregants is recognizable phenotypically because of the combination of characters of the single mutants; *hisA-1* fails to grow on histidinol in the normal time of 2 days but grows on a medium lacking histidine after 5–6 days; on the other hand *hisC-9* grows well on histidinol but makes no delayed growth in the absence of histidine or histidinol; the double mutant fails to grow on histidinol and makes no delayed growth. For the *cys* markers, the double mutant should not differ in phenotype from the *cysC* parental type, but its expected frequency is so low that it can be ignored except in a fine analysis.

3. RESULTS

List of known loci

In previous publications, loci were named by the alleles that first defined them. In this paper, locus designations of the type suggested by Demerec (1956) have

Table 1. *List of loci with their mutant alleles*

Locus	Alleles	Characteristics‡
acrA	*acr-9*, 50*	Resistant to acriflavine
acrB	*acr-3**	Resistant to acriflavine
adeA	*ade-3, 7, 22*†	Req. purines
ammA	*amm-1, 2, 3, 4, 5*	Req. —NH_4, or glutamic or aspartic acids
argA	*arg-1*	Req. arginine, or citrulline, or ornithine
argB	*arg-T74*	Req. arginine
athA	*ath* (formerly *ade*)*-2, 8, 10*, 11*, 12*	Req. purines plus thiamine
aurA	*aur*-1†	Req. arginine plus uracil
cysA	*cys-15*	Req. cysteine
cysB	*cys-4, 6, 22*	Req. cysteine or S_2O_3
cysC	*cys-3*	Req. cysteine or S_2O_3 or S_2O_4
cysD	*cys-5, 7, 9, 11, 13, 18*	Req. cysteine or S_2O_3 or S_2O_4 or S_2O_5
guaA	*gua-1*	Req. guanine
hisA	*his-1*	Req. histidine; acc. imidazole propanediol (?)
hisB	*his-12*	Req. histidine or histidinol; acc. imidazole glycerol phosphate
hisC	*his-9*	Req. histidine or histidinol; acc. no Pauly-positive material
hisD	*his-3, 4, 15*	Req. histidine or histidinol; acc. histidinol phosphate
hisE (*adeB*)	*his* (*ade*)*-6, 5*	Req. histidine or histidinol or purines
ilvA	*ilv-1*	Req. isoleucine plus valine
leuA	*leu-1*	Req. leucine or α-ketoisocaproic acid
leuB	*leu-5*†	Req. leucine or α-ketoisocaproic acid
metA	*met-2*	Req. methionine
metB	*met-3, 4, 5, 6*	Req. methionine or homocysteine
mthA	*mth-1* (formerly *hom-1*)	Req. methionine plus threonine
mthB	*mth-2*†	Req. methionine plus threonine; or homoserine
nicA	*nic-1, 3*	Req. nicotinamide
pdx	*pdx-1*	Req. pyridoxin
pheA	*phe-1*	Req. phenylalanine
proA	*pro-1*	Req. proline
redA	*red-1*	Produces red instead of red/blue indicator pigment
serA	*ser-1*	Req. serine or glycine
strA	*str-1*	Resistant to streptomycin
thiA	*thi-1*	Req. thiamine
thiB	*thi-3*	Req. thiamine or 4-methyl-5-β-hydroxyethylthiazole
thr	*thr-1*	Req. threonine
tyrA	*tyr-T98*	Req. tyrosine or phenylalanine
tyr	*tyr-1*	Req. tyrosine
uraA	*ura-1*	Req. uracil
ureA	*ure-1, 3, 20*	Urease-negative

* Isolated and mapped by G. Sermonti & I. Spada-Sermonti (personal communication).
† Isolated by L. Doležilová. ‡ Req. = require; acc. = accumulate.

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been assigned (Table 1). Where more than one mutant defines a locus, evidence of allelism has, except in three instances, been obtained as follows. Two strains, each bearing one of the mutants, were crossed. Spores were plated on a medium selecting for recombinants between the two mutants, and in parallel on a medium selecting between loosely linked or unlinked markers. Close linkage between the two mutants was indicated by a much lower colony count on the first medium than

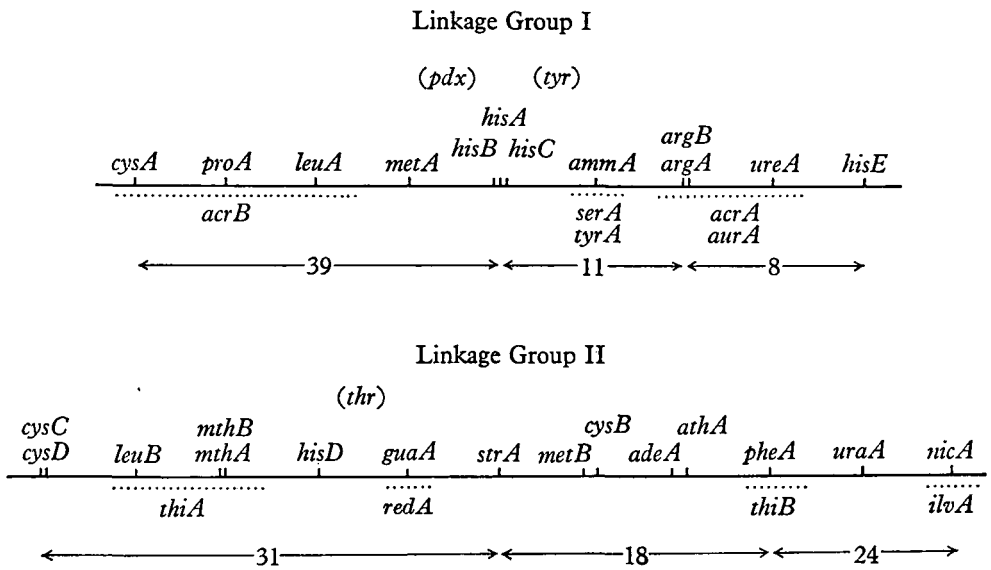

Fig. 1. Linkage map of *Streptomyces coelicolor* strain A3(2). For explanations of locus symbols, see Table 1. The map is not drawn to scale: loci are arbitrarily spaced at equal intervals, except for clusters, or possible clusters, of related loci (see text), which are indicated as closely spaced. Loci below the lines have not been ordered relative to the loci covered by dotted lines. Loci in brackets have been located only approximately in the linkage groups. The lengths of the intervals are indicated in percent recombination units as estimated by the analysis of heteroclones.

Sources of information on the location of loci are as follows: *metA*, *hisA*, *argA*, *hisD*, *strA*, *pheA*—Hopwood (1959) and Hopwood & Sermonti (1962); *hisB*, *hisC*, *adeA*, *uraA*—Hopwood & Sermonti (1962); *acrA*—G. Sermonti & I. Spada-Sermonti (personal communication) and the present paper; *acrB*—I. Spada-Sermonti (in Table V of Sermonti & Hopwood, 1964); data on the remaining loci are in the present paper.

on the second, while functional allelism was indicated by the absence of heteroclones on the first medium. For *acrA*, *ureA* and *nicA* this selective analysis was not possible (for *nicA* because the mutants grew well enough without added nicotinamide to cause excessive background growth); for these loci mutants have been provisionally assumed to be allelic on the basis of identical phenotype and similar linkage relations with other markers.

In this paper the linkage relations of the loci *metA*, *hisB*, *hisA*, *hisC*, *argA*, *hisD*, *strA*, *adeA*, *pheA* and *uraA*, as defined by previous data summarized in the review by Hopwood & Sermonti (1962), will be taken as a basis for the description and

interpretation of the new data. Where a new locus adds to the length of a linkage group, an estimate of its distance from previously located markers will be given. The linkage map, with a summary of sources of information on the location of the markers, appears in Fig. 1.

Data on the order of loci determined by three-point linkage tests in heteroclones

The analysis of segregation data from heteroclones is complicated by the fact that the heteroclones arise from incomplete heterogenotes (Morse, Lederberg &

Table 2. *Patterns of crossing-over required to produce the different genotypes of segregants in a three-point cross when the segregating heterozygote has deletions of chromosomal segments*

Deletions	Constitution of heterozygote*	Crossovers required to produce the genotypes: Parental		Single recombinants		Single recombinants		Double recombinants	
		a b c	*A B C*	*a B C*	*A b c*	*a b C*	*A B c*	*a B c*	*A b C*
None	*A B C* / 1 2 / *a b c*	—	—	1	1	2	2	1,2	1,2
One	*A B C* / *x* 1 2 / *a b c*	—	*x*	1	*x*,1	2	*x*,2	1,2	*x*,1,2
Two (*trans*)	*A B C* / *x* 1 2 *y* / *a b c*	*y*	*x*	1	*x*,1,*y*	2	*x*,2,*y*	1,2,*y*	*x*,1,2
Two (*cis*)	*A B C* / *x* 1 2 *y* / *a b c*	—	*x*,*y*	1,*y*	*x*,1	2,*y*	*x*,2	1,2	*x*,1,2,*y*

* Dotted lines represent deleted segments. $x, 1, 2, y$ represent the intervals in which crossing-over may occur.

Lederberg, 1956) lacking one or more terminal segments of chromosome in each linkage group (Hopwood & Sermonti, 1962; Hopwood, Sermonti & Spada-Sermonti, 1963; Sermonti & Hopwood, 1964). For analysing such data, three models have been described by Hopwood & Sermonti (1962), in which a linkage group has a single deletion, or two deletions at opposite ends in the *trans* or *cis* arrangements. In a three-point cross, the different situations can be recognized by comparing the

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Table 3. *Determination of the orders of new loci with respect to known loci in linkage group I by means of three-point data from heteroclones. (Various markers other than the three under investigation in each segregation have been ignored)*

New locus	Cross ● $\frac{A\ B\ C}{a\ b\ c}$			Numbers of segregants observed: Parental $a\ b\ c$	Parental $A\ B\ C$	Single recombinants $a\ B\ C$	Single recombinants $A\ b\ c$	Single recombinants $a\ b\ C$	Single recombinants $A\ B\ c$	Double recombinants $a\ B\ c$	Double recombinants $A\ b\ C$	Expected double recombinants* (no interference)
cysA	●*cysA-15*	*hisA-1*	+	124	10	83	4	26	1	14	0	
	+	*hisC-9*	*argA-1*	134		87		27		14		16
proA	● *proA-1*	+	+	132	15	21	2	14	4	5	0	
	+	*hisA-1*	*argA-1*	147		23		18		5		3
leuA	● *leuA-1*	+	+	48	9	22	0	13	2	0	0	
	+	*metA-2*	*argA-1* ●	57		22		15		0		—
ammA	● +	+	+	91	38	7	0	45	8	0	1	
	hisA-1	*ammA-5*	*argA-1* ●	129		7		53		1		—
serA	*hisA-1*	*serA-1*	+	155	38	22	39	29	12	4	12	
	hisC-9	+	*argA-1*	193		61		41		16		—
ureA	● +	+	*ureA-1*	11	37	122	10	5	3	0	1	
	proA-1	*argA-1*	+ ●	48		132		8		1		—
hisE	● +	+	*hisE-6*	41	32	11	5	3	4	1	0	
	proA-1	*argA-1*	+	73		16		7		1		1
Indicated order:†				*leuA proA cysA*	*metA*	*hisA/C*	*ammA serA*	*argA*	*hisE ureA*			

● This symbol is placed outside certain alleles to indicate deletions of chromosomal segments (see the general models in Table 2).

* This figure is calculable only when the heteroclone has a single deletion (see text).

† Positions of *metA*, *hisA/C*, *argA* already known.

Table 4. *Determination of the orders of new loci with respect to known loci in linkage group II by means of three-point data from heteroclones. (Various markers other than the three under investigation in each segregation have been ignored)*

New locus	Cross ● A B C / a b c			Numbers of segregants observed: Parental a b c	Parental A B C	Single recombinants a B C	Single recombinants A b c	Single recombinants a b C	Single recombinants A B c	Double recombinants a B c	Double recombinants A b C	Expected double recombinants* (no interference)
†*cysC/D*	● *strA-1*	+	*cysC-3*	126	8	10	0	27	4	5	0	
	+	*mthB-2*	*cysD-18*	134		10		31		5		3
†*mthB*	● *pheA-1*	*strA-1*	+	152	2	10	1	14	0	0	1	
	+	+	*mthB-2*	154		11		14		1		1
thiA	● *thiA-1*	+	+	110	33	12	1	24	1	1	0	
	+	*hisD-3*	*strA-1* ●	143		13		25		1		—
guaA	● *guaA-1*	+	+	50	28	3	1	53	7	0	0	
	+	*strA-1*	*pheA-1* ●	78		4		60		0		—
redA	● *hisD-4*	*redA-1*	*strA-1*	25	10	2	2	10	1	0	0	
	+	+	+ ●	35		4		11		0		—
metB	● +	+	*adeA-3*	9	3	23	0	12	0	1	0	
	strA-1	*metB-4*	+ ●	12		23		12		1		—
nicA	● +	+	*nicA-3* ●	100	11	3	8	6	2	1	0	
	adeA-3	*uraA-1*	+	111		11		8		1		—

mthB guaA

Indicated order:** *cysC/D* *thiA* *hisD* *redA* *strA* *metB* *adeA* *pheA* *uraA* *nicA*

● This symbol is placed outside certain alleles to indicate deletions of chromosomal segments (see the general models in Table 2).

* This figure is calculable only when the heteroclone has a single deletion (see text).

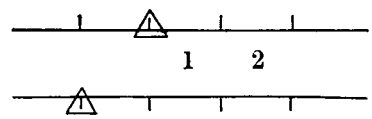
† The order of the three markers in these two segregations has been reversed so that the segregations correspond with the general model in Table 2.

** Positions of *hisD*, *strA*, *adeA*, *pheA*, *uraA* already known.

ratios between the frequencies of complementary genotypes, which always differ in the crossovers required to produce them, and therefore in frequency (Table 2).

Study of Table 2 shows the effects of deletions on the ordering of loci in a three-point cross, which have not previously been considered. It can be seen that, as in the classical case with no deletions, the combined frequencies of the two double recombinant classes are normally the smallest, since these classes are produced by the rarest crossover patterns, and so serve to define the order of the loci. The only

Table 5. *Determination of the orders of pairs of loci with respect to outside markers by means of selective analysis. Recombinants were recovered on a medium selective for wild-type alleles at the pair of loci under investigation, and scored for markers at the other two loci*

Pattern of cross: * 

Pair of loci	Cross	Numbers of non-selected crossovers in intervals: —	1	2	1,2
	cysA-15 + *hisA-1* +				
cysA	\+ *proA-1* + *argA-1*	63	6	1	1
proA	*cysA-15* + + *argA-1*				
	\+ *proA-1* *hisA-1* +	63	11	5	0
	cysA-15 + *hisA-1* +				
cysA	\+ *leuA-1* + *argA-1*	54	8	31	0
leuA	*cysA-15* + + *argA-1*				
	\+ *leuA-1* *hisA-1* +	44	3	7	0
	proA-1 + + *hisA-1*				
proA	\+ *leuA-1* *metA-2* +	74	7	6	0
leuA	*proA-1* + *hisA-1* +				
	\+ *leuA-1* + *argA-1*	80	1	4	0
mthB	*mthB-2* + + +				
hisD	\+ *hisD-3* *strA-1* *pheA-1*	72	10	11	1

Order of loci: *cysA* *proA* *leuA* *metA* *hisA* *argA* — *mthB* *hisD* *strA* *pheA*

* Triangles indicate selected alleles. 1 and 2 represent intervals in which non-selected crossovers may occur.

exception to this state of affairs occurs when there are two deletions in *cis*, *and* both x and y are small compared with the intervals 1 and 2; under these circumstances, one of the pairs of single recombinant classes is the least frequent, and ordering is ambiguous; such segregations, therefore, have to be excluded.

Three-point data for fourteen loci, seven in each linkage group, are given in Tables 3 and 4. (The very closely linked *cysC* and *cysD* are here considered as a single locus.) For five loci (*cysA*, *proA* and *hisE* in Table 3; *cysC/D* and *mthB* in

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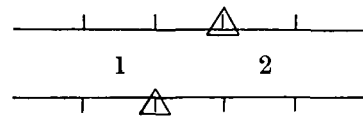
Table 4) the segregations fit the single deletion model; in the rest, the two deletions are in *trans* in all except one (*nicA*, Table 4), but in this one segregation the two deletions in *cis* are far enough from the trio of loci not to render the order of loci ambiguous.

The segregation locating *serA* (Table 3) is peculiar in showing reduced viability of the *serA-1* marker. The ratios within the parental and within the double recom-

Table 6. *Determination of the orders of pairs of loci with respect to outside markers by means of selective analysis. Recombinants were recovered on a medium selective for wild-type alleles at the pair of loci under investigation, and scored for markers at the other two loci*

Pattern of cross: * 

Pair of loci	Cross	Numbers of non-selected crossovers in intervals: —	1	2	1,2
	+ *metB-4* + +				
metB	*strA-1* + *cysB-6* *uraA-1*	64	5	6	4
cysB	*strA-1* *metB-4* + *uraA-1*				
	+ + *cysB-6* +	67	17	9	1
	+ *cysB-6* + +				
cysB	*strA-1* + *adeA-3* *uraA-1*	63	27	8	0
adeA	*strA-1* *cysB-6* + *uraA-1*				
	+ + *adeA-3* +	55	11	8	1
	+ *adeA-3* + +				
adeA	*strA-1* + *athA-8* *uraA-1*	23	10	15	2
athA	*strA-1* *adeA-3* + *uraA-1*				
	+ + *athA-8* +	29	9	10	2
	+ *athA-8* + +				
athA	*strA-1* + *pheA-1* *uraA-1*	41	1	5	0
pheA	*strA-1* *athA-8* + *uraA-1*				
	+ + *pheA-1* +	35	2	12	1

Order of loci: *strA* *metB* *cysB* *adeA* *athA* *pheA* *uraA*

* Triangles indicate selected alleles. 1 and 2 represent intervals in which non-selected crossovers may occur.

binant pairs of genotypes deviate from unity in opposite directions, and the same is true of the ratios within the two single recombinant pairs; in each case the genotype containing *serA-1* is in defect.

For the single deletion segregations, the combined frequencies of the double crossover classes expected in the absence of interference are indicated in Tables 3 and 4; they do not differ significantly from the observed values, indicating no

interference. Interference appears to be present in segregations from heterogenotes with two deletions, but is due to the patterns of crossing over necessitated by the presence of the deletions: deletions in *trans* result in a selection of single crossovers at the expense of doubles and therefore 'positive interference', while deletions in *cis* cause the opposite effect, and therefore 'negative interference'.

Data on the order of loci determined by selective analysis

The data in Tables 3 and 4 leave several orders of sets of loci to be resolved. (See the summaries at the foot of the tables.) Several of these orders, and those involving

Table 7. *Determination of the linkage relations of* acrA-50 *and* thiB-3 *by analysis of five heteroclones for each mutant*

(i) *acrA-50*

Genotypes			Numbers in heteroclone					Totals
			*a**	*b*	*c*	*d*	*e*	
Parental	*argA-1*	+	134	13	6	7	7	435
	+	*acrA-50*	53	85	44	42	44	
Recombinant	+	+	1	0	0	0	0	3
	argA-1	*acrA-50*	0	0	0	0	2	

(ii) *thiB-3*

Genotypes			Numbers in heteroclone					Totals
			p	*q*	*r*	*s*	*t*	
Parental	+	+	40	39	37	33	39	232
	thiB-3	*pheA-1*	8	4	12	12	8	
Recombinant	+	*pheA-1*	1	0	0	0	1	3
	thiB-3	+	1	0	0	0	0	

* The pattern of deletions in heteroclone *a* was different from that in *b*, *c*, *d* and *e*; therefore relative frequencies of genotypes differ.

further loci, have been determined by selective analysis with outside markers (Tables 5 and 6). For each cross, the expectation is as follows: one genotype with a high frequency, corresponding to the class requiring no extra (non-selected) crossover; two genotypes with intermediate frequencies (one non-selected crossover); and one genotype with a low frequency (two non-selected crossovers). The order of the pair of loci under investigation in each cross, relative to the two known loci, has been chosen so that the results obtained agree with this expectation. Crosses in which the coupling of the outside markers with respect to the selected loci was reversed give the same indication of order. Similar data not reported here established that *guaA* lies between *hisD* and *strA*, and *leuB* between *cysD* and *mthB*.

Data on other loci

Three loci have each been located very close to a known marker by means of heteroclone analysis, but the order relative to this marker is unknown. *Acr-9*, an

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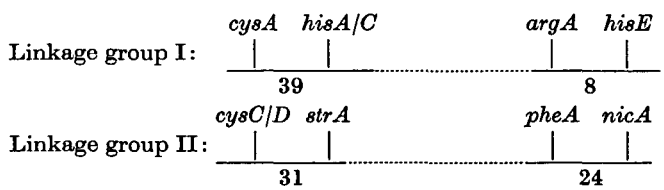
allele of *acrA*, was found by G. Sermonti and I. Spada-Sermonti (personal communication) to be closely linked to *argA*. Some heteroclone data showing very close linkage of another allele of this locus, *acrA-50*, to *argA* are given in Table 7. Similar data showing very close linkage of the locus *thiB* with *pheA* are also given in Table 7. A third locus, *aurA*, mutants at which require both arginine and uracil, has been found to be closely linked to *argA*, but instability of the only mutant so far available has prevented more precise location.

Table 8. *Calculation of recombination percentages between pairs of loci in heteroclones. Each row represents data from a different heteroclone, except as indicated by brackets*

	Segregants												
	Parental						Recombinant						
Pair of loci	Genotype†		No.	Genotype†		No.	Genotype†		No.	Genotype†		No.	Percent recombination
cysA hisA/C	+	hC	150	cA	hA	11	+	hA	97	cA	hC	4	39*
argA hisE	a	+	46	+	hE	43	+	+	5	a	hE	3	8*
cysC/D	cD	+	131	cC	s	8	cC	+	37	cD	s	4	23*
strA	cC	s	54	cD	+	44	cD	s	70	cC	+	9	39 ‡
	s	p	119	+	+	22	+	p	31	s	+	5	(18) ‡
													31
pheA	p	+	92	+	n	28	+	+	63	p	n	2	24 ‡
nicA	+	+	79	s	p	57	+	p	37	s	+	12	(18) ‡

Summary of new recombination percentages



† a = *argA-1*; cA = *cysA-15*; cC = *cysC-3*; cD = *cysD-18*; hA = *hisA-1*; hC = *hisC-9*; hE = *hisE-6*; n = *nicA-3*; p = *pheA-1*; s = *strA-1*.

* These heteroclones had a single deletion; therefore recombination percentages have been calculated directly.

‡ These heteroclones had two deletions in *trans*; therefore recombination percentages have been calculated by proportionality with the known figure of 18% for *strA – pheA* (see text).

Several further markers, for which information is incomplete, are indicated in Fig. 1.

Clusters of related loci

Three 'clusters', each consisting of a pair of loci, have been identified: *argA*, *argB*; *cysC*, *cysD*; and *mthA*, *mthB*. In each cluster the loci are distinguished by differences in the alternative growth requirements of their mutants (Table 1) and by complementation tests (production of heteroclones on media lacking the

appropriate growth factors). In each cluster the distance between mutant sites in adjacent loci is about 1 unit or less in a selective analysis (shorter in heteroclone analysis). Data on the order of three histidine loci, *hisA*, *hisB*, *hisC*, which were found (Hopwood, unpublished) to form a cluster have been reported by Hopwood & Sermonti (1962).

The length of the linkage map

The present data identify loci external to all four end markers previously described. The terminal loci are now *cysA* and *hisE* in linkage group I and *cysC/D* (here considered as a single locus) and *nicA* in group II. Estimates of the recombination percentages of these loci with loci already mapped are given in Table 8.

A single deletion in the heterogenote does not prevent the direct calculation of recombination percentages from heteroclone data (Hopwood & Sermonti, 1962). Data in Table 8 for the pairs *cysA* and *hisA/C*; *argA* and *hisE*; and one set of data for the pair *cysC/D* and *strA* are of this kind, and are indicated by an asterisk. The remaining data, one set for *cysC/D* and *strA* and the single set for *pheA* and *nicA* (indicated by ‡), are from heteroclones with two deletions in *trans*. Recombination percentages calculated directly would here be over-estimated, but can be corrected by proportionality if a pair of markers (*strA-1* and *pheA-1* in Table 8) giving a known recombination percentage are segregating in the same heteroclone (Hopwood, Sermonti & Spada-Sermonti, 1963). In Table 8, the recombination percentage between *strA-1* and *pheA-1* is taken as 18 (Hopwood & Sermonti, 1962) and the unknown distances are calculated from this figure.

The total lengths of the two linkage groups, based on the data of Table 8 and those in Table 11 of Hopwood & Sermonti (1962) are about 60 and 70 units respectively (Fig. 1).

4. DISCUSSION

The significance of two linkage groups

Two linkage groups, each represented by three markers, were identified by the first linkage studies in *Streptomyces coelicolor* (Hopwood, 1959). The lengths of these two groups have increased as more markers have been added to the map, but the two groups still remain separate, the markers in one group showing 50% recombination with those in the other. Since at present recombination analysis is the only means of detecting linkage in this organism, we cannot say whether the two linkage groups represent two separate structures ('chromosomes'), or sections of a single structure, separated by regions devoid of markers. It is relevant that in the linkage map of *Escherichia coli* (Hayes, 1964, Fig. 113) the loci so far identified (mainly, as in *Str. coelicolor*, by auxotrophic mutants) are non-randomly arranged; in particular there is a segment between the loci *try* and *his* which corresponds to about one-quarter of the total map and is completely devoid of markers. This segment is more than 50 units long, so that it could not have been bridged by recombination analysis alone. We must be prepared for a similar situation in *Str. coelicolor*.

The number of linkage groups in *Str. coelicolor* would be of no particular interest were it not for the fact that no mechanism is known in bacteria which could account for the regular distribution to daughter nuclei of haploid sets consisting of more than one chromosome, the function which in other cells is performed by mitosis. What evidence there is from other bacteria suggests that a single linkage group may indeed be the rule: *Escherichia coli* and *Salmonella typhimurium*, the only bacteria in which the total extent of the linkage map is known, have a single circular linkage group (Jacob & Wollman, 1958; Sanderson & Demerec, 1964) whose physical basis, in the case of *E. coli*, is a single closed loop of DNA (Cairns, 1963); furthermore it has been suggested, although the evidence is not conclusive, that all the known markers of *Bacillus subtilis* also are linked (Yoshikawa & Sueoka, 1963). However, we should not lose sight of the fact that the mechanism whereby the divisions of chromosome and cell are co-ordinated, even in a bacterium with a single chromosome, is not understood, so that we cannot assume that the possession of two chromosomes is incompatible with a bacterial type of cellular organization.

Clusters of related loci

The close linkage of some or all of the loci controlling successive steps in biosynthetic pathways is a feature of most of the bacteria that have so far been subjected to sufficiently extensive genetic analysis, and is extremely striking in the Eubacteria *Salmonella typhimurium*, *Escherichia coli* and *Bacillus subtilis*. On the other hand, in *Pseudomonas aeruginosa*, which has been placed in a different order of bacteria from the other three species (Breed, Murray & Smith, 1957), the absence of clustering has been noted (Holloway, Hodgins & Fargie, 1963). Only a single example of the phenomenon has been reported outside the bacteria, in *Neurospora crassa* (Giles, 1963), in spite of extensive mapping of loci defined by auxotrophic mutants, so that clustering seems to be essentially a bacterial characteristic.

The results reported in this paper indicate that *Streptomyces coelicolor*, which belongs to a different order of bacteria from any of the other species that have been analysed genetically, shows a significant amount of clustering. In addition to the trio of histidine loci previously identified, three pairs of closely linked loci are now known: *argA*, *argB*; *cysC*, *cysD*; and *mthA*, *mthB*. In addition *metB* and *cysB*, both controlling steps in the biosynthesis of methionine, may be adjacent, or may be separated by probably not more than one unknown locus, since the recombination percentage between the mutants *metB-4* and *cysB-22* is about 2·5 in a selective analysis. The neighbouring loci *adeA* and *athA* are farther apart (about 4 units for *adeA-3* and *athA-8*), so that if they are components of a cluster there must be several other intervening loci controlling purine biosynthesis to be discovered. The proximity of *aurA* (arginine plus uracil) to *argA/B* also may be significant.

It is already apparent from comparisons of known linkages in *Salmonella typhimurium* and *Bacillus subtilis* that, even in two organisms that show extensive clustering of functionally related loci, the precise arrangement of loci in clusters may differ. For example all the known histidine loci form a single cluster in *Salm. typhimurium* (Hartman, Loper & Serman, 1960), whereas in *B. subtilis* two histidine

loci are not closely linked (Nester, Schafer & Lederberg, 1963). *Streptomyces coelicolor* provides further examples of such differences. For the histidine system, the five loci so far identified are located as a trio and two single loci, which differs strikingly from the situation in *Salm. typhimurium*. For the leucine system, both *Salm. typhimurium* (Margolin, 1963) and *B. subtilis* (Anagnostopoulos, Borat & Schneider, 1964) show clustering of all known loci, while in *Str. coelicolor* the two leucine loci so far identified are unlinked. It is difficult to evaluate the significance of these differences between organisms until we can explain why it should confer a selective advantage on a bacterium for the loci controlling certain biosynthetic pathways to be closely linked, and those controlling others in the same organism to be at least partially dispersed throughout the linkage map.

SUMMARY

Linkage data, obtained by a combination of selective analysis of haploid recombinants and analysis of segregating heterozygotes, are given for twenty-eight loci in *Streptomyces coelicolor* A3(2). This brings the total known loci for the organism to thirty-nine. The two linkage groups previously recognized remain separate, and their lengths have been increased to about 60 and 70 recombination units respectively. Whether the two linkage groups correspond to two chromosomes remains an open question.

Three further examples of close linkage of pairs of functionally related loci have been found (a trio of such loci was already known), and three other pairs provide possible examples of the same phenomenon. Some loci which are clustered in *Salmonella* are unlinked in *Streptomyces*.

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