LINKAGE AND SEGREGATION OF A MATING TYPE SPECIFIC PHAGE AND RESISTANCE CHARACTERS IN NOCARDIAL RECOMBINANTS

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RECOMBINATION of auxotrophic and inhibitor resistant characters from crosses of *Nocardia canicruria* and *Nocardia erythropolis* have been previously reported (ADAMS and BRADLEY 1963; ADAMS 1964; BROWNELL and ADAMS 1967). Substrains derived from *N. canicruria* were observed to mate only with substrains derived from *N. erythropolis* while substrains of homologous origin were incompatible. From these observations it is apparent that some type of mating factor(s) controls compatibility in these organisms. To elucidate the mechanisms involved during recombination and gain knowledge of the gene(s) responsible for compatibility, construction of linkage maps was initiated (BROWNELL and ADAMS 1967). During those investigations, nocardioophage ϕ C was isolated from soil. This phage infected only *N. canicruria* strains and suggested a possible relationship between mating type and ϕ C sensitivity. The general characterization and conditions necessary for optimal reproduction of the ϕ C phage have been reported (BROWNELL, ADAMS and BRADLEY 1967). Since *N. erythropolis* is resistant to infection by ϕ C, recombinants obtained from matings of *N. canicruria* and *N. erythropolis* can be analyzed for segregation of ϕ C sensitivity and for their fertility in backcrosses to the parental types from which they were derived. Thus, the present work allowed a correlation of phage ϕ C sensitivity with mating behavior of recombinants and suggested a genomic location for the mating factors.

The addition of chloramphenicol resistance markers in strains previously mapped, permitted the confirmation and clarification of linkage relationships previously reported for these strains (BROWNELL and ADAMS 1967). With the clearer linkage map herein presented, and preliminary recognition of chromosomal location of mating factors responsible for fertility, a new tool for investigation of bacterial conjugational phenomena, nocardial recombination, is more firmly established.

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METHODS AND MATERIALS

Spontaneously occurring chloramphenicol resistant mutants were selected in previously described substrains JA-SD 3-48, of *N. canicruria* and JA-SD 2-82, of *N. erythropolis* (BROWNELL and ADAMS 1967). Mutants were selected by plating samples of sensitive populations of approximately 1×10^8 cells on supplemented minimal medium (SMM) containing 35 $\mu\text{g}/\text{ml}$ chloramphenicol (Chloromycetin, Parke, Davis & Co., Detroit, Mich.). Sensitive strains originating the resistant mutants were completely inhibited in the presence of 30 $\mu\text{g}/\text{ml}$ of chloramphenicol. Colonies of resistant cells which appeared on SMM were purified by three successive streak isolations on nutrient agar (Difco, Detroit, Mich.). These presumed mutants were tested on SMM plates containing increasing concentrations of chloramphenicol to determine their maximum resistance level.

Confirmed resistant mutants were codified and maintained as previously described (ADAMS 1964) except that strains now additionally bear laboratory identifications to maintain identities of strains isolated in different laboratories. The genotype and phenotype of the strains employed in these studies are shown in Table 1. The stability of the Cam-R phenotype for use as either a selective or as an unselective marker was tested. Saline suspensions of Cam-R mutants were prepared and appropriate dilutions were plated on SMM and on inhibitor-containing plates (IMM). After suitable incubation, comparisons were made of the number of colonies growing on the IMM and SMM plates. In addition, single colonies were transferred with sterile tooth-picks from SMM plates to fresh SMM plates. After 12 hrs of incubation these plates were replicated to SMM and IMM plates and examined for the presence of sensitive colonies. In a similar manner, master plates were replicated to peptone, yeast-extract medium (PY); and approximately 0.05 ml of phage ϕC in PY broth (at 1×10^9 plaque forming units/ml) was added to each fresh replicate. Following incubation the PY plates were examined for plaque formation. Thus, the occurrence of spontaneously occurring ϕC resistant clones was ascertained.

Mating techniques consisted of growing mixed cultures of appropriate parents on nutrient agar as previously described (BROWNELL and ADAMS 1967). Each parental strain was grown individually on identical medium to serve as controls. After 3 days incubation, saline suspensions were prepared from cross and control cultures. The population density of the cell suspension was determined and samples were plated on MM and incubated for 5-6 days. Prototrophic recombinants were picked and transferred to fresh MM plates. After 24 hrs incubation the recombinants were replicated to PY, IMM, and MM plates; the MM plate served as a replicating control. Recombinants replicated to PY were immediately covered with phage ϕC and scored for plaque formation following 12 hrs incubation. The IMM plates (BROWNELL and ADAMS 1967) were incubated for 5-6 days, and these sets of plates were compared to determine phenotypes of individual recombinants.

To correlate compatibility of F_1 recombinants with segregation of the ϕC resistance/sensitivity markers, backcrosses with selected F_1 recombinants were carried out. Prototrophic recombinants from the cross JA-SD 2-13 by GB-GA 3-52 containing at least one locus for inhibitor sensitivity were selected after scoring. These recombinants were purified by means of three successive single colony streak isolations on nutrient agar and tested on inhibitor containing medium to assure inhibitor sensitivity. Mating of these F_1 recombinants with both GB-GA 2-89 and GB-GA 3-52 were carried out as described above. Selection was made on IMM; thus auxotrophic parental types as well as the inhibitor-sensitive F_1 recombinants used in the crosses were selected against. The appearance of colonies on the selective IMM plates following these matings was considered as evidence of a fertile backcross.

In all instances, incubation was carried out at 30°C.

RESULTS

The reliability of the auxotrophic characters and their prototrophic alleles for selection of prototrophic recombinants was reported earlier (ADAMS and BRADLEY

TABLE 1
Mutant characteristics and designations

Strain*	Derived from	Mutant loci and mutational sites†										Relevant phenotype‡									
		<i>purA</i>	<i>purB</i>	<i>his</i>	<i>strA</i>	<i>strB</i>	<i>eryA</i>	<i>eryB</i>	<i>acr</i>	<i>tetA</i>	<i>tetB</i>	<i>camA</i>	<i>camB</i>	<i>φC</i>	Pur	His	Str	Ery	Tet	Cam	<i>φC</i>
2		+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	S	S	S	S	R
2-13	2-1	1	+	3	+	+	+	+	+	+	+	+	—	—	—	—	S	S	S	S	R
2-44	2-13	1	+	3	1	+	+	+	+	+	+	+	—	—	—	—	R	S	S	S	R
2-77	2-44	1	+	3	1	+	+	9	2	2	+	+	—	—	—	—	R	R	S	S	R
2-82	2-77	1	+	3	1	+	+	+	9	2	10	+	+	—	—	—	R	R	R	S	R
2-89	2-82	1	+	3	1	+	+	+	9	2	+	+	+	—	—	—	R	R	S	R	R
3		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S	S	S	S	S
3-3	3	+	2	+	+	+	+	+	+	+	+	+	+	+	+	+	S	S	S	S	S
3-7	3-3	+	2	+	+	2	+	+	+	+	+	+	+	—	—	—	R	S	S	S	S
3-39	3-7	+	2	+	+	+	+	7	11	+	+	+	+	+	+	+	R	R	S	S	S
3-48	3-39	+	2	+	+	2	+	2	7	+	+	+	+	+	+	+	R	R	R	S	S
3-52	3-48	+	2	+	+	2	2	2	7	+	9	+	+	+	+	+	R	R	R	R	S

* Strains of *Nocardia erythropolis* are prefixed with 2; strains of *N. canicruria* are prefixed with 3. The letter prefixes JA-SD and GB-GA used in the text indicate the laboratory in which the strain was isolated.

† Description of mutant loci, mutational sites, and relevant phenotypes are, insofar as possible, in conformity with the recommendation of DEMEREC *et al.* 1966.

‡ Symbols: +, synthesized; —, required; R, resistant; S, susceptible; Pur, purine; His, histidine; Str, streptomycin, 100 µg/ml; Ery, erythromycin, 5 µg/ml; Acr, acriflavine, 100 µg/ml; Tet, tetracycline, 3 µg/ml; Cam, Chloramphenicol, 35 µg/ml; *φC*, Nocardiphage *φC*.

1963; ADAMS 1964). Further tests were conducted to determine the suitability of auxotrophic and resistance markers for use in both selective and unselective methods (BROWNELL and ADAMS 1967). The stability of the chloramphenicol resistance loci, *camA1* and *camB2*, was established by examining 1,200 colonies for their spontaneous reversion to sensitivity at each locus. Since no sensitive colonies were obtained, reversion can be considered to be less than 8.3×10^{-4} and the markers are suitable for genetic analysis. The occurrence of spontaneous mutants of *N. canicruria* to ϕ C resistance (ϕ C-*r*) or *N. erythropolis* to ϕ C sensitivity (ϕ C-*s*) is based on the examination of about 500 colonies of each type for plaque formation. No exception to resistance or sensitivity in the examined colonies of the respective parental types was noted and the spontaneous occurrence of resistant or sensitive mutants must be less than 2.0×10^{-3} .

The patterns of segregation of sensitive and resistant loci among progeny for the crosses of *N. canicruria*, GB-GA 3-52, by *N. erythropolis*, JA-SD 2-13, are depicted in Table 2. The prototrophic alleles of genes determining dependence on purines, *purA1*, *purB2*, and on histidine, *his-3*, were used for selection. The location of the auxotrophic characters in relation to the unselected resistance loci was previously determined by both selective and unselective analysis (BROWNELL and ADAMS 1967). By employing selective techniques reciprocal class types from a cross cannot be recovered among the prototrophic recombinants with equal

TABLE 2

Segregation of unselected resistance markers and phage sensitivity from crosses of *N. erythropolis* JA-SD 2-13, ϕ C-*r* *his-3 purA1*, by *N. canicruria* GB-GA 3-52, *purB2* ϕ C-*s* *tetA9 camA1 eryA7 acr-11 strB2*

Recombinant phenotype						Number observed*	Fraction of population in percent	Postulated crossover regions		
ϕ C	Tet	Cam	Ery	Acr	Str					
S	R	R	R	R	R	439	82.2	I		
R	R	R	R	R	R	38	7.1	II		
S	S	S	R	R	R	2	0.4	I, II, IV		
R	S	S	R	R	R	27	5.0	IV		
S	R	R	R	R	S	14	2.6	I, VII		
R	R	R	R	R	S	5	0.9	II, VII		
R	S	S	R	R	S	3	0.6	IV, VII		
R	S	R	R	R	R	3	0.6	III		
R	S	S	R	S	R	2	0.4	IV, VI, VII		
S	R	R	S	S	S	1	0.2	I, V		
						534	100.0			
Linkage Model:†										
I		II		III		IV		V	VI	VII
+	+	+	+	+	+	<i>his-3</i>	<i>purA1</i>	+	+	+
<i>purB2</i>	ϕ C- <i>s</i>	<i>tetA9</i>	<i>camA1</i>	+	+	<i>eryA7</i>	<i>acr-11</i>	<i>strB2</i>		

* Prototrophic recombinants were selected on MM at a frequency of 1.5×10^{-5} .

† Linkage model is not scaled to relative map distances.

frequency since auxotrophic loci with their wild-type alleles in repulsion were used as selective characters. Thus, class types reciprocal for resistant alleles cannot originate as a result of similar numbers of crossover events when selecting for prototrophic recombinants if all markers are linked.

Since 82.2% (Table 2) of the recombinants were resistant to the inhibitors and ϕ C phage sensitive, these markers were considered to be more closely linked to the selective *his-3+* *purA1+* loci than to the *purB2* locus. The ϕ C-R phenotype showed the greatest segregation (14.6%) followed by: tetracycline sensitivity, *tetA9+*, (7.0%), chloramphenicol sensitivity, *camA1+*, (6.4%), streptomycin sensitivity, *strB2+*, (4.3%), acriflavine sensitivity, *acr-11+*, (0.6%) and erythromycin sensitivity, *eryA7+*, (0.2%). Based on the hypothesis that the best linear array results from the fewest crossover events and that all markers are linked, the loci have been arranged in the order shown at the bottom of Table 2. The inhibitor resistant, ϕ C-S, class type and inhibitor resistant, ϕ C-R, class type originated from single crossovers in regions I and II, respectively. The ϕ C-R Tet-S Cam-S represents a single crossover in region IV while the ϕ C-R Tet-S class, a single crossover in region III. It is possible to account for 94.9% of the recombinant class types on the basis of single crossover events while the remaining 5.1% can be considered as the products of double or triple crossovers. The most frequently occurring class types to originate from multiple crossovers are the ϕ C-S Str-S and ϕ C-R Str-S. Since *strB2* has been placed to the right of the *his-3+* *purA1+* loci, an additional crossover in region VII is necessary to obtain *strB2+* recombinants. Had the *strB2+* been placed to the left of the *his-3+* *purA1+* loci then the *strB2+* class types could have resulted only from triple crossing over.

Since only 0.2% of the recombinants were erythromycin sensitive, *eryA7* must be closely linked to the *his-3+* *purA1+* loci. It has been placed to the right of the *his-3+* *purA1+* characters based on the ϕ C-S Tet-R Cam-R Ery-S Acr-S Str-S class type which is postulated to have resulted from double crossover in region I and V. Had *eryA7* been placed to the left of the *his-3+*, the class could have been produced only from quadruple crossing over (Table 2). The *camA1* and *tetA9* markers segregated in a similar manner. Only one recombinant class type, ϕ C-R Tet-S Cam-R Ery-R Acr-R Str-R, at a frequency of 0.6%, represents crossing over between these *camA1* and *tetA9* loci. The *camA1* marker is, therefore, deduced to be closely linked to the right of the *tetA9* locus. Finally, *acr-11* is linked to the right of the *his-3+* *purA1+* loci.

In the cross of the resistant *N. erythropolis*, GB-GA 2-89, by *N. canicruria*, JA-SD 3-3, similar resistant phenotypes are available for analysis in the *N. erythropolis* mating type, with the exception of tetracycline resistance (Table 3). The most common progeny recovered employing prototrophic selection were ϕ C-S, inhibitor sensitive. Thus, these loci must be more closely linked to the counter selective *his-3 purA1* loci than to the selective *purB2+* locus. The ϕ C-R locus shows the greatest segregation (32.5%) followed by: *eryB9* (21.9%), *camB2* (8.4%), *strA1* (7.0%), and *acr-2* (3.4%). In order to account for the

TABLE 3

Segregation of unselected resistance markers and phage sensitivity from crosses of N. erythropolis GB-GA 2-89, ϕ C-r eryB9 camB2 his-3 purA1 acr-2 strA1 by N. canicruria JA-SD 3-3, purB2, ϕ C-s

Recombinant phenotypes					Numbers observed*	Fraction of population in percent	Postulated crossover regions
ϕ C	Ery	Cam	Acr	Str			
R	S	S	S	S	40	11.2	II
S	S	S	S	S	216	60.5	I
R	R	S	S	S	48	13.4	III
S	R	S	S	S	8	2.3	I, II, III
R	S	S	S	R	2	0.5	II, VI
S	S	S	S	R	12	3.4	I, VI
R	R	R	S	S	8	2.3	IV
S	R	R	S	S	2	0.5	I, II, IV
R	S	R	S	S	6	1.7	II, III, IV
S	S	R	S	S	3	0.8	I, III, IV
R	R	R	R	R	11	3.1	IV, V
S	R	R	R	R	0	0.0	I, II, IV, V
R	R	S	R	S	1	0.3	III, V, VI
S	R	S	R	S	0	0.0	I, II, III, V, VI
					357	100.0	
Linkage Model: †							
	I	II	III	IV	V	VI	
	+	ϕ C-r	eryB9	camB2	his-3 purA1	acr-2	strA1
purB2	+	+	+	+	+	+	+

* Prototrophic recombinants were selected on MM at a frequency of 7.8×10^{-4} .

† The linkage model is not scaled to relative map distances.

relatively high occurrence of recombinants containing *eryB9*, it is necessary to place this locus to the left of *his-3 purA1*. The Ery-R phenotype can then be accounted for by postulating a single crossover rather than multiple crossover events. Likewise, the *strA1* has been placed to the right of the *his-3 purA1* in order that the Ery-S Cam-S Str-R recombinant classes be realized from a double crossover. If *strA1* were placed to the left of the selective auxotrophic characters in *N. erythropolis*, this Ery-S Cam-S Str-R class type could have resulted only from triple crossover events involving consecutive regions. The *camB2* locus has been placed between *eryB9* and *his-3* and its resistant phenotype (Cam-R) may be recovered with ϕ C-R and Ery-R markers by a single crossover in region IV (Table 3). Acriflavine resistance resulting from crossovers in region V, are associated with Str-R recombinants, indicating their linkage to the right of the *purA1* locus. Only one recombinant (0.3%), phenotypically Acr-R Str-S, represents crossing over in regions V and VI simultaneously. The remaining 3.1% of the Acr-R recombinants did not show crossing over in region VI after having crossed over in region V (Table 3). From the cross of GB-GA 2-89 by JA-SD 3-3, a two-fold increase in recombinants with the ϕ C-R phenotype was observed when com-

TABLE 4

Segregation of unselected resistance markers and phage sensitivity from crosses of *N. erythropolis* GB-GA 2-89, ϕ C-r eryB9 camB2 his-3 purA1 acr-2 strA1 by *N. canicruria* GB-GA 3-52, purB2 ϕ C-s tetA9 camA1 eryA7 acr-11 strB2

ϕ C	Recombinant phenotypes					Numbers observed*	Fractions of population in percent
	Ery	Tet	Cam	Acr	Str		
S	R	R	R	R	R	236	82.8
R	R	R	R	R	R	29	10.2
S	R	S	S	R	R	0	0.0
R	R	S	S	R	R	17	6.0
S	R	S	R	R	R	0	0.0
R	R	S	R	R	R	2	0.7
S	R	R	R	R	S	0	0.0
R	R	R	R	R	S	1	0.3
						285	100.0

* Prototrophic recombinants were selected on MM at a frequency of 5.5×10^{-5} .

pared to the cross of GB-GA 3-52 by JA-SD 2-13 (32.5% and 14.6%, respectively). These results will be considered further below; but note can be taken of the postulated linear ordering of the genes in the *N. erythropolis* linkage group as illustrated at the bottom of Table 3.

Recombinant class types obtained from crosses of the inhibitor resistant strains (*N. erythropolis* GB-GA 2-89, by *N. canicruria* GB-GA 3-52) are presented in Table 4. There is no tetracycline resistance marker in GB-GA 2-89 and *tetA9* segregated in a manner similar to that observed in the cross of JA-SD 2-13 by GB-GA 3-52 (Table 2). Erythromycin or acriflavine sensitive recombinants were not recovered. On the basis of linkage, as determined from the previous crosses of resistant by sensitive strains, this behavior was anticipated. The recovery of 0.3% Str-S recombinants indicated that the *strA1* and *strB2* loci are not allelic and crossing over occurs between them. The occurrence of Cam-S recombinants at a level of 6% provides a partial explanation for the apparently exaggerated frequencies of occurrence of ϕ C-R phenotypes observed in the GB-GA 2-89 by JA-SD 3-3 cross when this cross was compared to the JA-SD 2-13 by GB-GA 3-52 cross. The Cam-S recombinants from the cross GB-GA 2-89 by GB-GA 3-52 indicate that the genes *camA1* and *camB2* are not allelic. Thus, in order for a relatively high proportion of Cam-S recombinants to occur among the recombinant progeny from this cross, the actual crossover distance between the *camA1* and *his-3+* alleles must be greater than the actual crossover distance between the *camB2* and *his-3* alleles. In this manner a single crossover between *camA1* and *camB2* loci allows the coupling of *camA1+* and *camB2+*, resulting in the Cam-S phenotype, with *his-3+* allele for which selection was made. Any other arrangement of these loci would require the recovery of Cam-S phenotypes in relatively high frequencies from the cross of GB-GA 2-89 by GB-GA 3-52 as the result of multiple crossover events. Further, normalization of the relative distance between

FIGURE 1.—Linkage maps of examined genes in *N. canicruria* and *N. erythropolis*. The solid line represents the linkage group of *N. erythropolis* with respective loci. The broken line represents the *N. canicruria* linkage group with its loci. The genes and their alleles are illustrated by vertical bands and are placed according to their relative distances from each other. Values for map distances between loci are given as percentage. 1 A) Linkage maps based on independent analysis of unselected phenotype from Tables 2 and 3. 1 B) A composite linkage map of the two strains with normalized relative distances of genes to the left of the *his-3*, *his-3+* alleles.

ϕ C-*r* and *his-3* as determined in the cross of GB-GA 2-89 by JA-SD 3-3 (30.2%) with the distance between ϕ C-*s* and *his-3+* as measured in the cross of JA-SD 2-13 by GB-GA 3-52 (13.8%) locates *camB2* at a distance of 3.5% to the left of *his-3+* (Figure 1B) while *camA1* is 6.0% to the left of *his-3+*; this leaves a distance between *camA1* and *camB2* of 2.5% allowing the recovery of a relatively high proportion of Cam-S phenotypes among the recombinant progeny from the cross. A third consideration allowing such normalization of results is further indicated in Table 4. All recovered Cam-S phenotypes are also phenotypically ϕ C-R Ery-R

Tet-S indicating that the loci controlling these factors were recovered from the *N. erythropolis* parent in the recombinant as the result of single rather than multiple crossover events.

Using the recovery fractions of unselected phenotypes from the data in Tables 2 and 3, the relative distances between loci were calculated, and a separate linkage map for each strain (Figure 1A) based on these relative distances was constructed. Relative distances are expressed as the percentile of the population examined exhibiting crossovers in the postulated crossover regions. By normalizing the linkage and relative distances of the markers as determined in strains GB-GA 2-89 and GB-GA 3-52 with selected allele, *his-3+*, and the unselected allele, ϕ C-*r*, a composite linkage map of the two strains with normalized relative distances is indicated in Figure 1B.

A few recombinants were selected from the cross of JA-SD 2-13 by GB-GA 3-52 and tested for their ability to backcross with the parental types (Table 5). Since the recombinants used were of diverse phenotype, the selective conditions were varied depending upon the strains used and the crosses were scored only for fertility. Two kinds of mating behavior were observed among these recombinants in backcrosses to the parental types: some selected recombinants were observed that backcrossed with *N. canicruria*, hence, contained an *N. erythropolis* type compatibility factor, while others failed to cross with either parental type. The factors controlling ϕ C sensitivity segregated from factors controlling mating since recombinants of both ϕ C-S and ϕ C-R phenotypes exhibited such mating behavior (Table 5). However, two correlations between mating behavior and

TABLE 5

Compatibility of recombinants obtained from the cross of N. canicruria GB-GA 3-52, purB2 ϕ C-s tetA9 camA1 acr-11 eryA7 strB2 by N. erythropolis JA-SD 2-13, ϕ C-r his-3 purA1 in backcrosses

Recombinant number	Phenotypes						Selection*	Compatibility	
	ϕ C	Tet	Cam	Acr	Ery	Str		3-52	2-89
R-36	S	S	S	R	R	R	MM + cam	NF	NF
R-41	R	S	S	R	R	S	MM + cam, + str	F	NF
R-46	S	S	S	R	R	S	MM + cam, + str	F	NF
R-61	R	S	S	S	R	R	MM + cam	NF	NF
R-67	S	S	S	S	R	R	MM + cam, + acr	NF	NF
R-200	R	S	S	R	R	R	MM + cam	NF	NF
R-205	R	S	S	R	R	S	MM + cam, + str	F	NF
R-215	R	S	S	R	R	S	MM + cam, + str	F	NF
R-217	R	S	S	R	R	R	MM + cam	NF	NF
R-220	R	S	S	R	R	R	MM + cam	NF	NF
R-227	R	S	S	S	R	R	MM + cam, + acr	NF	NF
R-232	R	S	S	R	R	S	MM + cam, + str	F	NF
R-235	S	S	S	R	R	R	MM + cam	F	NF

F = Fertile.

NF = Nonfertile.

* MM was used to select for prototrophy and the inhibitor, corresponding to the designated genotype, was used to select for resistance.

apparent genotype of unselected characters are suggested from the data. In all instances in which recombinants were fertile on backcrossing with the *canicruria* mating type, a correlation exists between the existence of the *strB2*⁺ allele and fertility. All recombinants which were fertile when backcrossed with GB-GA 3-52, excepting GB-GA R-235, contained the *strB2*⁺ allele and presumably the region to the right of this allele. GB-GA R-235 was streptomycin resistant but crossing over is known to occur to the right of the *strB2* and it is very likely that this strain contains the region to the right of the *strB2*⁺ allele. On the other hand, none of the recombinants chosen for these test crosses were fertile with the *erythropolis* mating type. In all of these recombinants, the *tetA9*⁺ and *camA1*⁺ alleles were present; thus, were donated by the *erythropolis* mating-type parent. However, crossing over to the left of these loci, resulted in the production of ϕC -s *tetA9*⁺ *camA1*⁺ recombinants. These recombinants were not fertile with the *erythropolis* mating type. It can be concluded that a factor preventing fertility of a strain with the *erythropolis* mating type is probably associated with the *tetA9*⁺ *camA1*⁺ loci but in any event is located to the right of the ϕC locus.

DISCUSSION

The present studies have served to increase the number of mapped genes in the nocardial linkage group and further substantiate the previously postulated linear arraying of genes in *N. erythropolis* and *N. canicruria*. When segregations of the markers originating in *N. erythropolis* strains are considered separately, as well as the markers in *N. canicruria*, segregation frequencies of specific phenotypes are found to be in close agreement with earlier results (BROWNELL and ADAMS 1967). However, when a composite linkage map is prepared for these two organisms on the basis of the present data in which additional markers were available for study, the composite map of this report (Figure 1B) compared to that of our earlier report (see Figure 1, BROWNELL and ADAMS 1967) is shown to be shifted somewhat. The reason for this shift was recognized from the data resulting from the cross of GB-GA 2-89 by JA-SD 3-3 in which the relative distance between the ϕC -r and *his-3* loci was two fold greater than the relative distance between the ostensibly allelic ϕC -s and *his-3*⁺ loci as measured in the cross of JA-SD 2-13 by GB-GA 3-52. Normalization of the distances between these regions, in order to construct the composite map, shifts the genes originating in *N. erythropolis* to the left of the *his-3* locus, more towards the *his-3* locus. Since GB-GA 2-89 was derived from JA-SD 2-13, and since GB-GA 3-52 was derived from JA-SD 3-3, a simple explanation of these results is not readily apparent. However, mating between GB-GA 2-89 and JA-SD 3-3 appeared to be more efficient than in other crosses. The frequency of recovery of recombinants in this cross in which the apparent anomaly was observed was approximately 50-fold greater than in any of the other crosses examined. It could be expected that with more efficient mating, and presumably better transfer of genetic information, differences in relative numbers of crossover events could occur. Without

the additional study of *camA1*, *camB2* and ϕC loci, this behavior could not be easily recognized and indeed was not in our earlier studies (BROWNELL and ADAMS 1967).

Until the mechanisms by which genetic information is exchanged in the Nocardiae are more precisely known, absolute map units cannot be calculated and map units must represent relative rather than absolute values (SERRA 1965). Nonetheless, these relative linkage maps are supported by internal consistency and use of individually mapped regions to predict multiple crossover events. For example, the frequency of multiple crossing over in *N. canicruria* involving regions I, II, and IV (Table 2) can be calculated from single crossover events involving these regions. The calculated frequency is 0.3% and the observed number of multiple crossovers in these regions, as indicated by the segregation of the selectable phenotypic segregant resulting from these multiple crossovers, is 0.4%. While the values for results observed with *N. erythropolis* (Table 3) do not correlate quite as well, a similar trend is observed. Moreover, it is quite likely that the increased frequency of recombinational events in GB-GA 2-89 by JA-SD 3-3 cross somewhat lowers this correlation.

While the relationship of phage ϕC specificity of infection of the *N. canicruria* mating type awaits the precise mapping of the factors responsible for compatibility, there is good evidence that the mating factors in these organisms are multiple and segregate (ADAMS 1964, 1967). The loci *C* and *E*, with alleles *c* and *e*, respectively, were postulated to control nocardial compatibility. Thus four genotypes for mating factors are combinationally possible: *cE*, *Ce*, *CE* and *ce*. The "standard" strain of *N. erythropolis* bears the *cE* loci; the "standard" strain of *N. canicruria* bears the *Ce* loci. All four genotypes should be recoverable among recombinants and have been recovered. The *CE* containing recombinants were first recognized (ADAMS 1964) by their ability to cross with both standard strains and *ce* containing recombinants by their inability to produce recombinants under any normal mating conditions (ADAMS 1967). In order for a cross to be fertile, one strain must bear the *C* allele and the other must bear the *E* allele; this explained the early recognized lack of compatibility of nutritionally complementary strains of homologous origin (ADAMS 1964). In the present study, two of the four proposed mating types, *cE* and *ce*, were obtained. They backcrossed, respectively, only with the *N. canicruria* or with neither parental type (Table 5). There was no correlation between the ability to backcross and segregation of sensitivity to phage ϕC . However, all of the *cE* containing recombinants, that is those crossing with the *N. canicruria* parental type, were found to contain most likely the region to the right of the *strB2*⁺ allele from *N. erythropolis*. Thus, *E* appears to be associated with the region to the right of the *strB2* locus. The thirteen recombinants chosen for further examination of mating behavior were chosen primarily on the basis of their segregation of alleles suitable for selection in backcrossing attempts and segregation of the ϕC locus which we had preliminarily associated with mating behavior (BROWNELL, ADAMS and BRADLEY 1967). None of these recombinants were capable of backcrossing with the *N. erythropolis* strain although a

positive correlation exists between the presence of the *c* allele in close association with the ϕC locus. Thus, phage sensitivity *per se* is not a presently useful technique for determining mating types of selected recombinants although the ϕC locus has brought to bear more information on the mapping of loci responsible for mating compatibility in these *Nocardiae*. The precise location of *C* and *E* and their alleles awaits the future availability of a large number of recombinants suitable for backcrossing and a simple technique employing tester strains but also allowing the carrying out of a large number of crosses. However, the present studies have fairly convincingly associated the mating factors with known genes of the nocardial chromosome.

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SUMMARY

The ordering of the genes in *N. erythropolis* was found to be: *purB2*+- ϕC -*eryB9-camB2-his-3-purA1-acr-2-strA1* and in *N. canicruria*: *purB2*- ϕC -*s-tetA9-camA1-his-3*+-*purA1*+-*eryA7-acr-11-strB2*. With the possible exception of genes controlling acriflavine resistance, loci in *N. erythropolis* were not allelic to genes controlling similar phenotypic resistance in *N. canicruria* and showed lateral displacement. Mating factors, believed to be multiple in nature, segregated in the recombinants to produce two mating types. One type backcrossed only to *N. canicruria* while the second failed to backcross with either of the parental strains. Genes controlling mating behavior segregated from those expressing phage sensitivity invalidating the use of phage ϕC for scoring mating type in the recombinants.

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