MITOCHONDRIAL GENETICS
VII. ALLELISM AND MAPPING STUDIES OF RIBOSOMAL MUTANTS RESISTANT TO CHLORAMPHENICOL, ERYTHROMYCIN AND SPIRAMYCIN IN *S. CEREVISIAE*

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ABSTRACT

We have isolated 15 spontaneous mutants resistant to one or several antibiotics like chloramphenicol, erythromycin and spiramycin. We have shown by several criteria that all of them result from mutations localized in the mitochondrial DNA. The mutations have been mapped by allelism tests and by two- and three-factor crosses involving various configurations of resistant and sensitive alleles associated in *cis* or in *trans* with the mitochondrial locus ω which governs the polarity of genetic recombination. A general mapping procedure based on results of heterosexual ($\omega^+ \times \omega^-$) crosses and applicable to mutations localized in the polar segment is described and shown to be more resolving than that based on results of homosexual crosses. Mutations fall into three loci which are all linked and map in the following order: ω–R_I–R_{II}–R_{III}. The first locus is very tightly linked with ω while the second is less linked to the first. Mutations of similar resistance phenotype can belong to different loci and different phenotypes to the same locus. Mutations confer antibiotic resistance on isolated mitochondrial ribosomes and delineate a ribosomal segment of the mitochondrial DNA. Homo- and hetero-sexual crosses between mutants of the ribosomal segment and those belonging to the genetically unlinked ATPase locus, O_I, have been performed in various allele configurations. The polarity of recombination between R_I, R_{II}, R_{III} and O_I decreases as a function of the distance of the R locus from the ω locus rather than as a function of the distance of the R locus from the O_I locus.

IN a preceding article of this series (BOLOTIN *et al.* 1971) we briefly reported some characteristics of mutants coded by the mit-DNA that conferred resistance either to erythromycin or to spiramycin or simultaneously to both macrolides. We also proposed a mapping procedure based on the polarity of recombination and presented an outline of the genetic map deduced from a study of a few antibiotic resistant mutants.

The purpose of this article is (a) to describe in detail the selection of a series of mutants conferring resistance to one or several macrolides; (b) to study their allelism systematically; (c) to perform a recombination analysis between genetic loci delineated by allelism tests; (d) to analyze the mapping relationship with

respect to chloramphenicol resistant mutants; (e) to analyze the mapping relationship of the linkage group uncovered in this manner to another mitochondrial locus specifying ATPase functions. A biochemical study, carried on in parallel, has shown that mutants belonging to the linkage group described here possess altered mitochondrial ribosomes and raised the possibility that they can be determined by modifications of ribosomal RNA (GRIVELL *et al.* 1973).

MATERIALS AND METHODS

TECHNICAL PROCEDURES

All procedures employed (standard cross, replica plating and aliquot plating) have been amply described elsewhere (COEN *et al.* 1970; AVNER *et al.* 1973).

MEDIA

For the composition of most media see COEN *et al.* (1970) and AVNER *et al.* (1973).

The three following media were not previously described.

Medium	Bactopeptone	Bacto-agar	Yeast extract	Glycerol	Ethanol	Buffer 0.05 M	Spira-mycin*	Chloram-phenicol
N3S5	1%	2.3%	1%	2%	–	pH 6.25 (Sörensen)	0.5%	–
N1C4S8	1%	2.3%	1%	–	2%	pH 6.25 (Sörensen)	0.8%	0.4%
N41S8	1%	2.3%	1%	–	2%	pH 5.3 (phosphate/ citrate McIlvaine)	0.8%	–

* Spiramycine Base, Rhône-Poulenc.

The N3S5 medium was used only in early experiments, being replaced in the later work by a more selective medium N41S8 which differs from the former by its pH, carbon source and drug concentration.

According to their level of resistance, the mutants studied in this paper show, when replicated on N41S8, a variety of growth patterns ranging from rapid and regular growth of the whole replica (e.g., S^R_{352}, E^R_{514}, E^R_{354}) to little and limited growth of the replica as a whole, accompanied by the emergence after a few days of numerous papillae (e.g., S^R_{551}). The presence of papillae results in no ambiguity in distinguishing between resistant and sensitive colonies since the latter shows no growth. A full description of the parameters of resistance at the cellular level is given in SLONIMSKI *et al.* (1974), and at the mitochondrial and ribosomal level in GRIVELL *et al.* (1973).

STRAINS

Two parental strains were used in the selection of the various mutants 55R5-3C and DP1-1B (see COEN *et al.* 1970).

All genetic studies in the present work were performed using the meiotic progeny of the diploids listed in Table 1. Some of these diploids (like IL233, IL240, IL249, IL262, etc.) were constructed by crossing the original mutants and isolating from selective medium (after ca. 20 mitotic generations) single colonies showing the desired phenotype. Other diploids (like IL287, IL288, IL292, etc.) were obtained from crosses involving spores of previous diploids (e.g., IL287 is obtained from a spore of IL262, see Table 1). Finally the construction of some of the diploids is described in other publications (IL16, IL17, IL102, etc., see COEN *et al.* 1974); IL778, etc., see AVNER *et al.* 1973).

The haploid strains obtained by sporulation of a diploid strain are denoted by adding to the name of the diploid a number (corresponding to the tetrad) and a letter A, B, C, D (corresponding to the spore).

TABLE 1

List of diploid strains

Name of the diploid	Isolated in the cross a × α			Phenotype	Mitochondrial genotype
IL16	55R5–3C/321 $\omega^-C^R_{321}E^S$	×	DP1–1B/514 $\omega^+C^SE^R_{514}$	C^RE^S	$\omega^-C^R_{321}E^S$
IL17	55R5–3C/321 $\omega^-C^R_{321}E^S$	×	DP1–1B/514 $\omega^+C^SE^R_{514}$	C^SE^R	$\omega^+C^SE^R_{514}$
IL102	55R5–3C/221 $\omega^-C^SE^R_{221}$	×	650–2C/36 $\omega^+C^R_{36}E^S$	C^SE^R	$\omega^-C^SE^R_{221}$
IL166	IL103–4D $\omega^+C^R_{36}E^S$	×	650–2C/36 $\omega^+C^R_{36}E^S$	C^SE^S	$\omega^+C^SE^S$
IL187	IL46–11C $\omega^+C^SE^S$	×	IL8–8C $\omega^+C^R_{321}E^R_{514}$	C^RE^S	$\omega^+C^R_{321}E^S$
IL216	IL126–1C $\omega^-C^R_{321}E^R_{221}$	×	DP1–1B $\omega^+C^SE^S$	C^SE^R	$\omega^+C^SE^R_{221}$
IL233	55R5–3C/353 $\omega^-S^SE^R_{353}$	×	DP1–1B/551 $\omega^+S^R_{551}E^S$	S^RE^R	$\omega^+S^R_{551}E^R_{353}$
IL240	55R5–3C/352 $\omega^-S^R_{352}E^S$	×	DP1–1B/553 $\omega^+S^SE^R_{553}$	S^RE^R	$\omega^+S^R_{352}E^R_{553}$
IL249	55R5–3C/354 $\omega^-C^SE^R_{354}$	×	DP1–1B/517 $\omega^+C^R_{517}E^S$	C^SE^R	$\omega^-C^SE^R_{354}$
IL251	55R5–3C/354 $\omega^-C^SE^R_{354}$	×	DP1–1B/517 $\omega^+C^R_{517}E^S$	C^RE^R	$\omega C^R_{517}E^R_{354}$
IL262	55R5–3C/321 $\omega^-C^R_{321}S^S$	×	DP1–1B/551 $\omega^+C^SS^R_{551}$	C^RS^R	$\omega^+C^R_{321}S^R_{551}$
IL287	55R5–3C/353 $\omega^-C^SS^SE^R_{353}$	×	IL262–2D $\omega^+C^R_{321}S^R_{551}E^S$	$C^RS^RE^R$	$\omega^+C^R_{321}S^R_{551}E^R_{353}$
IL288	55R5–3C/321 $\omega^-C^R_{321}S^SE^S$	×	IL233–3B $\omega^+C^SS^R_{551}E^R_{353}$	$C^SS^SE^R$	$\omega^+C^SS^SE^R_{353}$
IL290	IL166–4B $\omega^+C^SE^S$	×	IL251–6D $\omega^+C^R_{517}E^R_{354}$	C^SE^R	$\omega^+C^SE^R_{354}$
IL292	55R5–3C/324 $\omega^-C^R_{324}E^SS^S$	×	IL240–6D $\omega^+C^SE^R_{553}S^R_{352}$	$C^RE^RS^S$	$\omega C^R_{324}E^R_{553}S^S$
IL371	55R5–3C/321 $\omega^-C^R_{321}E^S$	×	IL8–8C/E41 $\rho^-\omega^0C^0E^R_{514}$	C^RE^R	$\omega^-C^R_{321}E^R_{514}$
IL442	55R5–3C/336/2 $\omega^-C^R_{336}E^R_{236}$	×	DP1–1B $\omega^+C^SE^S$	C^SE^R	$\omega^+C^SE^R_{236}$
IL778	55R5–3C/1 $\omega^-C^SO^R_1$	×	IL16–10B $\omega^-C^R_{321}O^S$	C^RO^R	$\omega^-C^R_{321}O^R_1$
IL781	55R5–3C/1 $\omega^-C^SO^R_1$	×	IL187–7A $\omega^+C^R_{321}O^S$	C^RO^R	$\omega^+C^R_{321}O^R_1$
IL841	55R5–3C/1 $\omega^-E^SO^R_1$	×	IL249–4D $\omega^-E^R_{354}O^S$	E^RO^R	$\omega^-E^R_{354}O^R_1$
IL842	IL836–2A $\omega^+E^SO^R_1$	×	IL290–3C $\omega^+E^R_{354}O^S$	E^RO^R	$\omega^+E^R_{354}O^R_1$
PS183	55R5–3C/321 $\omega^-C^R_{321}E^S$	×	IL8–8C/E41 $\rho^-C^0E^R_{514}$	C^RE^R	$\omega^-C^R_{321}E^R_{514}$

RESULTS

SELECTION AND CHARACTERIZATION OF MUTANTS

The macrolide group of antibiotics, particularly erythromycin and spiramycin are inhibitors of protein synthesis. In bacteria the acquisition of a high level of resistance to one of these drugs is usually accompanied by a cross resistance to the other.

In yeast it is known that spiramycin and erythromycin inhibit growth on non-fermentable substrates. We therefore set out to obtain mutants resistant to the action of these drugs on media containing glycerol or ethanol as sole carbon source.

A simple technique allows for the selection of mutants resistant to either one or both drugs according to the desired phenotype.

Selection procedure

All mutants were selected spontaneously. They were derived either from strains 55R5-3C or strain DP1-1B (COEN *et al.* 1970). A single colony of one of these strains grown on non selective medium is picked up and spread out entirely (*ca.* 10^8 cells) over the surface of a single Petri dish containing the selected antibiotic (see Figure 1). After about ten days a small number of colonies appear on the plate. These colonies are replicated on a medium containing the other antibiotic. This process allows one to distinguish three phenotypes : S^RE^S, S^SE^R and S^RE^R the latter being obtainable by a primary selection for either E^R or S^R. The distribution of mutant phenotypes according to the mother strain and to the primary selective medium used has not been systematically surveyed. Nevertheless, the data though preliminary, do outline some trends in the appearance of mutants.

When the primary selection is performed on N3E5 the mutants obtained from either 55R5–3C or DP1–1B fall mainly into the E^RS^R class. Such E^RS^S clones as 55R5–3C/353 or DP1–1B/553 are rare exceptions.

A different situation arises if the primary selection is performed on N3S5. In this, the two strains do not behave identically. All clones of 55R5–3C show a rather large number of S^RE^S mutants (like $S^R{}_{352}$) and only rare S^RE^R mutants. As for DP1–1B, two types of clones exist—one in which there is a majority of S^RE^S mutants and another in which this type of mutant is very rare. There is no indication as to whether the difference between the behavior of the two mother strains is due to difference in their mitochondrial or nuclear genomes.

Finally, only a single mutant is derived from a given sensitive clone. This restriction is of particular importance in order to be certain that the mutants of the same resistance phenotype derive from independent spontaneous mutagenic events.

Characterization procedure

Each mutant is then purified by subcloning on non-selective medium (N3). On this medium a single colony is picked up and tested for the retention of the phenotype of the original mutant colony. If the results are positive, the usual

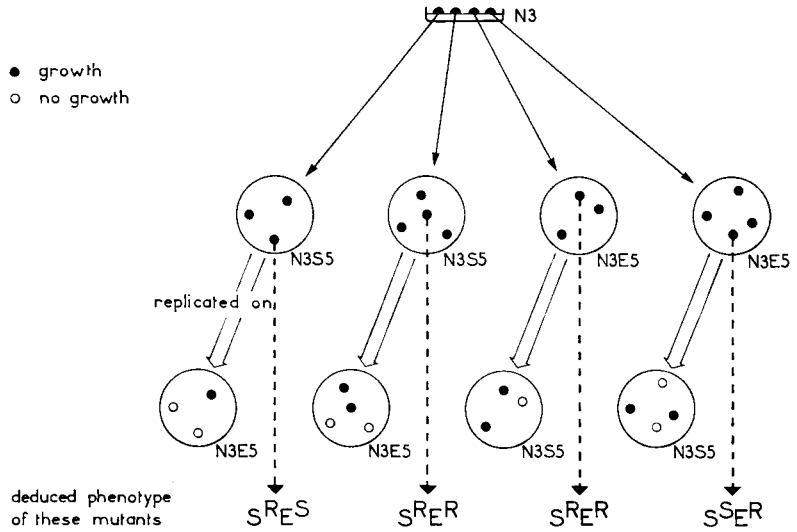


FIGURE 1.—Schematic representation of the isolation of *independently arising spontaneous* mutants resistant to Erythromycin and/or to Spiramycin (for details see text).

critera for testing mitochondrial inheritance patterns are used (COEN *et al.*, 1970; BOLOTIN *et al.* 1971; DEUTSCH *et al.* 1974): absence of meiotic segregation, existence of mitotic segregation, inclusion in the ρ^+ factor. The results are shown in Table 2.

All these mutants were obtained spontaneously by a single selective step. This leads us to believe that they result from a single mutational event. Nevertheless,

TABLE 2

Isolation and characterization of mutants resistant to erythromycin and/or spiramycin

Strain number	Phenotype	Mitotic segregation of Ant^R *vs.* Ant^S among diploids from the cross			Meiotic segregation in tetrads Ant^R *vs.* Ant^S
		ρ^+ Ant^R with ρ^+ Ant^S tester	ρ^+ Ant^R with ρ^- from Ant^S tester	ρ^- from Ant^R with ρ^+ Ant^S tester	
DP1-1B	E^SS^S	NO (all Ant^S)	NO (all Ant^S)	NO (all Ant^S)	0:4
DP1-1B/514	E^RS^R	YES	NO (all Ant^R)	NO (all Ant^S)	4:0
DP1-1B/551	E^SS^R	YES	NO (all Ant^R)	NO (all Ant^S)	4:0
DP1-1B/553	E^RS^S	YES	NO (all Ant^R)	NO (all Ant^S)	4:0
55R5-3C	E^SS^S	NO (all Ant^S)	NO (all Ant^S)	NO (all Ant^S)	0:4
55R5-3C/221	E^RS^R	YES	NO (all Ant^R)	NO (all Ant^S)	4:0
55R5-3C/352	E^SS^R	YES	NOT TESTED	NO (all Ant^S)	4:0
55R5-3C/353	E^RS^S	YES	NO (all Ant^R)	NO (all Ant^S)	4:0
55R5-3C/354	E^RS^R	YES	NO (all Ant^R)	NO (all Ant^S)	4:0
55R5-3C/336/236	E^RS^R	YES	NOT TESTED	NOT TESTED	4:0

The second column indicates the phenotype for which a mutant was selected for (see Figure 1). Diploids from the crosses indicated in the table were tested for mitotic segregation by replica plating onto N3, N3E and N41S8 media. All other procedures used including those for tetrad analysis are outlined in COEN *et al.* (1970).

the occurrence of two types of mutants expressing resistance to a single macrolide i.e., S^RE^S and S^SE^R raised the questions about the determinism of mutants exhibiting resistance to both macrolides i.e., S^RE^R.

One could suppose that these S^RE^R mutants were obtained in two mutational steps, at two different loci, the first one conferring only resistance to spiramycin, the second to erythromycin.

In order to test this possibility, we have crossed the S^RE^R mutants with sensitive strains and searched for the mitotic disjunction of S^R and E^R (i.e., the progeny of crosses was replicated on N3E5 and N4IS8 media). In the case of E^R_{221}, E^R_{354} and E^R_{514}, neither S^RE^S nor S^SE^R colonies were found amongst respectively 287. 186 and 354 colonies scored. This clearly shows that the double phenotype S^RE^R is conferred by a *single* pleiotropic mutation.

This fact does not rule out, of course, the possibility of constructing S^RE^R recombinants (e.g., IL233, IL240) by crosses between an S^RE^S strain and an S^SE^R strain but in this case, one can, very easily, dissociate the two phenotypes, as will be seen below (Tables 10 and 11).

Evidence for the Existence of Three Non-Allelic Loci—R_I, R_{II} and R_{III}

For the allelism test, we have adopted as a rule to search first for the wild-type i.e. antibiotic-sensitive recombinants. Obviously, in crosses between phenotypically identical mutants (for example $E^R \times E^R$), it is the only recombinant type that we can recognize. But even when the other recombinant type (e.g., S^RE^R, as in the $S^RE^S \times S^SE^R$ crosses) can be distinguished, we preferred to consider, first of all, the wild-type recombinants. In fact, it cannot be assumed, *a priori*, that the *cis association* of two resistance mutations gives rise to an unambiguous phenotype. One has to envisage the possibility of interactions between certain resistance-conferring mutations. An example of such interaction will be given. It has been observed previously that no ρ^+ C^RE^R recombinants could be found in crosses of C^R_{336} by E^R_{514} although ρ^+ C^SE^S recombinants were frequently observed (COEN *et al.* 1970). Subsequent studies have shown that the absence of cells expressing the double resistant phenotype was due to the fact that the C^R_{336} E^R_{514} and C^R_{336} E^R_{221} *recombination leads in a compulsory manner to the ρ^- mutation* which prevents, of course, the phenotypic expression of the resistance genotype. We have shown this in two ways: (a) a particular C^o E^R_{514} ρ^- mutant, IL8–8C/E41, transmits 83% of the E^R genetic marker to the diploid progeny (see FAYE *et al.* 1973) which contains in that case less than 5% of the ρ^- cells. This is true only when the ρ^+ E^S partner of the cross does not carry the C^R_{336} mutation. In the latter case the diploid progeny contains about 80% ρ^- cells and no single ρ^+ C^R E^R cell. This is precisely the result predicted if $C^R_{336}E^R_{514}$ recombinants give rise to ρ^- cells. (b) In a cross ρ^+ C^R_{336} $E^S \times \rho^+$ C^S E^R_{221} one isolates ρ^- diploid cells and by triploid crosses to a ρ^+ C^S E^S tester (technique described in details in MICHAELIS, PETROCHILO and SLONIMSKI 1973) one shows that many did contain simultaneously C^R_{336} and E^R_{221} genetic markers, while not a single ρ^+ cell could be found containing the two genetic markers together. It is probable that a combination of two modifications in the mitoribosome, none of which is individually deleterious, is so

harmful that mitochondrial protein synthesis is impaired and a ρ^- mutation ensues. This "mitochondrial lethality" is characteristic of some specific combinations of heteroalleles at the R_I and R_{III} loci, while other E^R mutations, like $E^R{}_{236}$, are compatible with $C^R{}_{336}$ (see Tables 2 and 14 of this paper and DUJON in preparation; SLONIMSKI *et al.* 1974). It should be stressed therefore that if no double-resistant recombinants are found, it may mean either that the single-resistant mutants are alleles, or that they are non-alleles, but the genetic recombinants between them are "mitochondrial lethals" or otherwise phenotypically not detectable. This is not the case for sensitive recombinants for which it has been shown that they are identical by all criteria employed to the reference wild-type strains (SLONIMSKI *et al.* 1974).

Two facts must be kept in mind when assessing the limits of the analysis based on the search for sensitive recombinants. The phenotype of the recombinants does not allow the use of a positive selection method. Sensitive colonies amongst a background of resistant ones must be identifed by replica plating; this is rarely performed on more than a thousand colonies. The absence of sensitive colonies in a total of n colonies tested can only be taken to mean that their frequency is less than $\frac{3.7^1}{n}$. In our case, then, a negative result means that the frequency of recombinants is less than 0.4%.

The selection of recombinants is also limited by several features of the mitochondrial genetic system which depends on the mitochondrial ω determinant (see BOLOTIN *et al.* 1971; COEN *et al.* 1974). One of the characteristics of heterosexual crosses (crosses between ω^- and ω^+ strains) is the existence of a polarity of recombination. If the following cross is performed: $\omega^+A^+B^+ \times \omega^-A^-B^-$ (where A and B stand for two loci), very unequal frequencies are obtained for the A^+B^- and A^-B^+ reciprocal recombinants. In such crosses one of the recombinants is always more numerous and therefore easier to detect. In homosexual crosses the reciprocal recombinants are equally represented but their frequency is less than that of the major recombinant in the corresponding heterosexual cross. Keeping these facts in mind, Figure 2 shows that the best possible configuration for detecting sensitive recombinants, in crosses of two resistant mutants of similar phenotype, is that of cross number 2 where such recombinants constitute the major recombinant class. In the homosexual crosses 3 and 4 they may also be detected but the conditions are less favorable.

Ideally, one would like to perform both types of heterosexual crosses as in the case of mutations $E^R{}_{514}$ and $E^R{}_{221}$ (cf., Table 3). Unfortunately, this is not always possible. A drug-resistant mutation isolated in a ω^- strain can be readily transferred to an ω^+ genome (as shown in COEN *et al.* 1974); the reverse however is not easy. To obtain a ω^- Drug^R strain from a ω^+ Drug^R one, one has first to dissociate the Drug^R mutation from the ω^+ locus. This can be done by obtaining a ρ^- ω^0 Drug^R petite, and thereafter by crossing this petite to a ρ^+ ω^- Drug^S grande, in order to isolate ρ^+ Drug^R diploids. After sporulation, genetic analysis of haploids shows that the mitochondrial genotype of such a diploid is ω^- Drug^R. This experi-

[1] 3.7 is the 95% confidence limit of the term 0 of a Poisson distribution.

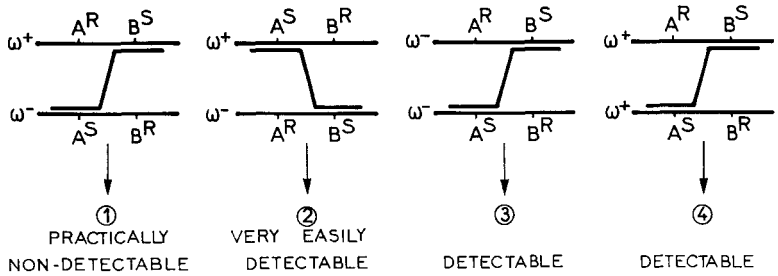


FIGURE 2.—Schematic representation of the facility of detection of antibiotic sensitive recombinants between two, A^R and B^R, antibiotic resistant non-allelic mutants as a function of the ω^+ *vs.* ω^- configuration (for details, see text).

ment was performed only with the E^R_{514} marker (BOLOTIN-FUKUHARA *et al.* 1974). Therefore most mutations isolated in ω^+ strains exist only in ω^+ genome with the exception of E^R_{514}.

It is apparent that the absence of sensitive cells in a cross of two resistant mutants may result from two distinct causes: either the mutants are alleles or the mutants are non-alleles, but the cross corresponds to the genetic configuration 1 of Figure 2. To solve this ambiguity we have proceeded as follows. A number of reference strains were constructed, every mutation being associated with ω^+ and ω^- ($\omega^+E^R_{221}$ = IL216, $\omega^-E^R_{221}$ = IL102; $\omega^+E^R_{514}$ = IL17, $\omega^-E^R_{514}$ = IL371; $\omega^+E^R_{354}$ = IL290; $\omega^-E^R_{354}$ = IL249; $\omega^+C^R_{321}$ = IL187; $\omega^-C^R_{321}$ = IL16, etc.). A new mutant is crossed by a ω^+ and by a ω^- reference strain carrying tester markers. Out of these two crosses *at least one* corresponds to the configuration 2,

TABLE 3

Allelism tests between erythromycin- and/or spiramycin-resistant mutants

Cross	$\omega^+E^R_{221} \times \omega^-E^R_{514}$				
			(E^R)	(E^S)	Colonies
IL216-1D × PS183-5B			304	0	304
IL216-1C × IL371-1D			380	0	380
IL216-1C × IL371-1D			139	0	139
Total			823	0	823
Cross	$\omega^+E^R_{514} \times \omega^-E^R_{221}$				
			(E^R)	(E^S)	Colonies
IL17-1D × IL102-9D			738	0	738
IL17-1D × IL102-9D			361	0	361
Total			1099	0	1099
Cross	$\omega^+E^R_{353}S^S \times \omega^-E^SS^R_{352}$				
	(E^RS^S)	(E^SS^R)	(E^RS^R)	(E^SS^S)	Colonies
IL288-1D × 55R5-3C/352	287	70	0	0	357
IL288-5C × 55R5-3C/352	160	28	0	0	188
Total	447	98	0	0	545

Standard cross conditions. Quantitative analysis by replica plating of random diploid cells onto N3, N3E and N41S8 media according to the markers present.

3 or 4 of Figure 2. *If neither of the two test crosses yields sensitive recombinants, the mutant is considered to be allelic to the tester.*

The situation is more favorable when the two mutants to be crossed have different phenotypes since both recombinant types can be detected barring possible phenotypic interactions in the double resistant recombinant (see above). For example, in the case of mutation S^R_{352} and E^R_{353} (Table 3), if these two mutations were not allelic, one of the recombinant types S^RE^R or S^SE^S ought to be detectable in a heterosexual cross between these mutants, because, regardless of the order of markers, one of the recombinant types has to be the major one.

Allelism tests between chloramphenicol-resistant mutants

In a previous publication (COEN *et al.* 1970) mitochondrial mutations conferring resistance to chloramphenicol, another bacterial protein synthesis inhibitor, were described. In order to find out whether these mutations concerned one or more loci the crosses described in Table 4 were performed; it can be seen that no sensitive recombinants were recovered irrespective of the configuration of the cross (homo- or heterosexual). Therefore mutations C^R_{517}, C^R_{321}, C^R_{324}, C^R_{325}, C^R_{336} belong to the same locus designated R_I. It has been shown previously that C^R_{36} is allelic to C^R_{321} and to C^R_{323} (COEN *et al.* 1970). The R_I locus contains therefore at least 7 independently isolated chloramphenicol resistant mutations.

Allelism tests between macrolide-resistant mutants

The fact that mutations E^R_{514} and E^R_{221} are allelic has already been mentioned (cf., Table 3). These two mutants which are E^RS^R have been used as partners in crosses with E^RS^S, E^SS^R or E^RS^R mutants, recombinants being characterized by the absence of growth on N3E5, N41S8 or both.

The results of the crosses performed are shown in Table 5. It can be seen that there are no E^SS^S recombinants when mutants S^R_{352}, E^R_{353} and E^R_{236} are crossed either to E^R_{221} (homosexual crosses) or to E^R_{514} (heterosexual crosses). This is in agreement with the finding that S^R_{352} and E^R_{353} when crossed to each other behave as alleles. We can therefore define a locus including mutations E^R_{221}, E^R_{236}, E^R_{514}, S^R_{352} and E^R_{353}. We have designated this locus R_{III}.

TABLE 4

Allelism tests between chloramphenicol-resistant mutants

	55R5–3C/321 $\omega^-C^R_{321}$	55R5–3C/324 $\omega^-C^R_{324}$	55R5–3C/325 $\omega^-C^R_{325}$	IL187–4C or 7A $\omega^+C^R_{321}$
DP1–1B/517	C^R = 1401 col.	C^R = 1374 col.	C^R = 1501 col.	C^R = 376 col.
$\omega^+C^R_{517}$	C^S = 0 col.	C^S = 0 col.	C^S = 0 col.	C^S = 0 col.
IL 16–5A	C^R = 775 col.	C^R = 342 col.	C^R = 616 col.	
$\omega^-C^R_{321}$	C^S = 0 col.	C^S = 0 col.	C^S = 0 col.	
55R5–3C/336				C^R = 1375 col.
$\omega^-C^R_{336}$				C^S = 0 col.

Conditions as in Table 3. Replicas onto N3 and N3C media.

TABLE 5

Allelism matrix of erythromycin- and spiramycin-resistant mutants

	E^R_{514}	S^R_{352}	E^R_{353}	E^R_{354}	S^R_{551}	E^R_{553}
E^R_{221}	—	—	—	+	not tested	not tested
	E^R_{514}	—	—	+	+	+
		S^R_{352}	—	+	+	+
			E^R_{353}	+	+	+
				E^R_{354}	—	—
					S^R_{551}	not tested

Summary of experiments performed as in Table 3.
(—): no recombinant E^S S^S colonies found in a sample of 1000 (or more) colonies replicated.
(+): presence of recombinant E^S S^S colonies.

Mutants E^R_{354}, S^R_{551} and E^R_{553} when crossed to mutants belonging to R_{III} always produce sensitive recombinants. Furthermore, when crossed to each other they do not give rise to sensitive cells. This means that they constitute another locus, we have called locus R_{II}.

Table 6 presents the results of a series of isonuclear crosses for which the values may be compared quantitatively as well as qualitatively. It can be seen that those mutants we have classified as alleles—i.e., S^R_{352} and E^R_{353} or S^R_{551} and E^R_{553} —behave in a very similar manner when crossed to the same partner.

In conclusion, mutations conferring resistance to the macrolide group of antibiotics can arise at two loci at least; one includes mutations E^R_{354}, E^R_{553} and S^R_{551}

TABLE 6

Frequency of wild-type $C^S E^S S^S$ *recombinants in isonuclear anisomitochondrial crosses*

		DP1-1B (α *hi tr*) ρ^+ ω^+ /514 E^R_{514}	/551 S^R_{551}	/553 E^R_{553}
55R5-3C (α *ur*) ρ^+ ω^-	/321 C^R_{321}	21.6% (1368/6332)*	1.7% (7/389)	5.8% (63/1078)
	/352 S^R_{352}	0% (0/587)	1.0% (15/1568)	1.1% (8/708)
	/353 E^R_{353}	0% (0/596)	1.0% (20/1988)	1.3% (9/697)
	/354 E^R_{354}	12.2% (126/1038)	0% (0/233)	0% (0/808)

Standard cross conditions. Quantitative analysis by replica plating of random diploid cells. The frequencies of wild type C^S E^S S^S diploids among total ρ^+ diploids are given and the number of colonies scored is given in parentheses.

* In a single cross ca. 200 diploid colonies are scored. The data give the sum of colonies scored in successive repeats of the cross. In this case there have been 25 repeats performed over a period of 3 years (see Table 5a, COEN *et al.* 1974).

(locus R_{II}); the other includes E^R_{221}, E^R_{514}, E^R_{236}, E^R_{353} and S^R_{352} (locus R_{III}). R_{II} and R_{III} can both be shown to be distinct from R_I by the occurrence of recombinants in crosses to mutants of that locus.

Topology of loci R_I, R_{II}, R_{III} *in relationship to each other and to the* ω *locus*

Two-factor heterosexual crosses

The demonstration of the existence of three loci gives no indication as to their location on the mitochondrial genome. To establish topological relationships among them, the influence of the mitochondrial determinant ω on recombination must be taken into account. It has already been stated that in heterosexual crosses involving two mutations at different loci, one of the recombinant classes is much more frequent than the other. One cannot explain this result by simply considering the distance between the markers since the reciprocal recombinants should be present in equal numbers, as indeed they are in homosexual crosses.

In heterosexual configurations, recombination depends not only on the distance *A–B* but also on the topology of *A* and *B* in respect to ω. As shown elsewhere (BOLOTIN *et al.* 1971; COEN *et al.* 1974), all recombinants are ω^+ in such crosses. To be present in a recombinant genome a resistance marker introduced in the cross by the ω^- parent must be separated not only from the other parental resistance marker—this event being dependant on the distance between the two resistance markers—but also from the ω^- allele; the latter event will happen all the more often as the distance between the resistance marker and the ω^- allele increases. A heterosexual cross involving two resistance markers *A* and *B* behaves in fact like a three factor cross involving loci ω, *A* and *B*, with a strong selection operating in favor of the ω^+ allele. These characteristics confer an orientation to the mitochondrial genome with respect to the ω locus. The frequency of recombinants in heterosexual crosses is therefore a function of the position of the alleles considered with respect to ω^+ and a function of the distance between the loci. Let us consider a cross of ω^+ $A^+B^+ \times \omega^-$ A^-B^-. *Both recombinants* A^+B^- and A^-B^+ will be ω^+. We would like to distinguish between orders ω–*A*–*B* and ω–*B*–*A*. If *A* is closer to ω than *B* the recombinant ω^+A^- will be less frequent than the recombinant ω^+B^-. Therefore

$$\frac{A^+ B^-}{A^- B^+} >> 1$$

Now, using the convention introduced in BOLOTIN *et al.* (1971), ω is placed to the left and the other markers are located with respect to ω by applying the following rule:

$$\frac{L^+ R^-}{L^- R^+} >> 1,$$

In this formula, *L* stands for the marker on the left and *R* for the marker on the right.

The methodology, outlined above, can be used to locate the three loci R_I, R_{II}, R_{III}, and the results will be given in the following paragraphs. Amongst all the

theoretically possible combinations of mutants, only a subset was studied. This is due to the fact that one must be able to detect phenotypically both recombinant types in order to apply the method. This precludes the two factor crosses between mutants of identical or similar phenotypes, since only one recombinant, the sensitive one, would be detectable in these crosses.

Crosses of mutants at the locus R_I *by the mutants at the locus* R_{II}

Table 7 gives the detailed results of crosses between E^R_{354} on the one hand and C^R_{321}, C^R_{324} and C^R_{517} on the other. Signs $R_I{}^+$ and $R_I{}^-$ stand for the alleles of locus R_I brought into cross by the ω^+ and ω^- genome respectively. This nomenclature holds no implication as to what type of resistance is involved (C, S or E), nor does it specify whether the allele referred to is the resistant or the sensitive one. The same nomenclature will be used for the other two loci.

The first remark one can make is that the homogeneity of the results of similar crosses is satisfactory. For instance, the eight crosses in the upper part of Table 7 give very similar results for both parental ($R_I{}^+ R_{II}{}^+$; $R_I{}^- R_{II}{}^-$) and recombinant ($R_I{}^- R_{II}{}^+$; $R_I{}^+ R_{II}{}^-$) categories, although the nuclear genotypes of the diploids vary from one cross to another.

Furthermore, there does not seem to be any selective advantage of one or the other cell types, resistant or sensitive, since it can be seen by comparing the two parts of Table 7 that each of the four genotypic classes $R_I{}^+ R_{II}{}^+$, $R_I{}^- R_{II}{}^-$, $R_I{}^+ R_{II}{}^-$ and $R_I{}^- R_{II}{}^+$ is present at comparable frequencies from one set of crosses to the other, independent of the actual phenotype—e.g., $R_I{}^+ R_{II}{}^+$ is $C^S E^R$ in the upper set of crosses and $C^R E^S$ in the lower one, etc.

Once the risk of distortion due to selective pressures has been eliminated, one can compare the frequencies of the recombinants: it appears that the recombinant carrying the plus allele at locus R_I and the minus allele at the site of E_{354} is always the more numerous; on the average

$$\frac{R_I{}^+ E^-_{354}}{R_I{}^- E^+_{354}} = \frac{280}{4} = 70 >> 1;$$

We therefore deduce that the order on the genome must be ω–R_I–E^R_{354}. The same analysis can be done on crosses of C^R_{321} with S^R_{551} (cf., Table 8) (notice that in one case, for IL262–2D, the resistant markers are in a *cis* configuration). Again little influence of nuclear genome or mitochondrial allele type is noticeable.

$$\frac{R_I{}^+ S^-_{551}}{R_I{}^- S^+_{551}} = \frac{22}{0} >> 1$$

This implies that the order is ω–R_I–S^R_{551}
Similarly it can be seen that (cf., Table 9)

$$\frac{R_I{}^+ E^-_{553}}{R_I{}^- E^+_{553}} = \frac{116}{1} >> 1$$

establishing that the order is ω–R_I–E^R_{553}.

The data obtained in these experiments do not conflict with those obtained

TABLE 7

Heterosexual two-point crosses: Respective order of ω, $C^{R}{}_{\substack{321\\324\\517}}$ *and* $E^{R}{}_{354}$

Cross	$\omega^{+}C^{S}E^{R}{}_{354} \times \omega^{-}C^{R}{}_{\substack{321\\324}}E^{S}$				
	$R^{+}{}_{I}R^{+}{}_{II}$ ($C^{S}E^{R}$)	$R^{-}{}_{I}R^{-}{}_{II}$ ($C^{R}E^{S}$)	$R^{+}{}_{I}R^{-}{}_{II}$ ($C^{S}E^{S}$)	$R^{-}{}_{I}R^{+}{}_{II}$ ($C^{R}E^{R}$)	Colonies
IL290-3C × 55R5-3C/321	203	16	5	0	224
IL290-5C × 55R5-3C/321	166	61	5	1	233
IL290-5B × IL16-10B	497	18	21	0	536
IL290-5C × IL778-3D	113	18	2	1	134
IL290-4B × IL778-2B	130	24	1	0	155
IL842-6A × 55R5-3C/321	84	20	2	0	106
IL842-6A × 55R5-3C/324	83	30	3	1	117
IL290-5C × 55R5-3C/324	97	47	4	1	149
Total	1373	234	43	4	1654
Average	83.0%	14.1%	2.6%	0.2%	

Cross	$\omega^{+}C^{R}{}_{\substack{321\\517}}E^{S} \times \omega^{-}C^{S}E^{R}{}_{354}$				
	$R^{+}{}_{I}R^{+}{}_{II}$ ($C^{R}E^{S}$)	$R^{-}{}_{I}R^{-}{}_{II}$ ($C^{S}E^{R}$)	$R^{+}{}_{I}R^{-}{}_{II}$ ($C^{R}E^{R}$)	$R^{-}{}_{I}R^{+}{}_{II}$ ($C^{S}E^{S}$)	Colonies
IL187-4C × IL249-4D	161	12	17	0	190
IL187-7A × IL249-2D	402	8	43	0	453
DP1-1B/517 × 55R5-3C/354	296	13	12	0	321
IL187-4B × IL841-2A	135	0	3	0	138
IL187-2D × IL841-3A	134	6	18	0	158
IL187-6D × IL841-1A	167	1	22	0	190
IL781-3A × IL249-7B	292	6	30	0	328
IL781-1A × IL249-8C	309	0	41	0	350
IL781-1A × IL249-4D	415	3	40	0	458
IL187-4C × IL841-2D	123	6	11	0	140
Total	2434	55	237	0	2726
Average	89.3%	2.0%	8.7%	0%	

Standard cross conditions. Quantitative analysis by replica plating of random diploid cells on to antibiotic containing media (N3, N3C, N3E, N3CE, N41S8, N1C4S8) according to the markers present in the cross. Each line corresponds to a different cross and the last column indicates the number of colonies replicated. The mitochondrial genotype of the two haploid parental strains is given in the headline in the same respective order as the names of the strains given in the first column. The symbols in brackets ($C^{S}E^{R}$, $C^{R}E^{S}$, etc.) represent the types of cells found amongst the diploid progeny and the corresponding numbers the frequency with which they are observed.

The symbols R_{I}, R_{II} and R_{III} designate the three ribosomal mitochondrial loci, and the superscript $^{+}$ or $^{-}$ designates that the allele at a given mitochondrial locus is derived from the ω^{+} or ω^{-} parent irrespective of whether they are resistant or sensitive.

TABLE 8

Heterosexual two-point crosses:
Experimental conditions and symbols as in Table 7.

Cross	$\omega^+C^SS^R_{551} \times \omega^-C^R_{321}S^S$				
	$R^+_IR^+_{II}$ (C^SS^R)	$R^-_IR^-_{II}$ (C^RS^S)	$R^+_IR^-_{II}$ (C^SS^S)	$R^-_IR^+_{II}$ (C^RS^R)	Colonies
IL233–3B × 55R5–3C/321	523	26	13	0	562
DP1–1B/551 × 55R5–3C/321	369	12	7	0	388
Total	892	38	20	0	950
Average	93.9%	4.0%	2.1%	0%	
Cross	$\omega^+C^R_{321}S^R_{551} \times \omega^-C^SS^S$				
	(C^RS^R)	(C^SS^S)	(C^RS^S)	(C^SS^R)	Colonies
IL262–2D × 55R5–3C/353	324	7	2	0	333
Total	97.3%	2.1%	0.6%	0%	

for the allelism tests. If the value for the major recombinant class is taken as a basis for an estimate of the genetic distance between locus R_I and locus R_{II} it is found to be 6.4%, 1.7% and 3.7% respectively for mutants E^R_{354}, S^R_{551}, E^R_{553} in combination with various locus R_I markers. These values are compatible with the order ω–R_I–R_{II} with the average value for the major recombinant class

TABLE 9

Heterosexual two-point crosses:
Respective order of ω, C^R_{321} *and* E^R_{553} *or* ^{324}
Experimental conditions and symbols as in Table 7.

Cross	$\omega^+C^SE^R_{553} \times \omega^-C^R_{\substack{321\\324}}E^S$				
	$R^+_IR^+_{II}$ (C^SE^R)	$R^-_IR^-_{II}$ (C^RE^S)	$R^+_IR^-_{II}$ (C^SE^S)	$R^-_IR^+_{II}$ (C^RE^R)	Colonies
DP1–1B/553 × 55R5–3C/321	435	18	16	0	469
DP1–1B/553 × 55R5–3C/321	208	6	10	0	224
DP1–1B/553 × 55R5–3C/321	303	45	37	0	385
DP1–1B/553 × 55R5–3C/324	655	17	22	1	695
IL240–6D × 55R5–3C/324	160	13	7	0	180
IL240–6D × 55R5–3C/324	244	17	3	0	264
IL240–8A × 55R5–3C/324	288	11	7	0	306
Total	2293	127	102	1	2523
Average	90.9%	5.0%	4.0%	0.04%	
Cross	$\omega^+C^R_{324}E^R_{553} \times \omega^-C^SE^S$				
	(C^RE^R)	(C^SE^S)	(C^RE^S)	(C^SE^R)	Colonies
IL292–6A × 55R5–3C/352	224	12	2	0	238
IL292–2B × 55R5–3C/352	315	34	12	0	361
Total	539	46	14	0	599
Average	90.0%	7.7%	2.3%	0%	

between these two loci being 4.8% as determined on 30 crosses and 8785 colonies. (Minor recombinant = 0.05%).

Crosses of mutants at the locus R_{II} *by the mutants at the locus* R_{III}:

In these crosses the following figures were obtained in *cis* or *trans* associations of resistance markers (Table 10).

$$\frac{E^{+}_{553}\, S^{-}_{352}}{E^{-}_{553}\, S^{+}_{352}} = \frac{716}{20} >> 1$$

In this case a fairly large fluctuation is observed for the major recombinant class frequency, but this does not blur the order of the markers which most probably is ω–E^{R}_{553}–S^{R}_{352}.

From Table 11 it can be shown that

$$\frac{S^{+}_{551}\, E^{-}_{353}}{S^{-}_{551}\, E^{+}_{353}} = \frac{280}{22} >> 1$$

The order ω–S^{R}_{351}–E^{R}_{353} is therefore probable.

These results point to the order ω–R_{II}–R_{III}. The average frequency is close to 18.9% for the major recombinant and to 0.80% for the minor recombinant as determined on 15 crosses and 5266 colonies.

Crosses of mutants at the locus R_I *by the mutants at the locus* R_{III}:

The crosses of Table 12 give the following results:

$$\frac{R_I^{+}\, S^{-}_{352}}{R_I^{-}\, S^{+}_{352}} = \frac{540}{7} >> 1$$

TABLE 10

Heterosexual two-point crosses:
Respective order of ω, E^{R}_{553} *and* S^{R}_{352}

Cross	$\omega^{+}E^{R}_{553}S^{S} \times \omega^{-}E^{S}S^{R}_{352}$ $R^{+}_{II}R^{+}_{III}$ ($E^{R}S^{S}$)	$R^{-}_{II}R^{-}_{III}$ ($E^{S}S^{R}$)	$R^{+}_{II}R^{-}_{III}$ ($E^{R}S^{R}$)	$R^{-}_{II}R^{+}_{III}$ ($E^{S}S^{S}$)	Colonies
Experimental conditions and symbols as in Table 7.					
DP1-1B/553 × 55R5-3C/352	257	5	20	2	284
DP1-1B/553 × 55R5-3C/352	308	36	74	6	424
DP1-1B/553 × 55R5-3C/352	300	42	80	5	427
IL292-6A × 55R5-3C/352	177	14	47	0	238
IL292-2B × 55R5-3C/352	192	42	123	4	361
Total	1234	139	344	17	1734
Average	71.2%	8.0%	19.8%	1.0%	
Cross	$\omega^{+}E^{R}_{553}S^{R}_{352} \times \omega^{-}E^{S}S^{S}$ ($E^{R}S^{R}$)	($E^{S}S^{S}$)	($E^{R}S^{S}$)	($E^{S}S^{R}$)	Colonies
IL240-6D × 55R5-3C/324	91	18	69	2	180
IL240-6D × 55R5-3C/324	154	20	90	0	264
IL240-8A × 55R5-3C/324	160	17	128	1	306
IL240-8A × 55R5-3C/1	289	17	85	0	391
Total	694	72	372	3	1141
Average	60.8%	6.3%	32.6%	0.3%	

TABLE 11

Heterosexual two-point crosses:
Respective order of ω, E^{R}_{353} *and* S^{R}_{551}

Experimental conditions and symbols as in Table 7.

Cross	$\omega^{+}E^{S}S^{R}_{551} \times \omega^{-}E^{R}_{353}S^{S}$				
	$R^{+}_{II}R^{+}_{III}$ ($E^{S}S^{R}$)	$R^{-}_{II}R^{-}_{III}$ ($E^{R}S^{S}$)	$R^{+}_{II}R^{-}_{III}$ ($E^{S}S^{S}$)	$R^{-}_{II}R^{+}_{III}$ ($E^{R}S^{R}$)	Colonies
DP1–1B/551 × 55R5–3C/353	337	25	1	48	411
DP1–1B/551 × 55R5–3C/353	576	26	16	95	713
DP1–1B/551 × 55R5–3C/353	212	3	2	29	246
DP1–1B/551 × 55R5–3C/353	324	6	1	24	355
DP1–1B/551 × 55R5–3C/353	210	16	0	37	263
Total	1659	76	20	233	1988
Average	83.5%	3.8%	1.0%	11.7%	
Cross	$\omega^{+}E^{R}_{353}S^{R}_{551} \times \omega^{-}E^{S}S^{S}$				
	($E^{R}S^{R}$)	($E^{S}S^{S}$)	($E^{R}S^{S}$)	($E^{S}S^{R}$)	Colonies
IL233–1C × 55R5–3C/1	301	53	2	47	403
Average	74.7%	13.1%	0.5%	11.7%	

TABLE 12

Heterosexual two-point crosses:
Respective order of ω, $C^{R}_{\substack{324\\517}}$ *and* S^{R}_{352}

Experimental conditions and symbols as in Table 7.

Cross	$\omega^{+}C^{R}_{\substack{324\\517}}S^{S} \times \omega^{-}C^{S}S^{R}_{352}$				
	$R^{+}_{I}R^{+}_{III}$ ($C^{R}S^{S}$)	$R^{-}_{I}R^{-}_{III}$ ($C^{S}S^{R}$)	$R^{+}_{I}R^{-}_{III}$ ($C^{R}S^{R}$)	$R^{-}_{I}R^{+}_{III}$ ($C^{S}S^{S}$)	Colonies
DP1–1B/517 × 55R5–3C/352	225	6	20	2	253
DP1–1B/517 × 55R5–3C/352	266	2	34	0	302
IL292–6A × 55R5–3C/352	177	12	49	0	238
IL292–2B × 55R5–3C/352	192	30	135	4	361
Total	800	50	238	6	1154
Average	74.5%	4.3%	20.6%	0.5%	
Cross	$\omega^{+}C^{S}S^{R}_{352} \times \omega^{-}C^{R}_{324}S^{S}$				
	($C^{S}S^{R}$)	($C^{R}S^{S}$)	($C^{S}S^{S}$)	($C^{R}S^{R}$)	Colonies
IL240–6D × 55R5–3C/324	93	13	74	0	180
IL240–6D × 55R5–3C/324	154	17	93	0	264
IL240–8A × 55R5–3C/324	160	10	135	1	306
Total	407	40	302	1	750
Average	54.3%	5.3%	40.3%	0.1%	

The order is ω–R_I–$S^R{}_{352}$

Similarly crosses of Tables 13 and 14 establish that

$$\frac{R_I{}^+ E^-{}_{353}}{R_I{}^- E^+{}_{353}} = \frac{201}{1} >> 1 \text{ and } \frac{R_I{}^+ E^-{}_{236}}{R_I{}^- E^+{}_{236}} = \frac{82}{4} >> 1$$

The orders are ω–R_I–$E^R{}_{353}$ and ω–R_I–$E^R{}_{236}$

If one considers the results obtained by COEN *et al.* (1974) (presented in Table 11) with other mutants at the R_{III} locus (especially $E^R{}_{221}$ and $E^R{}_{514}$) one obtains the following results:

$$\frac{R_I{}^+ R^-{}_{III}}{R_I{}^- R^+{}_{III}} = \frac{17057}{145} >> 1$$

The conclusion is therefore that the order is ω–R_I–R_{III}.

TABLE 13

Heterosexual two-point crosses: Respective order of ω, $C^R{}_{\substack{321\\517}}$ *and* $E^R{}_{353}$

Experimental conditions and symbols as in Table 7.

Cross	$\omega^+C^R{}_{\substack{321\\517}}E^S \times \omega^-C^SE^R{}_{353}$				
	$R^+{}_IR^+{}_{III}$ (C^RE^S)	$R^-{}_IR^-{}_{III}$ (C^SE^R)	$R^+{}_IR^-{}_{III}$ (C^RE^R)	$R^-{}_IR^+{}_{III}$ (C^SE^S)	Colonies
IL262–2D × 55R5–3C/353	316	7	10	0	333
DP1–1B/517 × 55R5–3C/353	144	3	12	1	160
DP1–1B/517 × 55R5–3C/353	266	4	28	0	298
DP1–1B/517 × 55R5–3C/353	360	4	40	0	404
Total	1086	18	90	1	1195
Average	90.9%	1.5%	7.5%	0.1%	
Cross	$\omega^+C^SE^R{}_{353} \times \omega^-C^R{}_{321}E^S$				
	(C^SE^R)	(C^RE^S)	(C^SE^S)	(C^RE^R)	Colonies
IL233–3B × 55R5–3C/321	425	26	111	0	562
Average	75.6%	4.6%	19.7%	0%	

TABLE 14

Heterosexual two-point crosses: Respective order of ω, $C^R{}_{321}$ *and* $E^R{}_{236}$

Experimental conditions and symbols as in Table 7.

Cross	$\omega^+C^SE^R{}_{236} \times \omega^-C^R{}_{321}E^S$				
	$R^+{}_IR^+{}_{III}$ (C^SE^R)	$R^-{}_IR^-{}_{III}$ (C^RE^S)	$R^+{}_IR^-{}_{III}$ (C^SE^S)	$R^-{}_IR^+{}_{III}$ (C^RE^R)	Colonies
IL442–4A × 55R5–3C/321	317	15	82	4	415
Average	76.4%	3.6%	19.7%	0.24%	

The average frequency is 25.0% for the major recombinant and 0.22% for the minor recombinant (71,574 colonies counted). The frequencies obtained with only the three mutants S^{R}_{352}, E^{R}_{353} and E^{R}_{236} were 20.2% for the major and 0.3% for the minor recombinant amongst 4076 colonies, results compatible with the overall frequency involving the five alleles at the R_{III} locus. Table 15 summarizes the data for two point crosses. All the results are consistent and lead to the unique order

$$\omega\text{–}R_I\text{–}R_{II}\text{–}R_{III}.$$

Two-Factor homosexual crosses.

Homosexual crosses $\omega^+ \times \omega^+$ and $\omega^- \times \omega^-$ do not exhibit a polarity of recombination and therefore do not yield any information on the situation of the markers with respect to ω. However these crosses do allow another estimate of the genetic distance between the markers by postulating that this distance is proportional to the frequency of the two recombinants which are approximately equal.

Homosexual crosses between E^{R}_{354} and the mutants of locus R_I (Table 16) call for a special comment: in both homosexual configurations $\omega^- \times \omega^-$ and $\omega^+ \times \omega^+$ there appears to be a deficit of $C^{S}E^{S}$ recombinants (0.2/3.3% or 0.3/1.3%). This deficit does not occur when the recombinants are $C^{R}E^{S}$ and $C^{S}E^{R}$, found with 2.1 and 1.5% frequencies (in crosses where the markers are in the *cis* configuration instead of the *trans* configuration). It is improbable that wild-type cells would be strongly selected against. The deficit could be due however to a very slow mitotic segregation in these crosses since in order to be scored as $C^{S}E^{S}$, a colony must be devoid of any $C^{R}E^{S}$ or $C^{S}E^{R}$ cells. Consequently, a late segregation could possibly lead to an overestimate of the $C^{R}E^{R}$ recombinant.

In order to minimize such effects, the frequency of recombinants in homosexual crosses shall be expressed as the sum of the frequencies of reciprocal recombinants.

TABLE 15

Summary of two-factor heterosexual crosses and deduced order in respect to ω

Data from:	ω	R_I	R_{II}	R_{III}
Table 7		$C^{R}_{321\ 324\ 517}$	E^{R}_{354}	
Table 8		C^{R}_{321}	S^{R}_{551}	
Table 9		$C^{R}_{321\ 324}$	E^{R}_{553}	
Table 10			E^{R}_{553}	S^{R}_{352}
Table 11			S^{R}_{551}	E^{R}_{353}
Table 12		$C^{R}_{324\ 517}$		S^{R}_{352}
Table 13		$C^{R}_{321\ 517}$		E^{R}_{353}
Table 14		C^{R}_{321}		E^{R}_{236}
COEN *et al.* (1974)		C^{R}_{321}		$E^{R}_{221\ 514}$
		and others		and others

TABLE 16

Homosexual two-point crosses involving R_I *and* R_{II} *loci*

Cross	$\omega^-C^R_{321}E^S$ (324, 325) × $\omega^-C^SE^R_{354}$				
	(C^RE^S)	(C^SE^R)	(C^SE^S)	(C^RE^R)	Colonies
55R5–3C/321 × IL249–4D	104	140	1	15	260
IL16–10B × IL249–2D	129	254	1	28	412
IL16–10B × 55R5–3C/354	175	187	2	7	371
IL778–3D × IL249–4D	70	104	0	6	180
IL778–1A × IL249–1A	79	151	1	6	237
IL778–2B × IL249–2A	115	134	1	9	259
55R5–3C/321 × IL249–1D	221	171	0	11	403
55R5–3C/324 × IL249–1D	279	136	0	10	425
55R5–3C/325 × IL249–1D	173	115	0	3	291
Total	1345	1392	6	95	2838
Average	47.5%	49.0%	0.2%	3.3%	
Cross	$\omega^+C^R_{321}E^S$ × $\omega^+C^SE^R_{354}$				
	(C^RE^S)	(C^SE^R)	(C^SE^S)	(C^RE^R)	Colonies
IL187–4C × IL290–3C	298	146	1	8	453
IL187–7A × IL290–5B	156	58	1	2	217
Total	454	204	2	10	770
Average	59.0%	26.6%	0.3%	1.3%	
Cross	$\omega^+C^R_{517}E^R_{354}$ × $\omega^+C^SE^S$				
	(C^RE^R)	(C^SE^S)	(C^SE^R)	(C^RE^S)	Colonies
IL251–6D × IL166–4B	314	98	5	7	424
IL251–7B × IL166–4B	158	72	5	7	242
Total	472	170	10	14	666
Average	70.9%	25.5%	1.5%	2.1%	
	Recombinants	137/4274 = 3.2%			

Experimental conditions and symbols as in Table 7.

This leads to a frequency of 3.2% between loci R_I and R_{II} as estimated on 4274 colonies in 13 crosses (Table 16).

The same operations can be done for homosexual crosses between loci R_{II} and R_{III} and lead to a frequency of 8.9% (Table 17).

The detailed results of homosexual crosses between locus R_I and locus R_{III} involving E^R_{221} or E^R_{514} for the latter locus are presented elsewhere (COEN *et al.*, 1974). In addition we now have the results for crosses involving E^R_{353}, S^R_{352} and E^R_{236} (Table 18). The recombination frequency between R_I and R_{III} stands at 9.0% in 5 crosses and 2188 colonies scored for these three mutants.

If one includes the data of COEN *et al.* (1974), (Table 11), the frequency of recombinants between R_I and R_{III} becomes 9.6% (63255 colonies scored).

In summary, the frequency of recombinants between R_I and R_{II} is 3.2%, that between R_{II} and R_{III} is 8.9%, while that between R_I and R_{III} is 9.6%. These

TABLE 17

Homosexual two-point crosses involving R_{II} *and* R_{III} *loci*

Cross	$\omega+E^{R}{}_{553}S^{R}{}_{352} \times \omega+E^{S}S^{S}$ (E^{R}S^{R})	(E^{S}S^{S})	(E^{R}S^{S})	(E^{S}S^{R})	Colonies
IL240–8A × IL166–4B	243	180	20	17	460
IL240–8A × IL166–4B	126	62	7	3	198
IL240–6D × IL166–7B	75	141	13	5	234
Total	444	383	40	25	892
Average	49.8%	42.9%	4.5%	2.8%	
Cross	$\omega+S^{R}{}_{551}E^{R}{}_{353} \times \omega+S^{S}E^{S}$ (S^{R}E^{R})	(S^{S}E^{S})	(S^{R}E^{S})	(S^{S}E^{R})	Colonies
IL287–7C × DP1–1B	176	256	22	20	474
IL287–10D × DP1–1B	175	228	28	26	457
Total	351	484	50	47	931
Average	37.7%	52.0%	5.4%	5.0%	
	Recombinants 162/1823 = 8.9%				

TABLE 18

Homosexual two-point crosses involving R_{I} *and* R_{III} *loci*

Cross	$\omega^{-}C^{R}{}_{321}E^{S} \times \omega^{-}C^{S}E^{R}{}_{353}$ (C^{R}E^{S})	(C^{S}E^{R})	(C^{S}E^{S})	(C^{R}E^{R})	Colonies
IL16–10B × 55R5–3C/353	233	201	16	44	494
Average	47.2%	40.7%	3.2%	8.9%	
Cross	$\omega+C^{R}{}_{321}E^{R}{}_{353} \times \omega+C^{S}E^{S}$ (C^{R}E^{R})	(C^{S}E^{S})	(C^{S}E^{R})	(C^{R}E^{S})	Colonies
IL287–7C × DP1–1B	175	257	21	21	474
IL287–10D × DP1–1B	175	228	26	28	457
Total	350	485	47	49	931
Average	37.6%	52.1%	5.0%	5.3%	
Cross	$\omega^{-}C^{R}{}_{321}S^{S} \times \omega^{-}C^{S}S^{R}{}_{352}$ (C^{R}S^{S})	(C^{S}S^{R})	(C^{S}S^{S})	(C^{R}S^{R})	Colonies
IL16–10B × 55R5–3C/352	268	178	4	14	464
Average	57.8%	38.4%	0.9%	3.0%	
Cross	$\omega+C^{R}{}_{321}E^{S} \times \omega+C^{S}E^{R}{}_{236}$ (C^{R}E^{S})	(C^{S}E^{R})	(C^{S}E^{S})	(C^{R}E^{R})	Colonies
IL187–4C × IL442–4A	131	144	8	16	299
Average	43.8%	48.2%	2.8%	5.3%	
	Recombinants 198/2188 = 9.0%				

results indicate that R_I and R_{II} are more closely linked than any other combination of loci and are therefore compatible with the order deduced from the heterosexual crosses—i.e., ω–R_I–R_{II}–R_{III}.

Three-factor heterosexual crosses

Although the results of all the two factor crosses are in good agreement, this type of cross suffers from the obvious disadvantage of not allowing the analysis of events at the three loci simultaneously. The great variety of nuclear and mitochondrial genomes used for the two factor crosses is a guarantee against possible experimental bias, nevertheless three factor crosses offer a necessary confirmation of the presumed order.

The derivation of the necessary double mutant strains (IL233; IL262; IL240) is described in Table 1. The results of the three point heterosexual crosses are summarized in Table 19.

The frequencies of genetically equivalent classes may vary considerably from one cross to another. Nevertheless in all cases the relative frequency, within any one cross, of the 8 cellular types follows the same rule:

$R^+_I R^+_{II} R^+_{III}$ greater than
$R^+_I R^+_{II} R^-_{III}$ greater than
$R^-_I R^-_{II} R^-_{III}$ greater than
$R^+_I R^-_{II} R^-_{III}$ greater than
$R^+_I R^-_{II} R^+_{III}$, $R^-_I R^+_{II} R^+_{III}$, $R^-_I R^+_{II} R^-_{III}$ and $R^-_I R^-_{II} R^+_{III}$.

Setting aside the parental classes, the *major recombinants* of each reciprocal class are $R^+_I R^+_{II} R^-_{III}$ (25.2%) followed by $R^+_I R^-_{II} R^-_{III}$ (1.9%) and $R^+_I R^-_{II} R^+_{III}$ (0.2%).

This result confirms the order R_I, R_{II}, R_{III}. The $R^+_I R^-_{II} R^+_{III}$ class represents the double recombinant, and its frequency is in agreement with the expected value which is the product of the frequency of the $R^+_I R^+_{II} R^-_{III}$ class (25.2%) by that of the $R^+_I R^-_{II} R^-_{III}$ *class* (1.9%).

The *minor recombinants* of each reciprocal class are present at very low frequencies. In certain configurations of the resistance markers (see Table 19) this low frequency can be determined by the aliquot plating on one type of selective media followed by replication on the other type. Amongst these minor recombinants the $R^-_I R^-_{II} R^+_{III}$ appears to be more frequent (0.088%) than the $R^-_I R^+_{II} R^-_{III}$ (0.049%), the $R^-_I R^+_{II} R^+_{III}$ being the least frequent one (0.0073%). Superficially these results may appear to be incompatible with the order R_I, R_{II}, R_{III}. As will be seen in a later section, results of this type reflect rather a general characteristic of recombinant frequencies in heterosexual crosses.

Three factor homosexual crosses

As expected from two point crosses, the frequency of recombinants between loci R_I and R_{II} is so low that an unambiguous order cannot be deduced from homosexual crosses. The data of Table 20 are nevertheless compatible with the order $R_I R_{II} R_{III}$.

TABLE 19

Heterosexual three-point crosses involving mutations at the R_I, R_{II} and R_{III} loci

Cross	$R^+_I R^+_{II} R^+_{III}$	$R^+_I R^+_{II} R^-_{III}$	$R^+_I R^-_{II} R^+_{III}$	$R^+_I R^-_{II} R^-_{III}$	$R^-_I R^+_{II} R^+_{III}$	$R^-_I R^+_{II} R^-_{III}$	$R^-_I R^-_{II} R^+_{III}$	$R^-_I R^-_{II} R^-_{I\,II}$	Colonies
$\omega^+ \times \omega^-$	($C^S E^R S^R$)	($C^S E^R S^S$)	($C^S E^S S^R$)	($C^S E^S S^S$)	($C^R E^R S^R$)	($C^R E^R S^S$)	($C^R E^S S^R$)	($C^R E^S S^S$)	
IL240–6D × 55R5–3C/324	91	69	2	5	0	0	0	13	180
$C^S E^R_{553} S^R_{352}$ $C^R_{324} E^S S^S$									
IL240–6D × 55R5–3C/324	154	90	0	3	0	0	0	17	264
$C^S E^R_{553} S^R_{352}$ $C^R_{324} E^S S^S$					0.0047%	0.018%	0.037%	8.3%	
IL240–8A × 55R5–3C/324	160	128	0	7	0	0	1	10	306
$C^S E^R_{553} S^R_{352}$ $C^R_{324} E^S S^S$					0.010%	0.080%	0.14%	2.8%	
	($C^R S^R E^S$)	($C^R S^R E^R$)	($C^R S^S E^S$)	($C^R S^S E^R$)	($C^S S^R E^S$)	($C^S S^R E^R$)	($C^S S^S E^S$)	($C^S S^S E^R$)	
IL262–2D × 55R5–3C/353	316	8	0	2	0	0	0	7	333
$C^R_{321} S^R_{551} E^S$ $C^S S^S E^R_{353}$									
	($C^S S^R E^R$)	($C^S S^R E^S$)	($C^S S^S E^R$)	($C^S S^S E^S$)	($C^R S^R E^R$)	($C^R S^R E^S$)	($C^R S^S E^R$)	($C^R S^S E^S$)	
IL233–3B × 55R5–3C/321	422	101	3	10	0	0	0	26	562
$C^S S^R_{551} E^R_{353}$ $C^R_{321} S^S E^S$									
	($C^R E^R S^S$)	($C^R E^R S^R$)	($C^R E^S S^S$)	($C^R E^S S^R$)	($C^S E^R S^S$)	($C^S E^R S^R$)	($C^S E^S S^S$)	($C^S E^S S^R$)	
IL292–6A × 55R5–3C/352	177	47	0	2	0	0	0	12	238
$C^R_{324} E^R_{553} S^S$ $C^S E^S S^R_{352}$			0.14%	1.0%					
IL292–2B × 55R5–3C/352	192	123	0	12	0	0	4	30	361
$C^R_{324} E^R_{553} S^S$ $C^S E^S S^R_{352}$			0.14%	2.4%					
Total	1512	566	5	41	0	0	5	115	2244
Average	67.4%	25.2%	0.2%	1.9%	0. %	0. %	0.2%	5.1%	
			0.14%	1.7%	0.0073%	0.049%	0.088%	5.6%	

Experimental conditions and symbols as in Table 7. Numbers underlined correspond to frequencies of minor classes determined by the aliquot plating procedure on selective media N3C or N3CE (see COEN *et al.* 1970) followed by replicating on to a second type of discriminating selective media. (See AVNER *et al.* 1973).

TABLE 20

Homosexual three-point crosses involving mutations at the R_I, R_{II} and R_{III} loci

a strain	×	α strain	$C^RS^RE^R$	$C^SS^SE^S$	$C^RS^RE^S$	$C^SS^SE^R$	$C^RS^SE^S$	$C^SS^RE^R$	$C^RS^SE^R$	$C^SS^RE^S$	Colonies
IL287–7C $\omega+C^R_{321}S^R_{551}E^R_{353}$	×	DP1–1B $\omega+C^SS^SE^S$	175	256	21	20	0	1	0	1	474
IL287–10D $\omega+C^R_{321}S^R_{551}E^R_{353}$	×	DP1–1B $\omega+C^SS^SE^S$	175	228	28	26	0	0	0	0	457
Total			350	484	49	46	0	1	0	1	931
Average			37.6%	52.0%	5.3%	4.9%	0. %	0.1%	0. %	0.1%	

Experimental conditions and symbols as in Table 7.

Mapping by transmission

It has been shown that the transmission of a given mitochondrial allele depends not only on the localization of this allele with respect to ω, but also on the nuclear genetic background (AVNER *et al.* 1973; COEN *et al.* 1974). Therefore, a meaningful comparison between various mitochondrial mutations has to be performed in a constant nuclear background. This has been done in crosses involving always 55R5–3C by DP1–1B. Summing up all the data from tables 7 to 13, one finds the following result:

Transmission of the alleles at the $R^{+}{}_{I}$ locus: 95.5% (± 0.8%)

Transmission of the alleles at the $R^{+}{}_{II}$ locus: 92.7% (± 0.7%)

Transmission of the alleles at the $R^{+}{}_{III}$ locus: 84.2% (± 1.1%)

Figures in brackets give 95% confidence limits of the sampling error. These results are in agreement with the previously deduced order ω^{+}–$R^{+}{}_{I}$–$R^{+}{}_{II}$–$R^{+}{}_{III}$

Relationships between the linkage group comprising loci R_I, R_{II}, R_{III} *and the locus* O_I

Until now we have considered the relationships between the three ribosomal loci, one with respect to the others. In this section we shall analyze the relation between these three loci, R_I, R_{II}, R_{III} and a gene the function of which is quite different from the ribosomal ones. We shall consider the locus O_I conferring the resistance to oligomycin which has been studied in details by AVNER *et al.* (1973). One shall find in this paper an exhaustive description of the present status of knowledge concerning the oligomycin-resistance in yeast. In addition to the experimental data obtained by us, we shall frequently use for comparison the

TABLE 21

Recombination between R_I, R_{II} *and* O_I *loci in homosexual crosses*

Cross a × α	$(R^{a}{}_{I}O^{\alpha}{}_{I} + R^{\alpha}{}_{I}O^{a}{}_{I})$	$(R^{a}{}_{II}O^{\alpha}{}_{I} + R^{\alpha}{}_{II}O^{a}{}_{I})$	Colonies
IL249–2A × IL778–2B $\omega^{-}C^{S}E^{R}{}_{354}O^{S}$ × $\omega^{-}C^{R}{}_{321}E^{S}O^{R}{}_{1}$	30 + 14	37 + 13	259
IL778–3D × IL249–1D $\omega^{-}C^{R}{}_{321}E^{S}O^{R}{}_{1}$ × $\omega^{-}C^{S}E^{R}{}_{354}O^{S}$	18 + 18	17 + 23	180
IL778–1A × IL249–1A $\omega^{-}C^{R}{}_{321}E^{S}O^{R}{}_{1}$ × $\omega^{-}C^{S}E^{R}{}_{354}O^{S}$	26 + 43	23 + 45	237
55R5–3C/1 × IL249–4D $\omega^{-}E^{S}O^{R}{}_{1}$ × $\omega^{-}E^{R}{}_{354}O^{S}$	—	7 + 41	167
Total of recombinants	74 + 75 = 149	84 + 122 = 206	843
Average	22.0%	24.4%	

Experimental conditions and symbols as in Table 7. Colonies replicated onto N3, N3C, N3E, N3CE, N3O, N3CO according to the markers present in the cross. Number of colonies of the corresponding recombinant type observed and the total of colonies replicated are given. The superscript ^{a} or $^{\alpha}$ designates that the allele at a given mitochondrial locus is derived from the *a* or α haploid parent irrespective of whether this allele is resistant or sensitive.

results obtained by AVNER *et al.* (1973) and by WOLF, DUJON and SLONIMSKI (1973).

Two factor homosexual crosses—R_1 *by* O_1, R_{II} *by* O_1, R_{III} *by* O_1

Numerous crosses have been made with these markers in different genetic configurations *cis* or *trans*, resistant and sensitive, involving various nuclear genetic backgrounds. Tables 21 and 22 give the results. In these crosses, which are all of the non-polar type, the total frequency of recombinants between R_I and O_I, R_{II} and O_I, R_{III} and O_I is respectively 19.3%, 23.4% and 19.6% (see Table 22). All these values are not significantly different. It is reasonable to conclude therefore that, in homosexual crosses, the loci R_I, R_{II} and R_{III} which are certainly linked (see above) are not genetically linked to locus O_I.

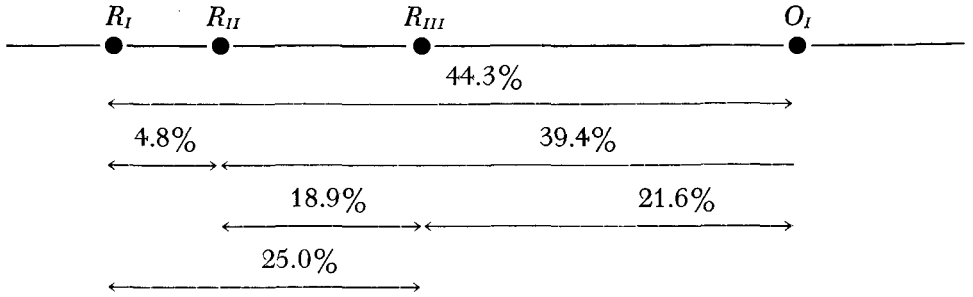
Three factor homosexual crosses

Table 23 shows the results obtained in crosses involving R_I, R_{II} and O_I. It is clear that two orders are possible R_I–R_{II}–O_I and O_I–R_I–R_{II}. On the other hand the order R_I–O_I–R_{II} can be eliminated, as shown in Table 24. This is in agreement with the results obtained by AVNER *et al.* (1973).

Two-factor heterosexual crosses

Major recombinants:

The frequencies of recombinants $R^+{}_IO^-{}_I$, $R^+{}_{II}O^-{}_I$ and $R^+{}_{III}O^-{}_I$ are 44.3%, 39.4% and 21.6% respectively (see Tables 22 and 25). Based on these frequencies, a genetic linkage could be postulated between loci R_I, R_{II} and R_{III} on the one hand, and locus O_I on the other hand. The order deduced from the values of major recombinants would be:



By this criterion, O_I seems to be linked to the ribosomal cluster of loci (additivity of frequencies is satisfactory), whereas O_I appeared to be independent in homosexual crosses.

Minor recombinants:

If we consider now the frequencies of recombinants $R^-{}_IO^+{}_I$, $R^-{}_{II}O^+{}_I$ and $R^-{}_{III}O^+{}_I$ in the previously analyzed crosses (Tables 22 and 25) we get 0.3%,

TABLE 22

Summary of recombination data from all the crosses involving the three ribosomal loci and the first oligomycin locus

	In homosexual crosses			In heterosexual crosses			
	Number of crosses	Recombinants between	Colonies	Number of crosses	Recombinant type	Colonies	Polarity and limits
		R_I and O_I			$R^+_IO^-_I$ $R^-_IO^+_I$		
This work	3	149	676	11	1092 4	2274	273 (136–546)
AVNER et al. (1973)	23	1916	9724	25	3623 31	8594	117 ((85–159)
WOLF, DUJON and SLONIMSKI (1973)	5	454	2631	10	2600 18	5655	144 (98–212)
Total	31	2519	13031	46	7315 53	16523	138 (108–176)
		19.3%			44.3% 0.3%		
		R_{II} and O_I			$R^+_{II}O^-_I$ $R^-_{II}O^+_I$		
This work	4	206	843	13	1226 28	3065	44 (32–60)
AVNER *et al.* (1973)	2	140	638	3	262 16	715	16 (11–25)
Total	6	346	1481	16	1488 44	3780	33 (26–44)
		23.4%			39.4% 1.2%		
		R_{III} and O_I			$R^+_{III}O^-_I$ $R^-_{III}O^+_I$		
This work	—	—	—	2	191 42	796	4.5 (3.4–6.0)
AVNER *et al.* (1973)	26	1795	9248	26	2289 659	9961	3.5 (3.2–3.8)
WOLF *et al.* (1973)	5	519	2584	8	717 227	4082	3.2 (2.8–3.6)
Total	31	2314	11832	36	3197 928	14839	3.4 (3.2–3.7)
		19.6%			21.6% 6.3%		

Symbols as in Table 7. Data from Tables 21, 25 of the present paper, Tables 7b and 12b of WOLF, DUJON and SLONIMSKI (1973), Tables 12c, 14, 17a, 18 of AVNER *et al.* (1973). One cross (IL922–1A × IL102–8D) from the latter work has not been taken into consideration.——Polarity of recombination is the ratio of the major recombinant to the minor one. 95% confidence limits of polarity were estimated on the basis of the number of colonies counted by the formula $\sigma^2 = (a/b^3)\ [a(n-b) + b(n-a)]/n$ (COEN *et al.*, in preparation) where a, b = number of recombinant colonies and n = total colonies.

TABLE 23

Homosexual three-point crosses involving mutations at the R_I, R_{II} *and* O_I *loci*

a strain × *α* strain	$R^a_I R^a_{II} O^a_I$	$R^a_I R^\alpha_{II} O^\alpha_I$	$R^\alpha_I R^a_{II} O^\alpha_I$	$R^\alpha_I R^\alpha_{II} O^a_I$	$R^a_I R^\alpha_{II} O^\alpha_I$	$R^a_I R^\alpha_{II} O^a_I$	$R^\alpha_I R^a_{II} O^\alpha_I$	$R^\alpha_I R^\alpha_{II} O^\alpha_I$	Colonies
IL778–1A × IL249–1A ω-$C^R_{321}E^SO^R_1$ ω-$C^SE^R_{354}O^S$	56	109	23	42	3	1	3	0	237
IL778–3D × IL249–4D ω-$C^R_{321}E^SO^R_1$ ω-$C^SE^R_{354}O^S$	53	86	17	18	1	0	5	0	180
IL249–2A × IL778–2B ω-$C^SE^R_{354}O^S$ ω-$C^R_{321}E^SO^R_1$	104	103	30	12	0	2	1	7	259
Total	213	298	70	72	4	3	9	7	676
Average	31.5%	44.1%	10.4%	10.7%	0.6%	0.4%	1.3%	1.0%	

Experimental conditions and symbols as in Tables 7 and 21.

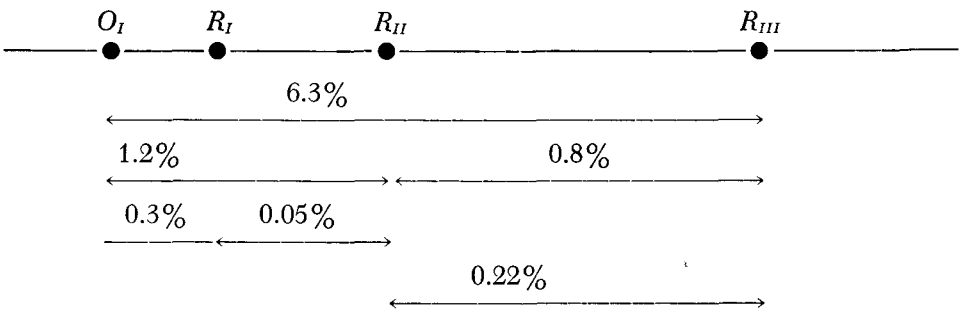
TABLE 24

Possible orders and observed frequencies of recombinants resulting from one or two crossovers in homosexual three-point crosses

Possible orders	Number of crossovers and resulting genotypes: One	One	Two
$R_I R_{II} O_I$	$R^a_I R^a_{II} O^\alpha_I = 10.4\%$ $R^\alpha_I R^\alpha_{II} O^a_I = 10.7\%$	$R^a_I R^\alpha_{II} O^\alpha_I = 0.6\%$ $R^\alpha_I R^a_{II} O^a_I = 0.4\%$	$R^a_I R^\alpha_{II} O^\alpha_I = 1.3\%$ $R^\alpha_I R^a_{II} O^\alpha_I = 1.0\%$
$R_I O_I R_{II}$	$R^a_I O^a_I R^\alpha_{II} = 1.3\%$ $R^\alpha_I O^\alpha_I R^a_{II} = 1.0\%$	$R^a_I O^\alpha_I R^\alpha_{II} = 0.6\%$ $R^\alpha_I O^a_I R^a_{II} = 0.4\%$	$R^a_I O^\alpha_I R^a_{II} = 10.4\%$ $R^\alpha_I O^a_{II} R^\alpha_I = 10.7\%$
$R_{II} R_I O_I$	$R^a_{II} R^a_I O^\alpha_I = 10.4\%$ $R^\alpha_{II} R^\alpha_I O^a_I = 10.7\%$	$R^a_{II} R^\alpha_I O^\alpha_I = 1.0\%$ $R^\alpha_{II} R^a_I O^a_I = 1.3\%$	$R^a_{II} R^\alpha_I O^a_I = 0.4\%$ $R^\alpha_{II} R^a_I O^\alpha_I = 0.6\%$

Data from Table 23.

1.2% and 6.3% respectively. Taking into account the values obtained previously between R_I, R_{II} and R_{III}, the most probable order would be:



This order is much less reliable than the preceding one, because the determination of frequencies of minor recombinants is less accurate and the additivity of frequencies is not satisfactory. The order is a circular permutation of the preceding one, in which O_I was placed on the right of the ribosomal segment. Although tempting, one should not hastily conclude from these data that the genetic map of mitochondrial DNA is a circular one.

Three factor heterosexual crosses between $R_I\ R_{II}$ *and* O_I *or between* $R_{II}\ R_{III}$ *and* O_I

For each of these two associations of markers, there are, *a priori*, three possible orders. We can ask whether at least one of these three orders is compatible with frequencies of the six recombinant types scored (Tables 26 and 27). To do this, we have to postulate that the recombinant type resulting from two recombination events should be less frequent than each of the two recombinant types resulting from a single recombination event. The predicted results and the observed frequencies in each case are listed in Table 28. It is apparent that the orders $R_I O_I R_{II}$ and $R_{II} O_I R_{III}$ can be excluded, for in these orders, the classes issued from two

TABLE 25

Recombinants between R_I, R_{II}, R_{III} *loci and* O_I *locus in heterosexual crosses*

Crosses $\omega^+ \times \omega^-$	$R^+_I O^-_I / R^-_I O^+_I$	$R^+_{II} O^-_I / R^-_{II} O^+_I$	$R^+_{III} O^-_I / R^-_{III} O^+_I$	Colonies
IL290–4B × IL778–2B $C^S E^R_{354} O^S$ $C^R_{321} E^S O^R_1$	71/1	70/1	—	155
IL290–5C × IL778–3D $C^S E^R_{354} O^S$ $C^R_{321} E^S O^R_1$	83/1	82/1	—	134
IL233–1C × 55R5–3C/1 $S^R_{551} E^R_{353} O^S$ $S^S E^S O^R_1$	—	122/3	102/28	403
IL240–8A × 55R5–3C/1 $E^R_{553} S^R_{352} O^S$ $E^S S^S O^R_1$	—	164/2	89/14	393
IL781–1A × IL249–4D $C^R_{321} E^S O^R_1$ $C^S E^R_{354} O^S$	237/0	201/4	—	458
IL781–1A × IL249–8C $C^R_{321} E^S O^R_1$ $C^S E^R_{354} O^S$	194/0	157/4	—	350
IL781–3A × IL249–7B $C^R_{321} E^S O^R_1$ $C^S E^R_{354} O^S$	149/0	122/3	—	328
IL842–6A × 55R5–3C/324 $C^S E^R_{354} O^R_1$ $C^R_{324} E^S O^S$	57/0	55/0	—	117
IL842–6A × 55R5–3C/321 $C^S E^R_{354} O^R_1$ $C^R_{321} E^S O^S$	41/1	39/1	—	106
IL187–6D × IL841–1A $C^R_{321} E^S O^S$ $C^S E^R_{354} O^R_1$	104/0	84/2	—	190
IL187–2D × IL841–3A $C^R_{321} E^S O^S$ $C^S E^R_{354} O^R_1$	65/1	52/6	—	158
IL187–4B × IL841–2A $C^R_{321} E^S O^S$ $C^S E^R_{354} O^R_1$	40/0	38/1	—	138
IL187–4C × IL841–2D $C^R_{321} E^S O^S$ $C^S E^R_{354} O^R_1$	51/0	40/0	—	140
Total of recombinants	1092/4	1226/28	191/42	
Polarity	273	44	4.5	
Limits	136 546	32 60	3.4 6.0	
Percent recombinants	48.0/0.18	40.0/0.9	24.0/5.3	

Experimental conditions and symbols as in Tables 7 and 21. Limits of polarity cf. Table 22.

putative recombination events are *always* more frequent than at least one class issued from a single putative recombination event. None of the remaining orders ($R_I R_{II} O_I$ and $O_I R_I R_{II}$ on the one hand, $R_{II} R_{III} O_I$ and $O_I R_{II} R_{III}$ on the other hand) is satisfactory: in each case, at least one putative double recombinant is found more frequently than at least one putative single recombinant—(e.g., $R^-_I R^+_{II} O^-_I = 0.10\% > R^-_I R^+_{II} O^+_I = 0.0046\%$, etc.).

TABLE 26

Heterosexual three-point crosses involving mutations at the R_I, R_{II} and O_I loci

Cross $\omega^- \times \omega^+$	$R^+_I R^+_{II} O^+_I$ ($C^S E^R O^R$)	$R^+_I R^+_{II} O^-_I$ ($C^S E^R O^S$)	$R^+_I R^-_{II} O^+_I$ ($C^S E^S O^R$)	$R^+_I R^-_{II} O^-_I$ ($C^S E^S O^S$)	$R^-_I R^+_{II} O^+_I$ ($C^R E^R O^R$)	$R^-_I R^+_{II} O^-_I$ ($C^R E^R O^S$)	$R^-_I R^-_{II} O^+_I$ ($C^R E^S O^R$)	$R^-_I R^-_{II} O^-_I$ ($C^R E^S O^S$)	Colonies
55R5-3C/321 × IL842-6A $\omega^- C^R_{321} E^S O^S$ × $\omega^+ C^S E^R_{354} O^R_1$	45	39	0	2	0 0.009%	0 0.13%	1 0.22%	19 15.0%	106
55R5-3C/324 × IL842-6A $\omega^- C^R_{324} E^S O^S$ × $\omega^+ C^S E^R_{354} O^R_1$	29	54	0	3	0 0.005%	1 0.065%	0 0.15%	30 9.5%	117
	($C^S E^R O^S$)	($C^S E^R O^R$)	($C^S E^S O^S$)	($C^S E^S O^R$)	($C^R E^R O^S$)	($C^R E^R O^R$)	($C^R E^S O^S$)	($C^R E^S O^R$)	
IL778-3D × IL290-5C $\omega^- C^R_{321} E^S O^R_1$ × $\omega^+ C^S E^R_{354} O^S$	32	81	0	2	0 0.0018%	1 0.14%	1 0.059%	17 8.8%	134
IL778-2B × IL290-4B $\omega^- C^R_{324} E^S O^R_1$ × $\omega^+ C^S E^R_{354} O^S$	60	70	0	1	0 0.0029%	0 0.066%	1 0.14%	23 8.1%	155
	($C^R E^S O^R$)	($C^R E^S O^S$)	($C^R E^R O^R$)	($C^R E^R O^S$)	($C^S E^S O^R$)	($C^S E^S O^S$)	($C^S E^R O^R$)	($C^S E^R O^S$)	
IL249-4D × IL781-1A $C^S E^R_{354} O^S$ × $C^R_{321} E^S O^R_1$	214	201	4	36	0	0	0	3	458
IL249-8C × IL781-1A $C^S E^R_{354} O^S$ × $C^R_{321} E^S O^R_1$	152	157	4	37	0	0	0	0	350
IL249-7B × IL781-3A $C^S E^R_{354} O^S$ × $C^R_{321} E^S O^R_1$	170	122	3	27	0	0	0	6	328
	($C^R E^S O^S$)	($C^R E^S O^R$)	($C^R E^R O^S$)	($C^R E^R O^R$)	($C^S E^S O^S$)	($C^S E^S O^R$)	($C^S E^R O^S$)	($C^S E^R O^R$)	
IL841-1A × IL187-6D $C^S E^R_{354} O^R_1$ × $C^R_{321} E^S O^S$	83	84	2	20	0	0	0	1	190
IL841-3A × IL187-2D $C^S E^R_{354} O^R_1$ × $C^R_{321} E^S O^S$	82	52	5	13	0	0	1	5	158
IL841-2A × IL187-4B $C^S E^R_{354} O^R_1$ × $C^R_{321} E^S O^S$	97	38	1	2	0	0	0	0	138
IL841-2D × IL187-4C $C^S E^R_{354} O^R_1$ × $C^R_{321} E^S O^S$	83	40	0	11	0	0	0	6	140
Total	1047	938	19	154	0	2	4	110	2274
Average	46.0%	41.2%	0.8%	6.8%	0.0% 0.0046%	0.1% 0.10%	0.2% 0.14%	4.8%	

Experimental conditions and symbols as in Tables 7 and 21.——Numbers underlined correspond to frequencies of minor classes determined by the aliquot plating procedure on selective media N3C or N3CE or N3CO followed by replicating onto a second type of discriminating selective media (see COEN *et al.* 1970; AVNER *et al.* 1973).

TABLE 27

Heterosexual three-point crosses involving mutations at the R_{II}, R_{III} and O_I loci

Cross $\omega^+ \times \omega^-$	$R^+_{II}R^+_{III}O^+_I$	$R^+_{II}R^+_{III}O^-_I$	$R^+_{II}R^-_{III}O^+_I$	$R^+_{II}R^-_{III}O^-_I$	$R^-_{II}R^+_{III}O^+_I$	$R^-_{II}R^+_{III}O^-_I$	$R^-_{II}R^-_{III}O^+_I$	$R^-_{II}R^-_{III}O^-_I$	Colonies
IL240–8A $\times$ 55R5–3C/1 $E^R_{553}S^R_{352}O^S$ $E^SS^SO^R_1$	200	89	12	73	0	0	2	15	391
IL233–1C $\times$ 55R5–3C/1 $S^R_{551}E^R_{353}O^S$ $E^SS^SO^R_1$	201	100	25	22	0	2	3	50	403
Total	401	189	37	95	0	2	5	65	794
Average	50.5%	23.8%	4.7%	12.0%	0. %	0.3%	0.6%	8.2%	

Data from Tables 26 and 27.

TABLE 28

Possible orders and observed percentage of recombinants resulting from one or two crossovers in heterosexual three-point crosses

Possible orders	Number of crossovers and resulting genotypes: One	One	Two
R_I–R_{II}–O_I	$R^+{}_IR^+{}_{II}O^-{}_I$: 41.2 $R^-{}_IR^-{}_{II}O^+{}_I$: 0.2	$R^+{}_IR^-{}_{II}O^-{}_I$: 6.8 $R^-{}_IR^+{}_{II}O^+{}_I$: 0.0046	$R^+{}_IR^-{}_{II}O^+{}_I$: 0.8 $R^-{}_IR^+{}_{II}O^-{}_I$: 0.10
O_I–R_I–R_{II}	$O^+{}_IR^+{}_IR^-{}_{II}$: 0.8 $O^-{}_IR^-{}_IR^+{}_{II}$: 0.10	$O^+{}_IR^-{}_IR^-{}_{II}$: 0.2 $O^-{}_IR^+{}_IR^+{}_{II}$: 41.2	$O^+{}_IR^-{}_IR^+{}_{II}$: 0.0046 $O^-{}_IR^+{}_IR^-{}_{II}$: 6.8
R_I–O_I–R_{II}	$R^+{}_IO^+{}_IR^-{}_{II}$: 0.8 $R^-{}_IO^-{}_IR^+{}_{II}$: 0.10	$R^+{}_IO^-{}_IR^-{}_{II}$: 6.8 $R^-{}_IO^+{}_IR^+{}_{II}$: 0.0046	$R^+{}_IO^-{}_IR^+{}_{II}$: 41.2 $R^-{}_IO^+{}_IR^-{}_{II}$: 0.2
R_{II}–R_{III}–O_I	$R^+{}_{II}R^+{}_{III}O^-{}_I$: 23.8 $R^-{}_{II}R^-{}_{III}O^+{}_I$: 0.6	$R^+{}_{II}R^-{}_{III}O^-{}_I$: 12.0 $R^-{}_{II}R^+{}_{III}O^+{}_I$: 0.0	$R^+{}_{II}R^-{}_{III}O^+{}_I$: 4.7 $R^-{}_{II}R^+{}_{III}O^-{}_I$: 0.3
O_I–R_{II}–R_{III}	$O^+{}_IR^+{}_{II}R^-{}_{III}$: 4.7 $O^-{}_IR^-{}_{II}R^+{}_{III}$: 0.3	$O^+{}_IR^-{}_{II}R^-{}_{III}$: 0.6 $O^-{}_IR^+{}_{II}R^+{}_{III}$: 23.8	$O^+{}_IR^-{}_{II}R^+{}_{III}$: 0.0 $O^-{}_IR^+{}_{II}R^-{}_{III}$: 12.0
R_{II}–O_I–R_{III}	$R^+{}_{II}O^+{}_IR^-{}_{III}$: 4.7 $R^-{}_{II}O^-{}_IR^+{}_{III}$: 0.3	$R^+{}_{II}O^-{}_IR^-{}_{III}$: 12.0 $R^-{}_{II}O^+{}_IR^+{}_{III}$: 0.0	$R^+{}_{II}O^-{}_IR^+{}_{III}$: 23.8 $R^-{}_{II}O^+{}_IR^-{}_{III}$: 0.6

Data from Tables 26 and 27.

Another point of interest concerning three point heterosexual crosses is shown in Table 29. The allele at the locus distant from ω, which is brought into a given recombinant type by the ω^- parent (for example $R^-{}_{III}$ in crosses involving R_I, R_{II} and R_{III}) is always more frequent than the allele at the same locus brought by the ω^+ parent. This is true for both minor and major recombinants between the two markers close to ω.

The constraints concerning apparent contradictions in the orders established from frequencies of major and minor recombinants (see Table 27) and

TABLE 29

Distribution of a third marker among different pair-wise recombinant classes for two others in heterosexual crosses

Among the recombinant type	Frequency of the recombinant carrying as third marker	
$R^+{}_IR^-{}_{II}$	O^+ : 0.8% O^- : 6.8%	$R^+{}_{III}$: 0.14% $R^-{}_{III}$: 1.7%
$R^-{}_IR^+{}_{II}$	O^+ : 0.0046% O^- : 0.10%	$R^+{}_{III}$: 0.0073% $R^-{}_{III}$: 0.049%
$R^+{}_{II}R^-{}_{III}$	O^+ : 4.7% O^- : 12.0%	
$R^-{}_{II}R^+{}_{III}$	O^+ : 0.0% O^- : 0.3%	

Data from Tables 19, 26 and 27.

those concerning the excess of the third allele brought by the ω^- parent are characteristics of all heterosexual mitochondrial crosses and have been found in other combinations of markers (see AVNER *et al.* 1973 and WOLF, DUJON and SLONIMSKI 1973). Any model attempting to explain the main features of the mitochondrial genetics have to take this into account. A model which explains the data will be presented elsewhere (DUJON, SLONIMSKI and WEILL 1974).

DISCUSSION

The aim of the present article is to demonstrate the existence of a linkage group involving three loci conferring resistance against certain antibacterial antibiotics to the mitochondrial ribosome. It is the first time in our knowledge, that a group of three genetically linked loci is shown to be located on the mitochondrial DNA. We have studied the topological relations in between these three loci and with respect to another locus, genetically not linked to them.

THREE RIBOSOMAL LOCI

Allelism tests based on the appearance of wild-type recombinants have allowed the definition of three loci on the basis of the following criteria: (1) two mutants belonging to the same locus do not give any wild-type recombinants among some thousand colonies scored (2) two mutants belonging to different loci give a significant frequency of wild-type recombinants.

It is important to stress that mutations at a given locus do not confer necessarily identical phenotypes. The main criteria which allow the distinction between mutants belonging to the same locus are cross-resistance to antibiotics other than the one used to select the mutant, and physiological and biochemical interactions with other mutations which do not belong to the same locus.

1. At the locus R_I there exist at least three different types of mutations: $C^R{}_{321}$ confers resistance to chloramphenicol, a low degree of resistance to spiramycin and does not interact with the mutants belonging to the locus R_{III}; $C^R{}_{324}$ confers resistance to chloramphenicol, does not confer cross-resistance to spiramycin and does not interact with the mutants belonging to the locus R_{III}; $C^R{}_{336}$ confers resistance to chloramphenicol and strongly interacts with certain mutants belonging to the locus R_{III}.

2. At the locus R_{II} there exist at least three different types of mutations: $E^R{}_{354}$ confers resistance to erythromycin and spiramycin and interacts with $C^R{}_{321}$; $E^R{}_{553}$ confers resistance to erythromycin but not to spiramycin; $S^R{}_{551}$ confers a weak resistance to spiramycin but not to erythromycin.

3. At the locus R_{III} there exists at least four different types of mutations: $E^R{}_{514}$ confers resistance to erythromycin, spiramycin and interacts strongly with $C^R{}_{336}$; $E^R{}_{236}$ confers resistance to erythromycin, spiramycin and does not interact with $C^R{}_{336}$; $E^R{}_{353}$ is resistant to erythromycin, weakly resistant to chloramphenicol and sensitive to spiramycin; $S^R{}_{352}$ is resistant to spiramycin, weakly resistant to chloramphenicol and sensitive to erythromycin. It is therefore *not possible to*

allocate a mutation to a given genetic locus on the basis of its resistance phenotype alone. A genetic analysis is necessary for this purpose.

The next question to be asked is whether phenotypically different mutations allocated to the same genetic locus affect the same nucleotide site of the mitochondrial DNA or not. Although it is theoretically possible by substituting the same site to obtain four phenotypically different homoalleles, it seems more probable that mutations are heteroallelic rather than homoallelic. Two lines of circumstantial evidence argue in favor of this position: (a) A quantitative analysis of the degree of resistance, cross-resistance and pleiotropic biochemical interactions during the exponential growth (SLONIMSKI *et al.*, 1974) discloses a much greater diversity of phenotypes which can be found at the same locus than the one observed here and which is based essentially on the plate growth. (b) An estimation of the physical length (i.e., number of base pairs) as related to the genetic distance (i.e., frequency of recombinants) discloses that the operational limit of allocating two mutations to the same genetic locus ($< 0.1\%$ recombinants) corresponds to some 20 to 50 base pairs. The latter value is computed from the kinetic complexity and DNA/DNA hybridization data concerning ρ^- *petites* which have retained the ribosomal loci R_I, R_{II}, R_{III} (see FAYE *et al.* 1973; MICHEL *et al.* 1974; LAZOWSKA *et al.* 1974).

The last question to be discussed concerns the relations between the genetic loci and the functions which they specify. A parallel study (GRIVELL *et al.*, 1973) has shown that representative mutations at each of the three loci alter the *in vitro* properties of purified mitoribosomes. In our opinion these results render improbable the claim advanced by BUNN *et al* (1970) and TREMBATH *et al.* (1973) that erythromycin-and/or spiramycin-resistant mutations are mitochondrial membrane alterations or result from mitochondrial membrane–ribosome interactions. It is not known whether the ribosomal loci R_I, R_{II} and R_{III} belong to the same unit of function or not. There is indirect evidence that at least one locus, R_{III}, either specifies or is very closely linked to the large ribosomal rRNA molecule (FAYE *et al.* 1973, FUKUHARA *et al.* 1974). In the first case, if the two other loci also specify the large rRNA, it would mean that there are three specific regions in this molecule where nucleotide sequence alterations selectively engender antibiotic resistance. It would also indicate that an intracistronic frequency of recombination could be exceedingly high (25% recombinants between R_I and R_{III}!). In the second case, if R_{III} is simply closely linked to the large rRNA cistron, the data would indicate that another component of the ribosome (a protein or a small rRNA) is specified by a cistron adjacent to the large rRNA one. Further work on biochemical characterization of various R_I, R_{II} and R_{III} mutants is obviously needed.

RIBOSOMAL LOCI IN RELATION TO ONE ANOTHER

Homosexual crosses

These crosses are instructive for allelism tests but not very informative for mapping the loci in relation to one another. The overall frequencies of recombinants are: 3.2% between R_I and R_{II}; 8.9% between R_{II} and R_{III}; 9.6% between

R_I and R_{III}. The order R_I–R_{III}–R_{II} can therefore be eliminated but the last two frequencies are not sufficiently different to discriminate between the orders R_I–R_{II}–R_{III} and R_{II}–R_I–R_{III}.

The transmission of mitochondrial alleles is variable from cross to cross and may be as low as 20% and as high as 80%. There is, however a *covariance of transmission*, that is when the allele of one locus from the *a* parent is highly transmitted to the progeny the allele of the other locus is also highly transmitted and *vice versa* (see Figure 3). This observation extends to the locus R_{II} the rule of covariance previously established for the alleles at the loci R_I and R_{III} (COEN *et al.* 1974) at the loci O_I, O_{II} and P (AVNER *et al.* 1973, WOLF, DUJON and SLONIMSKI 1973). Covariance of transmission is a strong argument in favor of the idea that all these genes are located at the same mitDNA molecule (see DUJON, SLONIMSKI and WEILL 1974, for the discussion).

Heterosexual crosses

The polarity of recombination and the Left-Right rule of assignment allows the deduction of an unambiguous order of loci in relation to one another and in relation to ω. Table 15 summarizes the results of all pairwise combinations studied. The results are compatible with the order ω–R_I–R_{II}–R_{III} *only*. The results are also coherent with the allocation through allelism tests of various mutations to different loci. Heterosexual crosses permit therefore the discrimination between map orders which could not be resolved by homosexual crosses alone. Taken together the two types of crosses allow the localization of R_{II} in a closer linkage to R_I (3.2% of recombinants in homosexual crosses) than to R_{III} (8.9% of recombinants).

This order and the respective distances have been confirmed by an independent method based on the study of linkage between mitochondrial genes in the petite mutation. It will be shown elsewhere (BOLOTIN-FUKUHARA *et al* 1974) that the

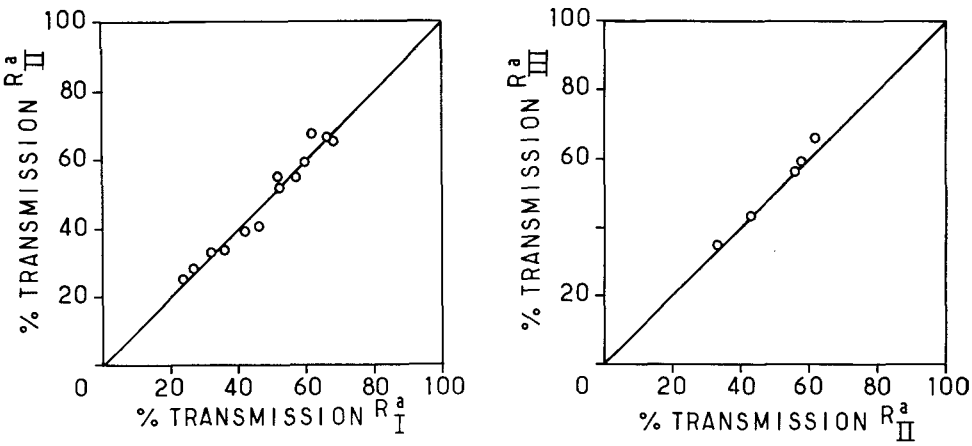


FIGURE 3.—Covariance of transmission in two point homosexual crosses involving R_I and R_{II} loci, or R_{II} and R_{III} loci.———Data from Tables 16 and 17 (for details see text). Each point represents one cross.

loci R_I and R_{II} are always deleted or retained together while the loci R_I and R_{III} can, although rarely, be separated by deletions covering one locus without impinging on the other (DEUTSCH *et al.* 1974; FAYE *et al.* 1973).

RIBOSOMAL LOCI IN RELATION TO THE ATPase LOCUS

Biochemical studies (GRIFFITHS *et al.* 1972; SOMLO *et al.* 1974) point out that mutations at the O_I locus alter the properties of the ATPase complex.

In homosexual crosses the locus O_I appears unlinked by recombination to the ribosomal segment. It can not therefore be localized by this method. In heterosexual crosses the Left-Right rule of assignment would place it at the right of the ribosomal segment. The existence of a single petite mutant that has retained loci R_{III} and O_I and lost all the other loci tested also argues in favor of this localization (see AVNER *et al.* 1973; BOLOTIN-FUKUHARA *et al.* 1974).

It may be of interest to analyze how the polarity of recombination changes with respect to various combinations between ribosomal loci and O_I. Table 22 gives the numerical values. The polarity is very high when a locus is close to ω

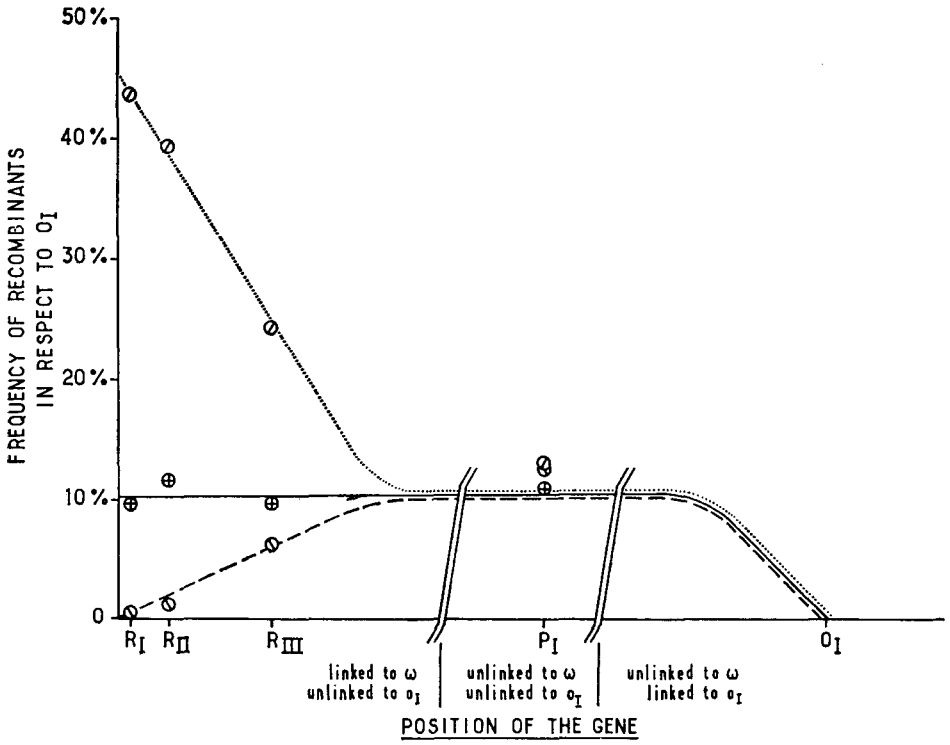


FIGURE 4.—Graphic representation of percentage of recombinants in homo and heterosexual crosses involving the O_I locus, as a function of the distance between ω and the second locus involved. Relative distances R_I–R_{II} and R_{II}–R_{III} on the abscissa were determined on the basis of the values obtained in homosexual crosses between R_I and R_{II} or R_{II} and R_{III} (3.2% and 8.9%) respectively (see Tables 16 and 17). Symbols ∅ and ⊘ represent recombinants in $\omega^+ \times \omega^-$ crosses. Symbol ⊕ represents recombinants in $\omega^- \times \omega^-$ or $\omega^+ \times \omega^+$ crosses.———Data from Table 22 and from WOLF, DUJON and SLONIMSKI (1973).

(138 on the average between R_I and O_I), intermediate when it is more distant from it (33 on the average between R_{II} and O_I) and finally moderate when it gets farther away (3.4 between R_{III} and O_I). This diminution of polarity results from a decrease in the frequency of the major recombinant concommitant with an increase in the frequency of the minor recombinant. Graphic presentation shown in the Figure 4 allows the comparison of the results of hetero- and homosexual crosses. Three cases are envisaged:

i) a gene is linked to ω and not linked to O. In this case heterosexual crosses yield different frequencies of recombinants from the homosexual ones. This difference decreases monotonously as the distance from ω increases.

ii) a gene is unlinked to ω and unliked to O. This case is exemplified by the paromomycin-resistance-conferring locus studied by WOLF, DUJON and SLONIMSKI (1973). Homo- and heterosexual crosses yield similar results. The frequency of recombinants is close to the limit to which the major and minor recombinants from the preceding case tend to.

iii) a gene is unlinked to ω and linked to O. As no case of this kind is known, this example is entirely hypothetical.

GENETIC MAP OF THE RIBOSOMAL SEGMENT OF MITOCHONDRIAL DNA

Main conclusions of this article are depicted by the following map:

ω R_I	$R_{I'}$	R_{III}
C^R_{36}	E^R_{354}	E^R_{221}
C^R_{321}	S^R_{551}	E^R_{236}
C^R_{323}	E^R_{553}	S^R_{352}
C^R_{324}		E^R_{353}
C^R_{325}		E^R_{514}
C^R_{336}		
C^R_{517}		

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