Inheritance of Mating Factors in *Nocardial* Recombinants

GEORGE H. BROWNELL AND KAROL L. KELLY

Department of Microbiology, Medical College of Georgia, Augusta, Georgia 30902

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The segregation of mating loci with other unselected genes was analyzed in recombinants obtained from matings of *Nocardia canicruria* and *N. erythropolis*. The loci *C/c* and *E/e* control nocardial compatibility. Four mating genotype combinations were observed: *cE*, *Ce*, *CE*, and *ce*. Strains of *N. erythropolis* bear the genotype *cE* and strains of *N. canicruria* bear the *Ce* alleles. The *CE* recombinant mating type is capable of mating with both organisms, whereas the *ce*-containing recombinant is nonfertile. The *E* locus was found to segregate with *StrA1* (streptomycin-resistance gene) on the *N. erythropolis* linkage group. The *C* locus appeared linked to *PurB2* (purine-requiring gene) on the *N. canicruria* linkage group. A few observed recombinants were capable of further segregation of unselected characters after colonial purification, suggesting a possible heterogenomic condition or multiple rounds of mating. Prior treatment of recombinants with acriflavine failed to alter their compatibility or the frequency at which recombinants were recovered. The segregation pattern of the mating loci allowed for specific recombinant class types to be compatible.

A comparison of linkage maps constructed from matings of *Nocardia canicruria* with *N. erythropolis* suggests an incomplete chromosome homology or oriented chromosome transfer during zygote formation (2, 4, 5). The continued expansion of the linkage maps with auxotrophic and resistance markers has to date failed to clarify the mechanisms or the extent of chromosomal involvement in zygote formation. An investigation into the compatibility factor(s) provides a different approach and possibly a more direct characterization of recombination in these organisms.

Since strains of homologous origin are incompatible, it is evident that some type of mating factor(s) controls compatibility in these organisms. Evidence of the multiple nature of the mating factors and their ability to segregate in the recombinant has been noted (5; J. N. Adams, Bacteriol. Proc., p. 55, 1967). The loci *C/c* and *E/e* have been postulated to control fertility. In a fertile cross, one strain must have the *C* allele at one locus while the other has the *E* allele at a second locus. Fertility was not believed to be affected when either of the remaining alternative alleles (*c* or *e*) is at the second locus. Three compatibility phenotypes have been recovered among recombinants: one is compatible with *N. erythropolis*, another is compatible with both parental

types, and the third is not compatible with either parental type (Adams, Bacteriol. Proc., p. 55, 1967).

The present investigation was initiated to determine the linkage and segregation of the mating alleles in relation to other unselected characters. Of particular interest was the stability of the recombinant mating genes and a location which could best explain their pattern of segregation. Owing to the time-consuming nature of obtaining and crossing *F*₁ recombinants and of analyzing the *F*₂ recombinants by present techniques, it was not possible to test a sufficient number of recombinants to allow the assignment of precise map distances to the mating loci. However, by comparing their behavior in relation to other selected and unselected characters, an approximate location of the mating loci was determined. The investigation thus serves to expand the linkage maps, to characterize further the mating genes, and to illustrate the genetic dissimilarities between these nocardiae and the eubacteria and streptomycetes.

MATERIALS AND METHODS

Recombinants were obtained from matings of *N. canicruria* and *N. erythropolis* substrains which contained previously mapped auxotrophic and resistance characters (1, 4, 5). The strains employed in this investigation with their genotypes and phenotypes are

listed in Table 1. The mutant strains are codified and maintained as previously described (3).

Standard mating techniques were employed (1). Mixed cultures of parental strains were grown on Nutrient Agar (Difco), and each parental strain was grown individually to serve as a control. After 3 days of incubation, saline suspensions were prepared from cross and control cultures. The population density of the cell suspensions was determined, and samples were plated on minimal medium (MM) plates and on inhibitor-containing supplemented minimal medium (ISM). After 4 to 6 days of incubation, recombinants were picked and transferred to fresh MM and ISM plates. After 24 hr of incubation, the prototrophic recombinants isolated on MM were replicated to peptone-yeast extract (PY), inhibitor-containing minimal medium (IMM), and MM plates; the MM plates served as replicating controls. Auxotrophic recombinants selected on ISM were replicated to PY, MM, supplemented minimal medium (SMM), and ISM plates. Recombinants replicated to PY were immediately covered with phage ϕ C (6) and were scored for plaque formation after 12 hr of incubation. The SMM, MM, and ISM or IMM and MM plates were incubated for 3 to 5 days, and these sets of plates were compared to determine phenotypes of individual recombinants.

To diagnose the mating type of the F_1 recombinants, test crosses were carried out with substrains of known mating type. After scoring, recombinants containing at least one locus for inhibitor sensitivity or auxotrophy were selected. These recombinants were purified by means of three successive single-colony streak isolations on Nutrient Agar, and after each streak isolation they were tested to assure sensitivity or auxotrophy and then test-crossed. The mating of these F_1 recombinants with parental-type strains was carried out as described above. Selection was made on MM to select against both auxotrophic parental and F_1 recombinant types, or on IMM to select against auxotrophic parental types and inhibitor-sensitive F_1 recombinants. The presence of colonies on the selective medium after these matings was considered evidence of a fertile test cross.

In some instances, to substantiate mating gene linkage from crosses involving similar nonallelic resistance markers, it was necessary to determine whether allelism existed in the F_1 recombinant and a parental-type organism. Likewise, when test crosses resulted in low recombination frequencies, F_2 recombinant phenotypes were determined by analysis similar to that carried out with the F_1 recombinants.

All plates were incubated at 30 C.

RESULTS

The reliability of the auxotrophic and resistance characters for selection of recombinants was reported earlier (1, 4). Class types of prototrophic recombinants obtained from crosses of *N. canicruria* 3-52 by *N. erythropolis* 2-13 which were selected for test-crossing are provided in Table 2. The number of each recombinant class type test-crossed, their frequency among the

recombinant population, and the selective mechanism used for the recovery of the F_2 recombinants are given in Table 2. One of the selective media, MM+C (35 μ g of chloramphenicol/ml), was employed for all but one of the class types test-crossed. Where they could be appropriately utilized, other inhibitor markers were used to substantiate compatibility. Compatibility of the F_1 recombinants with strains of *N. canicruria* (3-52) and *N. erythropolis* (2-89) and their proposed mating genotypes are also illustrated.

Two types of mating behavior were observed: fertility with *N. canicruria* (3-52) and nonfertility with both parental types. Since prototrophy was employed to select the recombinants, the compatibility genes, ϕ C sensitivity markers, and inhibitor-resistance markers were free to segregate as unselected characters, influenced only by their linkage to the selective auxotrophic characters. The only characters which seem to have segregated in a similar manner are streptomycin sensitivity (*StrB2*+) and the *N. erythropolis* mating gene (*E*). Acriflavine, erythromycin, and ϕ C markers could be found as either the sensitive or resistant allele in recombinants expressing either of the observed mating behaviors. Tetracycline and chloramphenicol behave similarly, existing at alternative alleles, in nonfertile recombinants (Table 2).

At the bottom of Table 2, the parental linkage groups are illustrated as they might have existed in the original zygote. A location of the mating genes accounting for the origin of recombinants possessing the particular mating behaviors is proposed. Two recombinant class type genomes exemplifying the mating behavior of the recombinants are shown. The first recombinant group (a) represents crossovers in region V and VIII, producing a genotype *c* ϕ C-*r tetA9* + *camA1* + *eryA7 acr-11 E strB2* + (i.e., test-crosses only with the *N. canicruria* parent). This class type comprises 0.6% of the prototrophic recombinants obtained. The second group (b) illustrates the genome resulting from a single crossover in region V, producing a genotype *c* ϕ C-*r tetA9* + *camA1* + *eryA7 acr-11 e strB2* (nonfertile with both parental class types). From prototrophic selection, this class type will comprise 5% of the recombinants.

When prototrophic recombinants were selected from the cross of a sensitive *N. canicruria*, 3-3, by a resistant *N. erythropolis*, 2-89, an additional mating type was obtained (Table 3). Five streptomycin-resistant (*StrA1*) recombinants (c) were found to be compatible with *N. canicruria* (3-52), whereas the streptomycin-sensitive (*StrA1*+) recombinants were incompatible. Fertility patterns from these results correlated with the fertility

TABLE 1. *Mutant characteristics and designations of strains employed*

Strain ^a	Derived from	Mutant designations ^b										Relevant phenotype ^c										
		purA	purB	his	strA	strB	eryA	eryB	acr	tetA	tetB	camA	camB	φC	Pur	His	Str	Ery	Acr	Tet	Cam	φC
2		+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	S	S	S	S	S	R
2-13	2-1	1	+	3	+	+	+	+	+	+	+	+	+	-	-	-	S	S	S	S	S	R
2-44	2-13	1	+	3	1	+	+	+	+	+	+	+	+	-	-	-	S	S	S	S	S	R
2-77	2-44	1	+	3	1	+	+	9	2	+	+	+	+	-	-	-	S	S	S	S	S	R
2-82	2-77	1	+	3	1	+	+	9	2	+	+	+	+	-	-	-	S	S	S	S	S	R
2-89	2-82	1	+	3	1	+	+	9	2	+	+	+	2	-	-	-	S	S	S	S	S	R
3		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S	S	S	S	S	R
3-3	3	+	2	+	+	+	+	+	+	+	+	+	+	+	+	+	S	S	S	S	S	S
3-7	3-3	+	2	+	+	2	+	+	+	+	+	+	+	+	+	+	S	S	S	S	S	S
3-39	3-7	+	2	+	+	2	7	+	11	+	+	+	+	+	+	+	S	S	S	S	S	S
3-48	3-39	+	2	+	+	2	7	+	11	9	+	+	+	+	+	+	R	R	R	R	R	S
3-52	3-48	+	2	+	+	2	7	+	11	9	+	1	+	+	+	+	R	R	R	R	R	S
3-55	3-52	+	2	+	+	2	7	+	11	9	+	1	+	+	+	+	R	R	R	R	R	S
3-56	3-55	+	2	+	+	2	+	+	11	+	+	+	+	+	+	+	R	R	R	R	R	S

^a Strains of *Nocardia erythropolis* are prefixed with 2; strains of *N. canicruria* are prefixed with 3.

^b Description of mutant loci, mutation sites, and relevant phenotypes are, insofar as possible, in conformity with the recommendation of Demerec et al. (7).

^c Symbols: +, synthesized; -, required; R, resistant; S, susceptible; Pur, purine; His, histidine; Str, streptomycin, 200 μg/ml; Ery, erythromycin, 6 μg/ml; Acr, acriflavine, 100 μg/ml; Tet, tetracycline, 6 μg/ml; Cam, chloramphenicol, 35 μg/ml; φC, nocardiphage φC.

TABLE 2. Compatibility of protrophic recombinants obtained from the cross of *N. canicruria* 3-52, *purB2* ϕ C-s *tetA9 camA1 eryA7 acr-11 strB2* by *N. erythropolis* 2-13, ϕ C-r *his-3 purA1* in test crosses

No. of recombinants tested	Recombinant phenotypes						Percentage of recombinant population	Selection ^a	Compatibility ^b		Mating genotype
	ϕ C	<i>Tet</i>	<i>Cam</i>	<i>Ery</i>	<i>Acr</i>	<i>Str</i>			3-52	2-89	
12	R	S	S	R	R	S (a)	0.6	MM + C, MM + S	F	NF	<i>cE</i>
6	S	S	S	R	R	S	0.4	MM + C, MM + S	F	NF	<i>cE</i>
2	R	S	S	S	S	S	<0.1	MM + C, MM + E, MM + T	F	NF	<i>cE</i>
15	S	S	S	R	R	R	0.4	MM + C	NF	NF	<i>ce</i>
7	S	S	S	R	S	R	0.6	MM + C, MM + A	NF	NF	<i>ce</i>
3	R	S	S	R	S	R	0.4	MM + C, MM + A	NF	NF	<i>ce</i>
16	R	S	S	R	R	R (b)	5.0	MM + C	NF	NF	<i>ce</i>
3	S	S	R	S	S	R	<0.1	MM + E, MM + T, MM + A	NF	NF	<i>ce</i>
2-13	+ ^c	(c)		ϕ C-r	+	+	+	His-3 PurA1	+	(E)	+
	I	II	III	IV	V	VI	VII	VIII	IX		
3-52	<i>PurB2</i>	(C)	ϕ C-s	<i>TetA9</i>	<i>CamA1</i>	+ ^c	+ ^c	<i>EryA7</i>	<i>Acr-11</i>	(e)	<i>StrB2</i>
a	+	(c)	ϕ C-r	+	+	+	+	<i>EryA7</i>	<i>Acr-11</i>	(E)	+
b	+	(c)	ϕ C-r	+	+	+	+	<i>EryA7</i>	<i>Acr-11</i>	(e)	<i>StrB2</i>

^a MM + C = minimal medium + 35 μ g of chloramphenicol (Parke, Davis & Co., Detroit, Mich.) per ml; MM + S = minimal medium + 200 μ g of streptomycin (Nutritional Biochemicals Corp., Cleveland, Ohio) per ml; MM + E = minimal medium + 6 μ g of erythromycin (Eli Lilly & Co., Indianapolis, Ind.) per ml; MM + T = minimal medium + 6 μ g of chlortetracycline HCl (American Cyanamid Co., Pearl River, N.Y.) per ml; MM + A = minimal medium + 100 μ g of acriflavine HCl (Allied Chemical Corp., New York, N.Y.) per ml.

^b F = fertile; NF = nonfertile.

^c Selective loci for F₁ recombinants.

patterns of the *strB2*+ recombinants obtained from the previous cross, supporting the location of the *E* locus (compatibility with *N. canicruria*) proximal to the *StrA1* character on the *N. erythropolis* linkage group. Twenty-seven recombinants possessing the ϕ C-s *eryB9*+ *camB2*+ *acr-2 strA1*+ genotype were test-crossed. Of these, five were fertile when crossed with *N. erythropolis* (2-89) and the remainder were nonfertile with both parental organisms. Unlike the *E* locus, the *C* locus, postulated to control fertility with *N. erythropolis*, shows no close linkage to any of the available unselected characters. Since the same class types produce both fertile and nonfertile recombinants with *N. erythropolis*, it is assumed that crossovers in the same regions, in relation to the unselected markers, produced the two types of mating behavior. In one instance (18.5%), crossing-over incorporated the *C* locus into the recombinant genome, producing the fertile recombinants; in the other (81.4%), crossing-

over incorporated the *c* allele, producing the nonfertile recombinants. The largest mapped crossover region is the distance from the selective *purB2*+ to the unselected ϕ C-s marker. The *C* locus would seem to be located in this area, more closely linked to the selective *purB2* than to the ϕ C-s marker.

A postulated zygote capable of producing the observed recombinants is illustrated in Table 3. Examples of the recombinant genomes exhibiting the three mating behaviors observed in the crosses are shown. The nonfertile recombinants (a) could originate from crossovers in regions II, VI, and VII, thus incorporating both the *c* and *e* loci into the genome. The second illustrated recombinant (b), although genotypically similar with regard to the unselected markers, could have originated from a crossover in a hypothetical region I closer to the *PurB2* locus, thus incorporating the *C* locus into the recombinant genome and allowing for fertility with *N. erythropolis*. The recombinant

TABLE 3. Compatibility of prototrophic recombinants obtained from the cross of *N. canicruria* 3-3, *purB2* ϕ C-s by *N. erythropolis* 2-89, ϕ C-r *eryB9* *camB2* *his-3* *purA1* *acr-2* *strA1* in test crosses

No. of recombinants tested	Recombinant phenotypes					Percentage of recombinant population	Selection ^a	Compatibility ^b		Mating genotype
	ϕ C	<i>Ery</i>	<i>Cam</i>	<i>Acr</i>	<i>Str</i>			3-52	2-89	
9	S	S	S	S	S	60.5	MM + C, MM + E, MM + S	NF	NF	<i>ce</i>
6	R	R	S	S	S	13.4	MM + C, MM + S	NF	NF	<i>ce</i>
22	S	S	S	R	S (a)	<0.3	MM + E, MM + S, MM + C	NF	NF	<i>ce</i>
5	S	S	S	R	S (b)	<0.3	MM + E, MM + S, MM + C	NF	F	<i>Ce</i>
6	R	S	S	R	S	<0.3	MM + E, MM + S	NF	NF	<i>ce</i>
4	S	S	R	S	S	0.8	MM + E, MM + S	NF	NF	<i>ce</i>
4	R	S	R	S	S	1.7	MM + E, MM + S	NF	NF	<i>ce</i>
2	S	R	S	S	S	2.3	MM + C, MM + S	NF	NF	<i>ce</i>
5	R	S	S	R	R (c)	<0.3	MM + E, MM + C	F	NF	<i>cE</i>
2-89	+ ^c	(c)	ϕ C-r	<i>EryB9</i>	<i>CamB2</i>	<i>His-3</i>	<i>PurA1</i>	<i>Acr-2</i>	<i>StrA1</i>	(E)
	I	II	III	IV	V		VI	VII	VIII	
3-3	<i>PurB2</i>	(C)	ϕ C-s	+	+	+ ^c	+ ^c	+	+	(e)
a	+	(c)	ϕ C-s	+	+	+	+	<i>Acr-2</i>	+	(e)
b	+	(C)	ϕ C-s	+	+	+	+	<i>Acr-2</i>	+	(e)
c	+	(c)	ϕ C-r	+	+	+	+	<i>Acr-2</i>	<i>StrA1</i>	(E)

^a See Table 2.
^b F = fertile; NF = nonfertile.
^c Selective loci for F₁ recombinants.

crossing with *N. canicruria* (c) could originate from a double crossover in regions III and VI, producing the *cE* genotype.

Recombinants obtained from the cross of a resistant *N. canicruria*, 3-55, by a resistant *N. erythropolis*, 2-89, were also suitable for test-cross analysis, owing to a high degree of nonallelism between phenotypically similar resistant markers in the two organisms which allowed for the recovery of their sensitive alleles in the F₁ recombinants (Table 4). From these crosses, members of a single class type were recovered exhibiting different mating behavior: nonfertility and fertility with *N. canicruria*. Since previous resistant by sensitive crosses (Tables 2 and 3) indicated close linkage of the *E* locus to the *strA1* marker in *N. erythropolis*, a variation in mating behavior of streptomycin-resistant recombinants from a *strA1* by *strB2* cross could originate as illustrated in Table 4. The nonfertile recombinant (a) is produced after a single crossover in region II, resulting in a *c strB2 e* class type, whereas the type

fertile with *N. canicruria* (b) would require a double crossover in regions II and X, producing a *c strA1 E* genome. Thus, streptomycin resistance in the nonfertile recombinant should result from the incorporation of the *N. canicruria strB2* locus, whereas the recombinant fertile with *N. canicruria* would owe its resistance to the *strA1* locus from *N. erythropolis*. To substantiate this relationship and confirm the location of the *E* locus in the proximity of *strA1* in *N. erythropolis*, the F₂ recombinants obtained from test-crossing *N. canicruria* (3-52) and recombinant (R-b) were scored for streptomycin resistance. As shown in Table 5, 1.4% of the F₂ progeny were streptomycin-sensitive, indicating recombination between the nonallelic *strA1* and *strB2* loci. Other resistance markers, which could have originated from the *N. erythropolis* genome (*camB2*, *acr-2*) and thus be nonallelic to similar phenotypic markers in the *N. canicruria* linkage group, failed to segregate sensitive phenotypes. *N. canicruria* appears to be the origin of these

TABLE 4. Compatibility of prototrophic recombinants obtained from the cross of *N. caniculatus* 3-55, *purB2* ϕ C-s *tetA9 camA1 acr-11 strB2* by *N. erythropolis* 2-89, ϕ C-r *eryB9 camB2 his-3 purA1 acr-2 strA1* in test crosses

No. of recombinants tested	Recombinant phenotypes						Percentage of recombinant population	Selection ^a	Compatibility ^b		Mating genotype
	ϕ C	<i>Ery</i>	<i>Tet</i>	<i>Cam</i>	<i>Acr</i>	<i>Str</i>			3-52	2-89	
1	R	S	R	R	R	R	10.2	MM + E	NF	NF	ce
3	S	S	R	R	R	R (a)	82.8	MM + E	NF	NF	ce
1	S	S	R	R	R	R (b)	82.8	MM + E	F	NF	cE
5	S	S	R	S	R	R	<0.3	MM + E	NF	NF	ce
1	R	S	R	S	R	R	<0.3	MM + E, MM + C	NF	NF	ce
2	R	S	R	S	S	R	<0.3	MM + C	NF	NF	ce
2	S	S	R	R	S	R	<0.3	MM + E	F	NF	cE
5	R	R	S	S	R	S (c)	<0.3	MM + C, MM + S	NF	NF	ce
<hr/>											
2-89	+ ^c	(c)	ϕ C-r	EryB9	+	+	+	PurA1	+	StrA1	+
	<hr/>										
	I II III IV V VI VII VIII IX X XI XII										
PurB2	(C)	ϕ C-s	+	TetA9	CamA1	+	+	+ ^c	+	Acr-11	+
	<hr/>										
3-55	+	(c)	ϕ C-s	+	TetA9	CamA1	+	+	+	Acr-11	+
	<hr/>										
a	+	(c)	ϕ C-s	+	TetA9	CamA1	+	+	+	StrA1	+
	<hr/>										
R-b	+	(c)	ϕ C-r	EryB9	+	+	+	+	+	Acr-11	+
	<hr/>										
c	+	(c)	ϕ C-r	EryB9	+	+	+	+	+	Acr-11	+
	<hr/>										

^a See Table 2.
^b F = fertile; NF = nonfertile.
^c Selective loci for F₁ recombinants.

TABLE 5. Segregation of inhibitor resistance characters and compatibility loci in F_2 recombinants obtained from test-crossing *N. canicruria* 3-52 by R-b

Recombinant phenotypes						No. observed	Selection ^a	Compatibility ^b		Mating genotype		
<i>ϕC</i>	<i>Tet</i>	<i>Cam</i>	<i>Ery</i>	<i>Acr</i>	<i>Str</i>			3-52	2-89			
S S	R R	R R	R R	R R	R S (a)	138 3	— MM + E	— NF	— NF	— <i>ce</i>		
R-b	+ ^c	(c)	ϕC-s	TetA9	CamA1	+	+	+	Acr-11	StrA1	(E)	+
	I	II	III	IV	V		VI	VII	VIII	IX	X	
3-52	PurB2	(C)	ϕC-s	TetA9	CamA1	+	+	EryA7 ^c	Acr-11	+	(e)	StrB2
a	+	(c)	ϕC-s	TetA9	CamA1	+	+	EryA7	Acr-11	+	(e)	+

^a MM + E = minimal medium plus 6 μ g of erythromycin per ml.^b NF = nonfertile.^c Selective loci for F_2 recombinants.

characters, and these results support the cross-overs suggested to produce the F_1 recombinants (Table 4, R-b). When streptomycin-sensitive F_2 recombinants were test-crossed, they were incompatible with both parental-type strains, indicating an origin as illustrated at the bottom of Table 5.

A streptomycin-sensitive class type (*strA1* +, *strB2* +) showing incompatibility with both parental types was recovered from the resistant by resistant cross (Table 4, c). As illustrated in Table 4, with respect to the unselected resistance characters, a crossover in region VI and regions XI or XII would produce this recombinant genome. Since the genotype was *strA1* + *e strB2* +, it follows that the *e* locus is linked to the left of *strB2*. This class type originates from double rather than triple crossover events. Cross-overs involving the proposed region XII producing *strA1 e* genotypes were not observed. The failure of the *E* locus to segregate from the *strA1* indicates that the *e* allele is closely linked to either side of the *strA1* + locus on the *N. canicruria* linkage group (Table 4).

Some F_1 recombinants, when crossed after a single streak purification, produced results contrary to those previously observed. However, continued streak purification, phenotype scoring, and test-crossing indicated that the original crossed organisms had not completed their segregation or were a heterogeneous population. Further growth produced different class types in regard to both the unselected markers and the mating genes (Table 6). One streptomycin-sensitive recombinant (a) was observed to cross with both parental organisms and appeared to be of the *CE* genotype. On the basis of the linkage map,

excluding the proposed location of the mating loci, the recombinants originated from multiple cross-overs involving regions I, III, V, VI, and IX. Further growth that accompanied additional streak purification on Nutrient Agar produced a second streptomycin-sensitive class type (b) crossing only with the *N. canicruria* parent. This class type originated from a double crossover in regions II and VI in contrast to the multiple crossover required to explain the genotype of its precursor. When several single-colony isolates from this same streak purification were tested and again streak-purified, the isolates were found to be phenotypically similar and failed to show further segregation. A second recombinant which also appeared to be phenotypically unstable was scored first as *C tet-s cam-s E str-r* and stabilized as a *c tet-r cam-r e str-r* after additional purification (Table 6).

To substantiate the linkage of the C locus to the *PurB2* locus in *N. canicruria*, adenine-requiring recombinants were obtained (Table 7). When mixed cultures of *N. canicruria* (3-56) and *N. erythropolis* (2-89) were plated on MM supplemented with adenine and containing chloramphenicol, a single adenine-requiring class type suitable for test-crossing was recovered. To help substantiate which adenine locus (*PurB2* or *PurA1*) from the parental organisms was responsible for the adenine requirement of the recombinant, selection of F_2 recombinants was made on MM and ISM. All recombinants crossed with *N. erythropolis* (2-89) on both MM and MM + Ad + E + C (MM + adenine + erythromycin + chloramphenicol). Since F_2 recombinants were recovered on MM, it is apparent that the

TABLE 6. Compatibility of prototrophic recombinants obtained from the cross of *N. canicruria* 3-52, *purB2* ϕ C-s *tetA9* *camA1* *eryA7* *acr-11* *strB2* by *N. erythropilis* 2-13, ϕ C-r *his-3* *purA1* following each of three streak purifications^a

Colony isolation and test cross	Recombinant phenotypes						Compatibility ^b			Mating genotype	Crossovers required
	φC	Tet	Cam	Ery	Acr	Str	3-48	2-59			
1	S	S	S	S	S	R (a)	F	F	CE	I, III, V, VI, IX	
2	S	R	R	S	S	S (b)	F	NF	cE	II, VI	
3	S	R	R	S	S	S	F	NF	cE	II, VI	
1	R	S	S	S	S	R	F	F	CE	I, II, V, VI, IX	
2	R	R	R	S	S	R	NF	NF	ce	III, VI, VIII	
3	R	R	R	S	S	R	NF	NF	ce	III, VI, VIII	
a	PurB2+	(C)	φC-s	TetA9+	CamA1+	His-3+	PurA1+	EryA7+	Acr-11+	(E)	StrB2
b	PurB2+	(c)	φC-s	TetA9	CamA1	His-3+	PurA1+	EryA7+	Acr-11+	(E)	StrB2+

^a Selection = minimal medium plus 6 μ g of erythromycin per ml.

^b F = fertile; NF = nonfertile. Shaded area = genes showing further segregation.

TABLE 7. Compatibility of auxotrophic recombinants obtained from the cross of *N. canicruria* 3-56, *purB2* ϕ C-s *acr-11 strB2* by *N. erythropolis* 2-89, ϕ C-r *eryB9 camB2 his-3 purA1 acr-2 strA1* in test crosses

No. of recombinants tested	Recombinant phenotypes									Cross	Selection ^a		Mating genotype
											MM	MM + Ad + E + C	
1	Pur ⁻	ϕC-s	Ery-s	Cam-r	Acr-r	Str-r (a)				2-89 3-48	F NF	F F	CE
17	Pur ⁻	ϕC-s	Ery-s	Cam-r	Acr-r	Str-r (b)				2-89 3-48	F NF	F NF	Ce
2-89	+	(c)	ϕC-r	EryB9	CamB2 ^b	His-3	PurA1	Acr-2	+	StrA1		(E)	+
	I	II	III	IV	V	VI		VII	VII	IX	X		
3-56	PurB2	(C)	ϕC-s	+	+	+	+	+	Acr-11	+	(e)		StrB2
a	PurB2	(C)	ϕC-s	+	CamB2	+	+	+	Acr-11	StrA1	(E)		+
b	PurB2	(C)	ϕC-s	+	CamB2	+	+	+	Acr-11	+	(e)		StrB2

^a Selection: MM = minimal medium; MM + Ad + E + C = minimal medium plus 10 μ g of adenine, 6 μ g of erythromycin, and 35 μ g of chloramphenicol per ml. F = fertile; NF = nonfertile.
^b Selective loci for F₁ recombinants.

F₁ recombinants were *PurB2 C PurA1* + (Table 7). As anticipated, no recombinants were recovered when these same F₁ recombinants were crossed to *N. canicruria* (3-48) and selected on MM. Allelism, which had invalidated selection on MM as a test of fertility, would not be expected to prevent recombination with this strain when selection was made on the MM + Ad + E + C medium. Indeed, one F₁ recombinant did cross with *N. canicruria* when selection was made on this medium (Table 7, a). Since all 18 of the *PurB2* recombinants were observed to cross with the *N. erythropolis* mating type, linkage of the *C* locus to the *PurB2* locus in *N. canicruria* is apparent. Seventeen of the recombinants (b) failed to cross with *N. canicruria*, indicating a *Ce* genotype, and these recombinants owe their streptomycin resistance to the *strB2* of the *N. canicruria* genome. The zygote from which the auxotrophic F₁ recombinants are believed to have originated is illustrated in Table 7. The recombinants exhibiting the two mating behaviors crossed over in regions IV and V owing to the selection of *camB2* from the *N. erythropolis* genome. Recombinant (a) was produced from an additional crossover in region VIII, whereas no additional crossover occurred in recombinant (b).

The numbers of recombinants of varying origin that have been test-crossed are not adequate to

allow precise measurement of the postulated cross-over regions. However, the predictability of recombinant mating behavior on the basis of inheritance of the unselected characters (e.g., *StrA1-E*, *StrB2-e*, *PurB2* + *c*, and *PurB2* - *C*) allows good location of the genes controlling compatibility in relation to these unselected characters exhibiting similar patterns of segregation. Figure 1 shows linkage maps of the two organisms as determined by independent analysis from resistant by sensitive crosses. An approximate location of the loci controlling compatibility, as determined by their unselective segregation patterns in the various F₁ recombinants, is shown.

DISCUSSION

The present investigation has served to substantiate the multiplicity of the mating factors in these nocardial organisms and to characterize their pattern of segregation in the recombinants. The two loci with alleles *C/c* and *E/e* control nocardial compatibility. Four mating genotype combinations are possible: *cE*, *Ce*, *CE*, and *ce*. Strains of *N. erythropolis* bear the *cE* loci and strains of *N. canicruria* bear the *Ce* loci. All four genotypes have been recovered among recombinants. The *CE*-containing recombinant is recognized by its ability to cross with both parental organisms and the *ce*-containing recombinant by

FIG. 1. Linkage map 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. The maps are based on independent analysis of unselected phenotypes (5). The relative location of the mating loci based on their segregation with other unselected characters is illustrated.

its inability to produce recombinants under any normal condition.

The proposed locations of the mating loci correlate well with the known locations of selective characters. When prototrophic selection is employed for the auxotrophic markers used, the most frequently encountered mating type is the nonfertile *ce* recombinant. All recombinants containing *strB2* carried the *e* locus and failed to cross to the *N. canicruria* parent. The only exception was those recombinants which showed continuing segregation (Table 6), but once their segregation was completed they did reflect this relationship. The alternative alleles of these characters, *strB2*⁺ and *E*, showed a similar inheritance pattern. The nonallelic streptomycin-resistance marker of *N. erythropolis* (*strA1*) behaved in a reciprocal manner; i.e., the *strA1* and *E* loci, and the *strA1*⁺ and *e* loci, showed closely linked inheritance (Table 3). Crossing-over was observed between the *strB2* and *e* loci in crosses of Str-R parents, as reflected by the recovery of the Str-S, nonfertile recombinant class type (Table 4). Consequently, the *strA1* and *E* loci must be more closely linked than are the *strB2* and *e* loci. To account for the *strA1*⁺ *e* *strB2*⁺ recombinant type on the basis of the fewest number of crossovers, the *e* locus is linked to the left of the *strB2* locus. The linkage of the *strA1* and *E* loci was further substantiated by the analysis of F₂ recombinants, which revealed

that fertile test crosses of Str-R *N. canicruria* by Str-R F₁ recombinants produced Str-S recombinants (Table 5). Therefore, the Str-R phenotype of the F₁ recombinants could have originated only from the *strA1* marker on the *N. erythropolis* genome.

The inheritance patterns observed for the *C* and *c* alleles were less obvious from analysis of selected prototrophic recombinants. Recombinants obtained from the crosses of a resistant *N. canicruria* with both sensitive and resistant *N. erythropolis* (Tables 2 and 4) contained only the *c* locus. These results suggest that the *C* locus in *N. canicruria* is in close proximity to the selective *PurB2* locus. There is a curious discrepancy in the cross of the sensitive *N. canicruria* by the resistant *N. erythropolis* (Table 3), where 5 recombinants among 27 of the same class type contained the *C* locus or crossed to *N. erythropolis*. Why the *C* locus should appear to be less closely linked to *PurB2* when the unselective resistance markers are on the *N. erythropolis* genome is not understood. However, a similar pattern of behavior has been observed for all unselected markers on the *N. erythropolis* genome (4, 5). There is considerable lateral displacement of genes on the *N. erythropolis* genome with genes expressing similar resistance phenotypes of *N. canicruria* (Fig. 1). Based on independent analysis, most unselected characters on the *N. canicruria* genome appear

more closely linked to the *his-3* + locus than are similar markers to the *his-3* allele in *N. erythropolis*.

Several recombinants were demonstrated to be capable of further segregation following prototrophic selection, and the segregation involved the *C* and *c* alleles (Table 6); heterozygosity between the *PurB2*/*PurB2* + and *C/c* alleles may be involved.

Selection that allowed for the recovery of *purB2* recombinants provided evidence for the close linkage of the *PurB2* and *C* loci (Table 7). All of these recombinants crossed with the *N. erythropolis* parental type when selected on both MM and on adenine-supplemented IMM. As a result, the adenine requirement of the *F*₁ recombinant was due to the *PurB2*, from the *N. canicruria* parent, and not to *PurA1*. Of 18 auxotrophic recombinants, one recombined with the *N. canicruria* parent, but only when a selective medium containing adenine was employed. Since a crossover is required to the right of the *his-3* + to obtain the *E* locus, a minority of the recombinants would be expected to possess fertility with *N. canicruria*. Crossovers involving this region occur in about 5% of the prototrophic recombinants (4, 5), whereas crossovers in the same unselected regions from the auxotrophic selection occur at about 18% based on the segregation of the *E* locus in the proposed region (Table 7).

The genetic characters utilized for recombinant selection in test crosses were not of equal advantage for analysis. The inhibitor markers are more closely linked to the auxotrophic characters in *N. erythropolis* (*his-3*, *purA1*) than to the *purB2* in *N. canicruria*. As a result, when prototrophic sensitive recombinants are test-crossed to auxotrophic resistant parental organisms and selection is made for prototrophic resistant *F*₂ recombinants, the crossover regions potentially involved include 80% of the mapped linkage group in *N. canicruria* (Fig. 1). In *N. erythropolis* the selective crossover regions between *his-3* and a given resistance locus vary from 4 to 30%. Thus, the recombination frequency in a fertile test cross with *N. erythropolis* is lower than with a fertile *N. canicruria*. The decreased recombination frequency is still well above the anticipated reversion frequencies of the selective characters.

The ability of a few *F*₁ recombinants to segregate further after growth was demonstrated (Table 6). Heterozygosity may be responsible for the phenotypic instability. The markers involved (alleles *C/c*, *tetA9* +/*tetA9*, *camA1* +/*camA1*, and *strB2* +/*strB2*) are all unselective (Table 6, a and b). Dominance of the *C*, *tetA9* +, and

camA1 + alleles could explain their behavior. Since the streptomycin marker segregates from Str-R to Str-S, phenotypic lag of the *strB2* + locus resulting from its transfer from *N. erythropolis* to the resistant cytoplasm of *N. canicruria* may occur. It has been postulated that the *N. erythropolis* genome is transferred to the *N. canicruria* recipient (2).

In light of the linkage maps, it seems unlikely that heterozygosity is limited to those characters that demonstrated further segregation. It is possible that a complete diploid for the mapped area exists, thereby allowing for growth on MM. Undoubtedly, segregation of these heterozygotes, although relatively few in number, has been forced by replication to various IMM plates, and may have produced some erroneous information as to the segregation of unselected characters. They may, however, provide a valuable method for future analysis.

Another consideration is the possible existence of multiple mating rounds between specific recombinants. For example, the cross of the resistant *N. canicruria* by the sensitive *N. erythropolis* produced *strB2* + recombinants which were compatible with the *strB2* *N. canicruria* parent. An additional mating would lead to a reduced number of recoverable *strB2* + recombinants and thus reduce the accuracy of the linkage maps.

The data of the present report combined with other efforts would suggest that the mating factors controlling nocardial recombination are dissimilar from those of *Escherichia coli* or *Streptomyces*. Treatment of parental and recombinant mating types with acriflavine has failed to affect the compatibility of the tested strains or to alter the frequency at which they produce recombinants under standard mating methods. The formation of a heterogenomic zygote, controlled by the existence of complementary mating loci *C* and *E*, may persist for several generations in an unselective environment. Subsequent replication of incompletely segregated organisms for phenotypic classification, as well as multiple mating by specific recombinant class types, could well account for previously described chromosome inhomologies.

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