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# Phase-Shift and Other Mutants in the First Part of the <latex>$r$</latex>II B Cistron of Bacteriophage T4

Leslie Barnett, S. Brenner, F. H. C. Crick, R. G. Shulman and R. J. Watts-Tobin

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# PHASE-SHIFT AND OTHER MUTANTS IN THE FIRST PART OF THE *r*II B CISTRON OF BACTERIOPHAGE T4

By LESLIE BARNETT, S. BRENNER, F.R.S., F. H. C. CRICK, F.R.S., R. G. SHULMAN† and R. J. WATTS-TOBIN

*Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, England*

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† John Simon Guggenheim Fellow 1961 to 1962. Permanent address: Bell Telephone Laboratories, Murray Hill, New Jersey, U.S.A.

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The mutants considered consist of over 200 of the phase-shift type and 16 of the base-substitution type. A set of 61 of these has been mapped with precision and the rest have been located approximately. The results of all the crosses needed to locate the various mutants are listed. The sites of mutation are spread fairly evenly over the genetic map, with only a few appreciable gaps. A sign has been allocated to all the phase-shift mutants except for a group of nine at the extreme left-hand end of the gene. All but two of the base-substitution mutants are ochres or ambers.

A complete index is given of all the multiple mutants so far constructed. The phenotype of most of these combinations follows the rules previously proposed. No combination gives the wild or pseudo-wild phenotype which is not expected to do so.

The nature of the 'barriers' produced in certain pairs of mutants of unlike sign has been explored. Some of these barriers are ochres or ambers, but at least four of them fall into neither of these classes. All of them can be removed by a mutation of the base-substitution type.

Certain combinations of double mutants of the type (+ +) have been shown to produce minute plaques on K. Most of these are associated with two of the barriers.

## 1. INTRODUCTION

### (*a*) *Previous work*

Small mutations, as opposed to large deletions, are basically of two types:

(i) Base-substitution mutants, in which the total number of bases is unchanged, but in which one base is substituted for another. Such mutations are typically produced by base analogues, such as 2-aminopurine or bromouracil.

(ii) Phase-shift mutants, in which one or a small number of bases are either added or deleted. If the number is not a multiple of three, a phase-shift is introduced into the reading of the genetic message. Such mutants are produced in bacteriophage T4 when infected host bacteria are treated with an acridine such as proflavin.

Our study has been mainly concentrated on the left-hand end of the B cistron of the *r*II region of phage T4. As described in our original paper (Crick, Barnett, Brenner & Watts-Tobin 1961) we have produced many different phase-shift mutants by isolating intragenic suppressors of existing phase-shift mutants. In all, about 180 mutants have been obtained in this way. In addition, we have included some fifty-three mutants occurring in this region picked up by other means. Of these, roughly two-thirds are of the phase-shift type, and one-third of the base-substitution type.

Our original paper (Crick *et al.* 1961) suggested that in making a protein the (copy of the) genetic message was read from a fixed point in non-overlapping triplets of bases, and


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that the genetic code was probably highly degenerate. Recent work on the genetic code has fully confirmed these predictions (see papers in *Cold Spring Harbour Symposium of Quantitative Biology*, vol. **31**, 1966). Another prediction, that for one of our double mutants of the (+ with −) type 'a string of amino acids would be altered, corresponding to the region of the polypeptide chain between the two mutants', has also recently been confirmed by the work of Streisinger and his colleagues using the lysozyme of phage T4 (Okada *et al.* 1966; Streisinger *et al.* 1966). Thus our basic ideas now need little defence, and the object of the present paper is rather to make available to others the very extensive studies we have made of these mutants, and to discuss certain difficulties and anomalies which we have encountered.

### (*b*) *Basic theory*

Since the genetic code is a triplet code any message can be read in three ways, one of which is in the correct phase and the other two out of phase. In the same way it should be possible to allocate to any mutant the phase zero, plus or minus, corresponding respectively to no change in the number of bases, to the addition of one base, or to the subtraction of one base. As far as phase is concerned a mutant which has lost two bases would have phase plus, or a mutant which has gained two bases would have phase minus. A mutant which gained or lost three bases would have phase zero, but in general we would not expect in our region that such a 'mutant' would show the mutant phenotype.

We still cannot determine which of our two classes of phase-shift mutants is actually plus, and the allocation of sign is purely conventional, FC0 being arbitrarily taken as (+). Thus the strict definition of our symbolism is

$$+ \text{ represents } +m, \text{ modulo } 3 \qquad - \text{ represents } -m, \text{ modulo } 3,$$

when $m$ is either 1 (if our convention is correct) or $-1$ (if our convention is the reverse of the true state of affairs). *Added in proof*: Crick & Brenner (1967) suggest $m = +1$.

A frame shift $\leftarrow$ is defined as that between a (+ −) pair, and the $\rightarrow$ shift that between a (− +) pair. In other words, the head of the arrow points towards the plus.

### (*c*) *Suppression: a note on terminology*

In our original paper (Crick *et al.* 1961) we used the word 'suppressor' for intragenic suppressors of the phase-shift type. We continue this usage here, mainly for convenience.

In the meantime a large class of *extra*genic suppressors has been much studied, especially those which suppress the ochre triplet (UAA) or the amber triplet (UAG) (Weigert, Gallucci, Lanka & Garen 1966; Stretton, Kaplan & Brenner 1966). We shall never refer to these here simply as suppressors, but will use 'extragenic suppressor' or 'amber suppressor' or 'ochre suppressor', as the case may be.

## 2. Materials and methods

### (*a*) *Bacterial strains*

The following strains of *Escherichia coli* were used:

$B_W$, a derivative of *E. coli* B, referred to as B.

BB, Berkeley strain of *E. coli* B. On this strain rII mutants have $r^+$ phenotype.




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KB, standard strain of *E. coli* K12($\lambda$) (Benzer & Champe 1961), referred to as K.

KB1, a mutant of KB, permitting growth of a class of leaky *r*II mutants (subset III) (Benzer & Champe 1961).

D0, Hfr Cavalli met$^-$($\lambda$), restricting growth of a class of leaky *r*II mutants which grow on K.

CA244, a derivative of Hfr H($\lambda$) is the standard *su*$^-$ strain (Brenner & Beckwith 1965).

CA266, CA180 and CA265, derivatives of Hfr H($\lambda$) contain the amber suppressors $su_{\mathrm{I}}^+$, $su_{\mathrm{II}}^+$ and $su_{\mathrm{III}}^+$ respectively (Brenner & Beckwith 1965).

CA165, CA167 and CA248, derivatives of Hfr H($\lambda$) contain the ochre suppressors $su_{\mathrm{B}}^+$, $su_{\mathrm{C}}^+$ and $su_{\mathrm{D}}^+$ respectively (Brenner & Beckwith 1965).

QA1, CR63($\lambda$h), contains the amber suppressor $su_{\mathrm{I}}^+$.

### (*b*) *Bacteriophage strains*

P13 and P61 used for isolation of suppressors labelled FC are proflavin-induced mutations of T4B isolated in this laboratory (Brenner, Benzer & Barnett 1958). Other non-FC mutants were either isolated in this laboratory or given to us by Professor S. Benzer and Dr J. Drake as indicated in table A 1 (*a*).†

### (*c*) *Media*

M9 medium contains per litre: 5·8 g $Na_2HPO_4$, 3 g $KH_2PO_4$, 0·5 g NaCl and 1 g $NH_4Cl$. After autoclaving, this is supplemented with 0·8% glucose and $MgSO_4$ to 0·002 M.

M9 buffer contains per litre: 6 g $Na_2HPO_4$, 3 g $KH_2PO_4$, 0·25 g $MgSO_4,7H_2O$ and 5 g NaCl.

B broth contains per litre: 10 g Bacto-tryptone (Difco) and 5 g NaCl.

Top agar contains per litre: 8 g Bacto-agar (Difco), 10 g Bacto-tryptone (Difco) and 8 g NaCl.

Bottom agar contains per litre: 10 g Bacto-agar (Difco), 10 g Bacto-tryptone (Difco) and 8 g NaCl.

### (*d*) *Summary of the main experimental methods*

(i) *Spot test crosses* (*Benzer* 1961)

*E. coli* B was grown with aeration in B broth to $2\times10^8$/ml. and diluted with an equal volume of M9 buffer. Eight drops (about 0·4 ml.) of this suspension were added to one drop (about 0·05 ml.) of a standard stock ($2\times10^9$/ml.) of each parent phage in a tube and adsorption allowed to proceed at room temperature for 10 min. The multiply infected cells were then streaked on plates containing *E. coli* K12($\lambda$) in the top agar. Each plate accommodated 10 to 15 streaks. Recombination is easily detected between mutants which give 0·01% recombinants in standard crosses.

(ii) *Standard crosses*

*E. coli* B was grown with aeration in B broth plus 0·2% glucose to $2\times10^8$/ml., centrifuged, resuspended in M9 buffer, starved for 10 min and 20 $\mu$g/ml. L-tryptophan added. Parental phages were added in an equal volume of M9 buffer, each at a multiplicity of

† The A series of tables refers to the Appendices to this paper (§12).

about seven. After adsorption for 8 min at 37 °C the complexes were diluted $10^{-3}$ into B broth, incubated at 37 °C for 1 h and lysed with $CHCl_3$. K/B ratios were measured and recombination frequency was calculated taking into consideration the fact that crosses of phase-shift mutants may generate suppressed doubles as well as true wild.

(iii) *U.v. crosses*

A culture of *E. coli* B at $3 \times 10^8$/ml. in M9 medium containing 20 $\mu$g/ml. L-tryptophan was infected with the two parental phages each at a multiplicity of about seven. After incubation at 37 °C for 10 min, the mixedly infected cells were diluted 1:8 with M9 medium, and irradiated with u.v. light, the dose corresponding to about 20 T4 lethal hits. The irradiated complexes were incubated at 37 °C and lysed after $1\frac{1}{2}$ h with $CHCl_3$.

(iv) *Orgy crosses* (*S. Benzer, privately published*)

A rapid test was used to detect recombinants, in addition to true wild type, in crosses between *r* mutants. Instead of crossing each independently with true wild type, ten recombinants were mixed, crossed together in an orgy, and the progeny scored for *r* segregants. To do this one drop was taken from each of the ten stocks, mixed together and titrated on B and the percentage of *r* plaques among the $r^+$ noted. Four drops of this mix were then adsorbed to B and irradiated as for a u.v. cross. The growth was titrated and the percentage of *r* compared with that in the mix before crossing. If the mix contained both pseudo-wild and true wild the percentage of *r*'s increased considerably.

(v) *Phage stocks*

Standard stocks were grown in small tubes containing 3 ml. B broth inoculated with about $10^6$ phage and one drop of a saturated culture of BB. The tubes were incubated without shaking at 37 °C for 2 h or overnight and then lysed with chloroform. The lysates contained 2 to $5 \times 10^9$ phages/ml. Larger quantities, up to 7 ml., were grown in the same manner and had roughly the same titres.

To prepare high-titre stocks, 10 ml. cultures of *E. coli* BB were grown in M9 at 37 °C with aeration to $10^8$ cells/ml. and inoculated with about $10^6$ phages/ml. Aeration was continued for $4\frac{1}{2}$ h and the cultures lysed with $CHCl_3$. These lysates contained about $2 \times 10^{11}$ phages/ml.

### (*e*) *Induction of mutants*

(i) *2-aminopurine or 5-bromodeoxyuridine* (*Champe & Benzer* 1962*a*)

*E. coli* BB was grown in M9 with aeration to $2 \times 10^8$/ml. 0·5 ml. was added to tubes containing 1 ml. of M9 supplemented with 30 $\mu$g L-tryptophan/ml. and either 1 mg 2-aminopurine/ml. or 100 $\mu$g 5-bromodeoxyuridine/ml. The tubes were inoculated with about $10^2$ phages, incubated overnight at 37 °C without shaking, and lysed with $CHCl_3$. Controls were always run for comparison omitting the base-analogue mutagens. A mutant known to revert with base-analogues was also included as a positive control in each series.

(ii) *Proflavin*

*E. coli* BB was grown in M9 with aeration to $3 \times 10^8$/ml., and supplemented with L-tryptophan to 20 $\mu$g/ml. Proflavin was added to a final concentration of 8 $\mu$g/ml. and

the bacteria infected with phage at a multiplicity of five. After aeration at 37 °C for 8 min the bacteria were superinfected at a multiplicity of five with the same phage to produce lysis inhibition. The cultures were grown at 37 °C for a further hour, then diluted 1:50 into B broth, and the dilutions incubated for 60 min at 37 °C without aeration, when the cells were lysed with $CHCl_3$. Care was taken to minimize exposure of these cultures to light.

(iii) *Hydroxylamine* (*Freese, Bautz & Bautz-Freese* 1961)

A solution with final concentrations 1·25 M $NH_2OH$, HCl, 1 M NaCl, 0·001 M $MgSO_4$ and 0·075 M $Na_2HPO_4$ adjusted to pH 7·5 with NaOH was freshly prepared just before use. High-titre phage stocks were diluted 1:50 in M9 buffer. 0·2 ml. of these suspensions containing about $2 \times 10^9$ phages/ml. were added to 0·8 ml. of the hydroxylamine solution and the tubes incubated at 37 °C for 2 h. The reaction was stopped by a 1:20 dilution into M9 buffer containing 0·5% tryptone and 2% acetone. This solution must be buffered to prevent the fall of pH.

(*f*) *Mixed indicator techniques*

(i) Two different host bacteria were sometimes mixed together in the ratio of 1:2 (permissive:non-permissive) and two drops used in top agar for plating.

(ii) Two different host bacteria were sometimes used in two separate layers of top agar one upon the other.

In both these techniques the top agar was diluted by addition of 20% of its volume of B broth. Plaques were scored as turbids if they were overgrown by one of the strains.

## 3. The origin of the mutants

### (*a*) *The two main methods*

The mutants which fall into our region have been isolated by two main methods.

(i) *Mutants have been picked because they give the* r *phenotype on B*

Some of these were spontaneous mutants, while others were isolated after treatment with mutagens. These mutants are listed in table A 1 (*a*) which also gives their sources.

It is not reasonable to include in our set *every* independent mutant isolated in every laboratory, because in some cases a particular mutant has apparently been isolated very many times. Thus some criteria are necessary for accepting mutants or leaving them on one side.

We have accepted *all* mutants available to us which we have reason to believe are of the phase-shift type, whether they appear to be repeats of existing mutants or not. Mutants of the base-substitution type have been accepted if they map at a site at which no other mutant of this type occurs in our set. In addition, a mutant of this type has been accepted, even if it does map at the site of a base-substitution mutant already included, if it has a different extragenic suppression pattern. There is one unimportant exception to this rule. We have included both 360 and 739, which by the above criteria are identical, because by the time we realized they were the same we had done substantial work on each of them.

By these means we hope we have included *all* mutants of the phase-shift type and *one* example of each mutant of the base-substitution type. However, since we have not thoroughly screened every mutant which has occurred, but only a biased sample of them,

chosen in the light of the origin of each mutant, we may have missed a few which should strictly have been included.

(ii) *Mutants have been isolated as suppressors of a previously existing mutant in this region*

The technique for doing this is outlined below. All such mutants were derived from either P13 (now called FC0), either as its suppressors, or as suppressors of its suppressors, etc.; or, in similar ways, from P61.

All such mutants have been labelled FC mutants and are listed in table A 1 (*b*). Mutants listed with numbers between FC0 and FC153 are derived ultimately from P13; all those with numbers greater than FC200 are ultimately derived from P61. Table A 1 (*b*) shows the origin of each mutant and certain details of the isolation procedure. It also indicates whether it has been checked *directly* that it suppresses the mutant which it was presumed to suppress during its isolation. The origin and the approximate map position for each mutant is also displayed in figure 1.

FC0 is arbitrarily defined as having sign (+); its suppressors are given sign (−); their suppressors sign (+), and so on. In the same way P61 is called (+), its suppressors (−), and so on. That these two independent sets of signs are mutually consistent is shown in § 5 (*a*).

### (*b*) *The isolation of FC mutants*

The method of isolation of FC mutants was, in outline, as follows: for spontaneous revertants the mutant chosen was plated on B and a number of separate *r* plaques (usually about ten) was picked and grown in separate tubes. A portion of each tube was then plated on K and *one* plaque was picked, purified on B, and a standard stock grown. (In the case of the revertants of P13 *three* plaques were picked from each K plate—this is discussed in § (*d*) (i) below.)

When an acridine was used as a mutagen to obtain revertants it was not added to ten separate tubes but to a single tube (see § 2 (*e*) (ii) for details). The burst was plated on K and the appropriate number of plaques picked, purified on B and grown. Because of the enhanced mutation rate and the high dilution the chances of isolating two revertants which originated in the same act are very slight, and the different revertants can be considered independent. This is, of course, not true for spontaneous mutants which may have arisen mainly from a 'jackpot' in the early stages of the growth of a culture.

The stock of the revertant was then titrated, and its plaque-type on B and K noted. If, because of some accident of picking, it would not grow on K it was rejected. In three cases (two by accident, one by design) the revertant picked was a minute; that is, it had a small sharp plaque on K, and incidentally had an *r* plaque on B. The suppressors found in this way were FC20 (from FC1), FC34 (from FC6) and FC58 (from FC9), as noted in table A 1 (*b*).

Each revertant was then crossed against standard wild type, using u.v. to increase recombination (see § 2 (*d*) (iii)). The result of the cross was usually plated on B, and the number of *r* plaques noted. If the revertant had in fact been a double (the original mutant and its suppressor) then this percentage gave a rough idea of their distance from each other. A number (usually about ten) of the *r* plaques was picked, purified on B, and then crossed using the standard spot-cross (§ 2 (*d*) (i)). Each was back-crossed to itself (to check background) and to some other (distant) mutant to check that phage was present. In general


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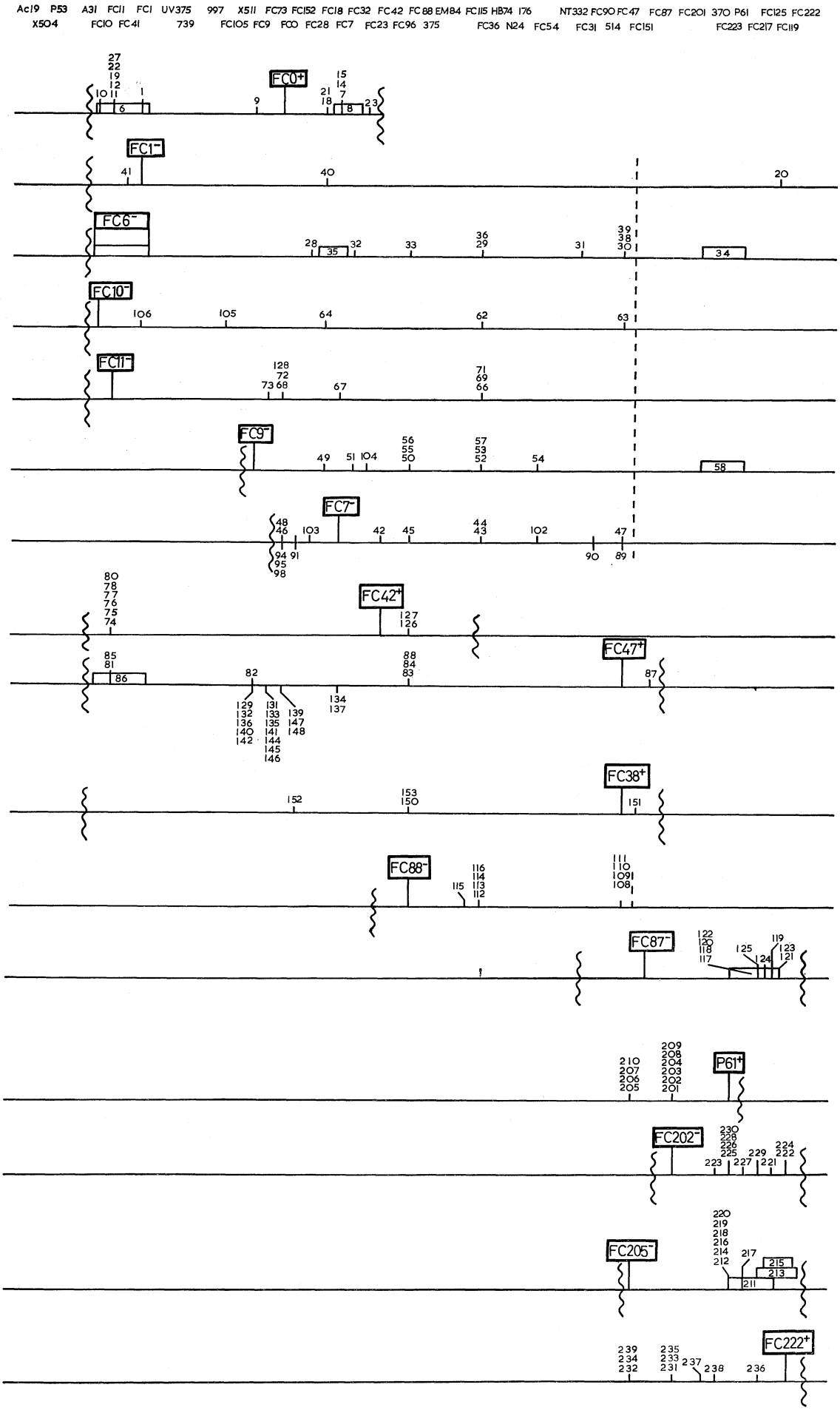


FIGURE 1. For legend see facing page.


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some of these *r*s would not recombine with the original mutant and were therefore assumed to be identical with it. These are labelled 'p' in table A 1 (*b*), column 4. The remaining *r*s (provided they had a low background) were crossed against one of their number or sometimes all against all. Typically, most of these mutants would not recombine with each other. They were presumed to be different copies of the suppressor, and were labelled 's' (table A 1 (*b*), column 5). One was chosen and given a new FC number. In some cases one or two of the *r*s were different from the majority. These were presumed to be spontaneous mutants and were discarded.

In general one would expect roughly equal numbers of the original mutant and its suppressor, but in certain cases recombination appeared to be grossly unequal, and too few of either the original mutant or its suppressor were obtained. We have taken as our criterion of acceptance that the *r*s picked on B from the u.v. cross must have contained at least two copies of the original mutant and at least three copies of the new suppressor. If this criterion was not obeyed, or if for some other reason there was any doubt that the two mutants were suppressing each other, then this was checked directly by crossing them together. If this has been done there is a tick in column 6, table A 1 (*b*).

### (*c*) *The nature of the phenotypes*

In some cases the revertant (which, it should be recalled, was picked on K) turned out to have a phenotype on B very far from wild, and sometimes very like an *r* phenotype, although it could nevertheless grow on K. (In Benzer's original classification (Benzer 1956) this would be called an *r*III.) In order to pick out the true *r*s from the revertant itself the product of the cross was plated on a mixture of B and K (see § 2 (*f*)). With care the two types—both *r* on B, but one growing on K, the other not—could be distinguished on such a plate. Approximate descriptions of the phenotypes are listed in tables A 4 (*a*) and (*c*) (see § 7 (*d*)).

In every case where we have been able to recover a suppressor it has turned out to be a non-leaky *r*. With one exception (P61/13), we have never had a case in which a suppressor appeared to be present but did not have *r* phenotype. If this were to occur we should find that the *r*s produced by the u.v. cross of the revertant against wild were all the original *r* (except for an occasional spontaneous mutant). Such an event may *appear* to occur, either by chance (which is unlikely) or because of unequal recombination, which is not infrequent. In such cases we have repeated the cross, and obtained further *r*s, until the suppressor was discovered. In doubtful cases, as already explained, we have checked that the presumed suppressor does indeed suppress the original mutant (see column 6, table A 1 (*b*)). In the case of P61/13 we were unable to recover a suppressor, but it is probable

FIGURE 1. The family tree of FC suppressors isolated. The boxed FC number on each line indicates the parent phage. Along each line the suppressors isolated from the parent phage are distributed in approximate map positions as points or with extent as appropriate. Where more than one set of suppressors was isolated from the same parent (FC7 and FC47) these have been separately disposed above and below the line. The vertical wavy lines show the limits of suppression in each particular set. The dashed vertical line gives the position of the minute barrier (see § 8 (*h*)). The list of mutants at the top of the figure is given in map order to assist in orienting the rest of the figure.




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that this phage contains a small duplication (see § 11 (*b*)). It is thus not a true exception to our rule that the suppressor is always a non-leaky *r*.

### (*d*) *Bias in the isolation procedure*

In general all revertants were *independent* isolates and the selection of revertants was unbiased. There are two exceptions to this statement.

(i) Due to the inexperience of one of us (F.H.C.C.) the revertants of P13 were picked in sets of three. Each set of three was independent of every other set, but the three members of each set were not necessarily independent.

It would be strictly good practice to accept only one mutant from each set. Instead we have accepted mutants from the same set if (by mapping or otherwise) they have been clearly shown to be distinct from each other. Otherwise only one was retained and the rest discarded. Mutants discarded in this way are FC13, FC16, FC17, FC24, FC25, FC26, FC100 and FC101, as noted in table A 1 (*b*).

(ii) The revertants of FC38 (FC149 to FC153) are a biased sample. They were obtained because we wished to isolate mutants of minus sign in the region of the map between FC88 and FC47. Extensive reversion of FC47 (which maps at the same site as FC38) had not thrown up any suppressors in this region but had produced many mutants at positions near FC6 or FC9. It was decided to produce a large number of revertants and to attempt to screen out those having suppressors on the left of the map.

Fifty independent spontaneous revertants were picked on K, purified on B and grown. Each was then spot-crossed against the double mutant (FC6+FC38), which has the wild phenotype. Any revertant which has its suppressor under or very close to FC6 is unlikely to give *r* recombinants in this cross. Of the 50 mutants, 28 failed to give a single *r* among about 2000 wild-type plaques. The remaining 22 were crossed against (FC9+FC38). Only eight gave any *r* plaques. These eight were then crossed against (FC21+FC38). Three failed to give any *r*s, but five gave at least one. These five revertants were then treated in the usual way and their suppressors isolated as FC149 to FC153. FC149 was discarded as being a high reverter.

It should be noted that this screening procedure did apparently eliminate suppressors on the far left of the map, although it allowed FC152 to slip through. Unfortunately, the only new site obtained by this procedure was FC151, and no new suppressor, of minus sign, was found between FC88 and FC38.

### (*e*) *Failure to obtain a usable suppressor*

There are three circumstances in which we have been unable to obtain a satisfactory suppressor.

(i) The *revertant* (picked on K) may throw off *r*s at a high rate, in spite of repeated purification. It has been shown that these unstable revertants are the result of a duplication of part of the *r*II B cistron which is eliminated at a high rate to generate the original *r* mutant (R. P. Freedman & S. Brenner, manuscript in preparation).

(ii) Some suppressors have the normal *r* phenotype, but revert to wild at a sufficiently high rate to make their mapping difficult. As is common practice, these mutants have been left on one side.


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(iii) Some revertants appear to be either true wild, or to have an extremely close suppressor. It is operationally very difficult to distinguish between these two alternatives and we have not always attempted to do so. To be accepted into this class the phenotype of the revertant must be indistinguishable from true wild; when crossed against wild very few *r*s must appear and most if not all of these *r*s must recombine with the original mutant. That is, most of them must not be the original mutant or one very close to it, but, presumably, the normal background of spontaneous mutants.

The number of suppressors isolated and the number of cases in which an acceptable suppressor was not found are listed in table 1.

TABLE 1. SUMMARY OF SUPPRESSORS

Abbreviations used: supp., suppressors isolated from doubles which were wild on K; d. min., suppressors isolated from doubles which were minute on K; wild, parent had apparently reverted to true wild; 'wild', suppressor very close indeed to parent: not isolated; r. rev., the suppressor had a high spontaneous reversion rate: not isolated; d. rev., the double had a high spontaneous reversion rate to the parent: not used; anom. anomalous (see § 11 (*b*)).

| parent | mutagen† | supp. | d. min. | wild | 'wild' | r. rev. | d. rev. | anom. |
|---|---|---|---|---|---|---|---|---|
| P13 ≡ FC0 | . | 16‡ | . | 1 | . | . | 1 | . |
| FC1 | . | 2 | 1 | . | . | . | . | . |
| FC6 | . | 10 | 1 | 1 | . | . | . | . |
| FC7 | . | 9 | . | 2 | . | 1 | . | . |
| FC7 | acridine yellow | 8 | . | . | 2 | . | . | . |
| FC9 | . | 10 | 1 | . | . | . | . | . |
| FC10 | . | 5 | . | . | 6 | 1 | . | . |
| FC11 | . | 8 | . | 2 | . | . | . | . |
| FC38 | . | 4¶ | . | . | . | 1 | . | . |
| FC42 | . | 8 | . | . | 1 | . | . | . |
| FC47 | . | 8 | . | . | . | 1 | . | . |
| FC47 | proflavine | 8 | . | . | . | 2 | . | . |
| FC47 | amino-acridine | 10 | . | . | . | . | . | . |
| FC87 | . | 9 | . | . | . | . | . | . |
| FC88 | . | 9 | . | . | . | . | . | . |
| P61 | . | 10 | . | . | . | . | . | 1 |
| FC202 | . | 10 | . | . | . | . | . | . |
| FC205 | . | 10 | . | . | . | . | . | . |
| FC222 | . | 9 | . | . | . | . | . | . |

† Spontaneous unless indicated otherwise.
‡ Obvious duplicates discarded.
¶ Others systematically discarded.

## 4. MAPPING THE MUTANTS

### (*a*) *The canonical set*

In mapping the mutants we have not attempted to place each and every mutant in a *precise* site on the genetic map, as the work involved in doing this would be very great, and would not be justified by the results. Instead we have mapped with precision a set of sixty-one of our mutants, which we shall call the canonical set. All other mutants have been *approximately* located on the map formed from the canonical mutants.

We have accepted a mutant as a canonical mutant if its known mapping behaviour is clearly different from all other mutants in the canonical set. In other words, each mutant not in the canonical set is, as far as we can tell, in a very similar position to one already in it.




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### (*b*) *Mapping the canonical set*

The map of the canonical mutants, shown in figure 2, has been constructed as follows. The mutants were originally crossed with each other until it was found which mutants were close together. This presented no special difficulty. The problem was then to order the mutants precisely.

It will be seen from the map that the mutants tend to fall into groups, the members of which are clearly near to each other. With one exception, no mutant in one group is *very* close to any mutant in another group, although the gaps between groups are not really very big. The exception concerns groups 3A and 3B. The group 997 . . . EM84 fell into two groups (997 . . . FC0 and FC28 . . . EM84) before FC152 was mapped. FC152 overlaps FC28 but is outside deletion PB296. It is also very close to FC0, and thus links the two groups together. As the resulting single group would be rather large we have arbitrarily maintained the original division into two groups but have called them A and B.

The groups are:

| | |
|---|---|
| 1 | Ac19 . . . . . P53 |
| 2 | A31 . . . . . 739 |
| 3A | 997 . . . . . FC0 |
| 3B | FC152 . . . . . EM84 |
| 4 | FC115 . . . . . FC54 |
| 5 | NT332 . . . . . FC47 and FC151 and FC87 |
| 6 | FC201 and 370 . FC222 |

We have included EM84 in group 3B because two-point crosses show that it maps closer to FC88 than to FC115 (see note added in proof, p. 532). Let us leave the mutants FC151, FC87 and FC201 temporarily to one side.

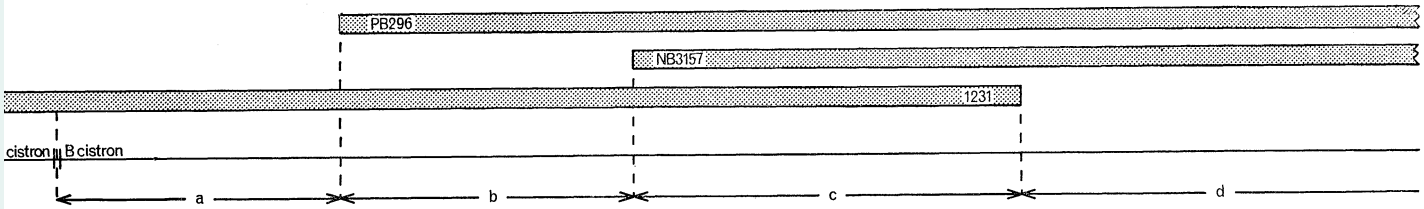


FIGURE 3. Deletion mutants which divide the genetic map up into regions a, b, c and d.

The major mapping of the groups has been done using the three deletions 1231, PB296 and NB3157. This divides the region into four segments called here a, b, c and d, as shown in figure 3. *All* the canonical mutants have been crossed against the relevant deletions. The results show the following.

(i) Group 6 is mainly in segment d, but FC34 and 370 are in segment c. This fixes the position and orientation of group 6.

(ii) Group 5 has NT332 in segment b and FC47 in segment d. This fixes the position and orientation of group 5.

(iii) Group 4 lies in segment b.

(iv) Group 3B is mainly in segment b but has FC152 in segment a. This fixes the position and orientation of group 3B.

(v) Groups 1, 2 and 3A all lie in segment a.


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URE 2. The genetic map of part of the B cistron of the *r*II locus in phage T4 Benzer. The canonical set (see § 4 (*a*)) are shown boxed and are given the same extent unless known to cover multiple sites. As far as possible distances between mutants are taken from the result of u.v. crosses. With unreliably small distances (recombination $\sim 1 \times 10^{-5}$) the two mutants are butted up against one another. With unreliably large distances (recombination $> 50 \times 10^{-5}$), or a spot-cross plus result, the gap shown is of arbitrary length. All the other mutants used in this study are listed at the site of a canonical mutant with which they show recombination of $< 10^{-5}$ in a u.v. cross; FC mutants above the line, others below. The group numbers 1 to 6, the regions a, b, c and d (see § 4 (*b*)) and some of the mapping deletions are indicated.

(*Pull-out to face p.* 498)

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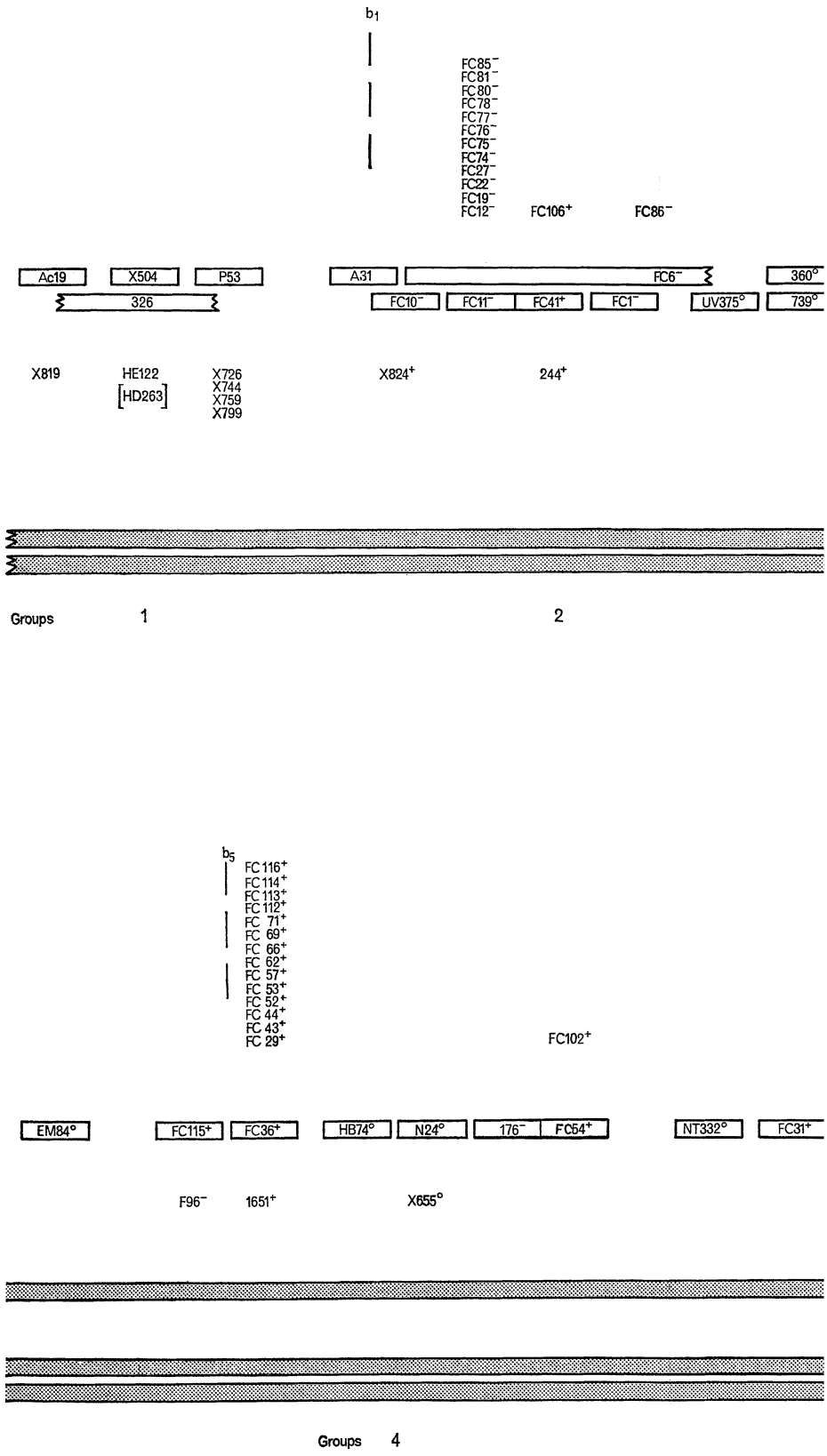
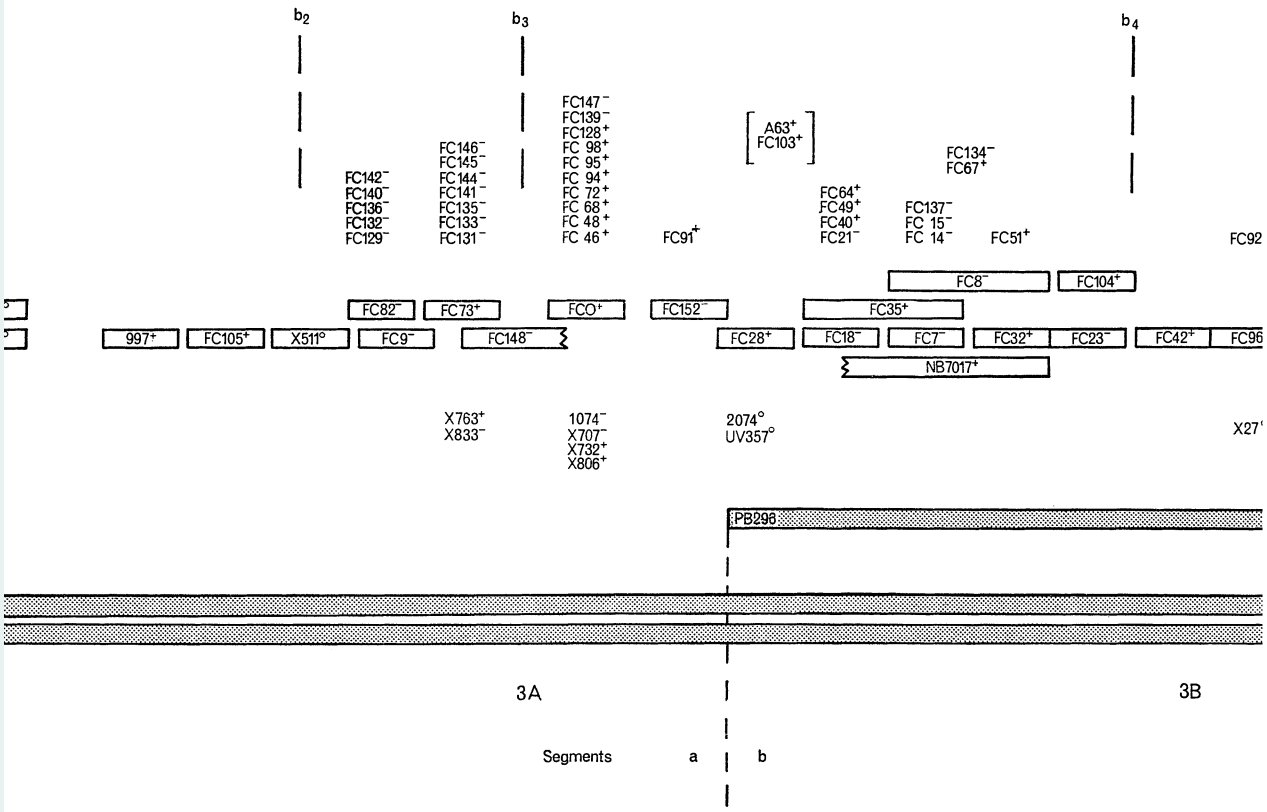
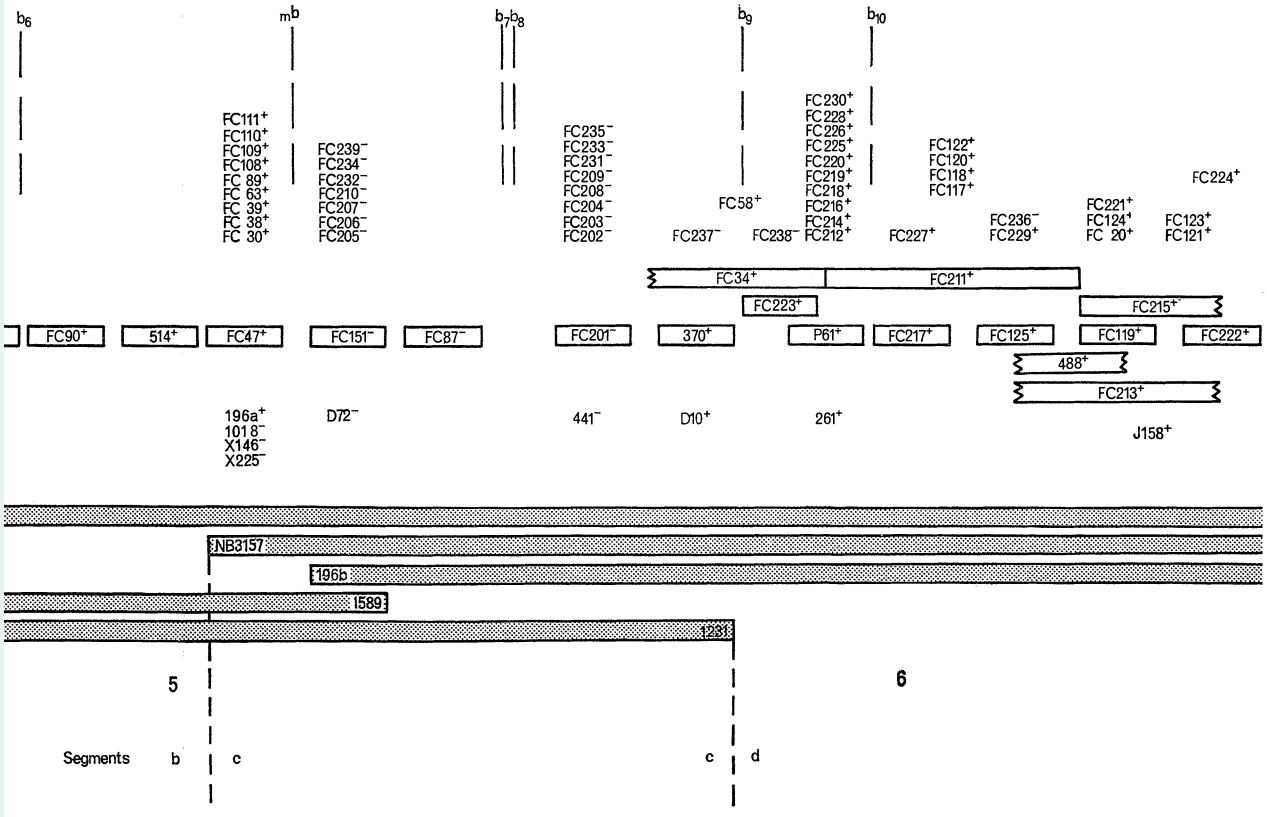

b1
FC85⁻
FC81⁻
FC80⁻
FC78⁻
FC77⁻
FC76⁻
FC75⁻
FC74⁻
FC27⁻
FC22⁻
FC19⁻
FC12⁻
FC106⁺
FC86⁻
Ac19
X504
P53
326
A31
FC6⁻
360°
FC10⁻
FC11⁻
FC41⁺
FC1⁻
UV375°
739°
X819
HE122
[HD263]
X726
X744
X759
X799
X824⁺
244⁺
Groups
1
2
b5
FC 116⁺
FC 114⁺
FC 113⁺
FC 112⁺
FC 71⁺
FC 69⁺
FC 66⁺
FC 62⁺
FC 57⁺
FC 53⁺
FC 52⁺
FC 44⁺
FC 43⁺
FC 29⁺
FC102⁺
EM84°
FC115⁺
FC36⁺
HB74°
N24°
176⁻
FC54⁺
NT332°
FC31⁺
F96⁻
1651⁺
X655°
Groups
4



FC153 −
FC150 −
FC143 −
FC127 −
FC126 −
FC 84 −
FC 83 −
FC 56+
FC 55+
FC 50+
FC 45+
FC 33+

2+

6+ FC88− 375°

7°

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The position of groups 1 to 3B and the orientation of groups 2 to 3B have been established by three-point crosses, the details of which are set out in table 2. They show (i) FC9 is between FC1 and FC23, (ii) P53 is *not* between FC1 and FC9. This establishes the order:

| FC1 | FC9 | FC23 |
|---|---|---|
| (group 2) | (group 3A) | (group 3B) |

and since FC23 is in segment b, whereas the first two are in segment a, this establishes the *order* of groups 2, 3A and 3B. Further three-point crosses (table 2) show: (i) FC55 is between FC40 and FC54, (ii) FC36 is between FC55 and FC54, (iii) FC54 is between FC36 and FC31. This gives the order:

| FC40 | FC55 | FC36 | FC54 | FC31 |
|---|---|---|---|---|
| (group 3B) | (group 3B) | (group 4) | (group 4) | (group 5) |

TABLE 2. TWO- AND THREE-POINT CROSSES WHICH ESTABLISH THE POSITION AND ORIENTATION OF SOME OF THE GROUPS WITHIN THE GENETIC MAP (§ 4(*b*))

| mutants crossed | recombination (%) |
|---|---|
| FC9 *v.* (FC1+FC23) | 0·004 |
| FC9 *v.* FC1 | 0·11 |
| FC9 *v.* FC23 | 0·08 |
| P53 *v.* (FC1+FC9) | 0·3 |
| P53 *v.* FC1 | 0·2 |
| P53 *v.* FC9 | 0·32 |
| FC55 *v.* (FC40+FC54) | 0·003 |
| FC55 *v.* FC40 | 0·08 |
| FC55 *v.* FC54 | 0·09 |
| FC36 *v.* (FC55+FC54) | 0·002 |
| FC36 *v.* FC55 | 0·28 |
| FC36 *v.* FC54 | 0·02 |
| FC54 *v.* (FC36+FC31) | 0·0009 |
| FC54 *v.* FC36 | 0·02 |
| FC54 *v.* FC31 | 0·14 |

All these mutants are in segment b, but FC31 is rather close to segment c. This establishes the position and orientation of groups 3B and 4. Thus we have established the position of all the groups except group 1 and the orientation of groups 3A, 3B, 4, 5 and 6, since 3A and 3B are linked together by FC152.

We can now place the three mutants FC151, FC87 and FC201. They all fall into segment c, and must thus lie between sets 5 and 6. Their relative positions have been found by two-point crosses (see table 3), bearing in mind that FC47 is in group 5 and FC34 in group 6.

In addition we have attempted to establish the orientation of the first three groups by the two-point and three-point crosses set out in table 4. The results are reasonably satisfactory for groups 2 and 3, but only moderately so for group 1. Apart from this the broad features of the map are well established. Note that the crosses in table 4 establish quite clearly the position of group 1.

The order of the mutants within the groups has been found by two methods. Where possible the mapping has been done by the overlapping-deletion method. For example

inspection of the map shows that the order of mutants from FC18 to FC23 (in group 3B) or for most mutants between 370 and FC222 (in group 6) can be fixed in this way. In other parts of the map we have been forced to rely on two-point crosses of the following type. For three mutants A, B and C it has been found that A is very close to B, and B very close to C, but that A and C are separated by a distance perhaps ten times or more as great. This has been taken as evidence that B is between A and C. Many examples can be found in table A 2(*a*). This technique has also been used by Tessman (1965). By this means we have been able to map the order of almost all the mutants without ambiguity.

TABLE 3. TWO- AND THREE-POINT CROSSES WHICH ESTABLISH THE POSITION OF MUTANTS NOT ORDERED BY DELETIONS (SEE § 4(*b*))

| mutants crossed | recombination (%) |
|---|---|
| FC151 *v.* FC47 | 0·01 |
| FC151 *v.* FC87 | 0·04 |
| FC47 *v.* FC87 | 0·09 |
| FC201 *v.* FC87 | 0·09 |
| FC201 *v.* FC34 | 0·004 |
| FC87 *v.* FC34 | 0·11 |

TABLE 4. TWO- AND THREE-POINT CROSSES WHICH ORIENT THE FIRST THREE GROUPS OF THE MAP (SEE § 4(*b*))

| mutants crossed | recombination (%) |
|---|---|
| Ac19 *v.* FC10 | 0·26 |
| Ac19 *v.* 739 | 0·44 |
| P53 *v.* FC10 | 0·06 |
| P53 *v.* 739 | 0·14 |
| 739 *v.* (FC1+FC9) | 0·0004 |
| 739 *v.* FC1 | 0·024 |
| 739 *v.* FC9 | 0·024 |
| FC105 *v.* (FC0+FC41) | 0·02 |
| FC105 *v.* FC0 | 0·10 |
| FC105 *v.* FC9 | 0·012 |
| FC105 *v.* FC41 | 0·05 |
| FC105 *v.* 739 | 0·008 |

The details of the genetic crosses of the canonical set are given in table A 2(*a*). '+' implies that the two mutants clearly show recombination using the usual spot cross. The figures show the recombination values, in units of $10^{-5}$, for standard u.v. crosses. A close study of this table will show that the map is nowhere seriously ambiguous. However, it has not always been possible to decide whether the recombination between two mutants is really zero or a genuine rather small number. Such uncertainty has little effect on the map except in the immediate vicinity of the two mutants. Whenever such details are important the table should always be consulted rather than the map, and when necessary further crosses should be done to establish the true recombination frequency. With this reservation we believe the map to be correct.

### (*c*) *Mapping the non-canonical set*

The relevant mapping information for each of the non-canonical mutants is set out in table A 2(*b*). In addition every non-canonical mutant has been mapped against the

(relevant) deletions mentioned earlier (results not shown in the tables). Table A 2(*b*) shows, for every non-canonical mutant, the canonical mutant it overlaps (using a u.v. standard cross) and the two canonical mutants, one to its left and one to its right with which it recombines in a spot-test cross. In some cases the table also shows which canonical mutants it is close to, but different from, as judged by a u.v. cross. This data is sufficient to place each non-canonical mutant in an approximate position on the map, as shown in figure 2. To define its position precisely, further crosses may or may not be needed, but in all cases it can be deduced from the map and the tables which further crosses are needed.

In addition we have crossed *every* mutant at or very near the end of a group with all mutants at or very near the adjacent end of the next group, to make sure that no mutant has been missed which might help to fill the gaps between groups. The details of these spot-test crosses are not tabulated here, as no further overlaps were found.

### (*d*) *Anomalies of mapping*

(i) *FC57* appears to give rather larger recombination distances with mutants near or on either side than one would expect from the estimated map lengths. This is also true to some extent of FC36. Moreover the map distance between FC57 and FC54 is 0·12%, whereas between FC36 and FC54 it is only 0·02%. In addition in certain triples having FC57 in the middle, such as (FC55+FC57+FC47), FC57 segregates more often than might be expected. This is not true of FC36. We have not explored these discrepancies further.

(ii) *A63 and FC103.* These two mutants, which each have sign plus, map together. However, careful crossing shows that A63 is close to FC35 ($4\times10^{-5}$, using u.v.), but some distance from FC152, FC28, FC18 and all other mutants in this region. This suggests that it lies between FC28 and FC35 on the map. In such a position, however, it should be very close to FC28, which it is not. Thus A63 appears to be somewhere in this region (it is under the deletion PB296, as are FC28, FC18 and FC35, whereas FC152 is not) but at abnormally large distances from other mutants. For this reason we have not included it in our canonical set.

### (*e*) *Comparison with previous map*

The map of figure 2 differs in two important respects from that given in figure 2 of our previous publication (Crick *et al.* 1961). In the first place the actual order of the mutants in that map was not entirely correct. The map given there is accurate, within the limitations there stated, from its left-hand end as far as FC33. The order of the mutants FC31, FC54 and FC36 (and their associated mutants) should be reversed, as should the order of FC34, FC87 and FC39 (and their associated mutants). Part of this confusion was caused by the peculiar behaviour of the deletion *r*196, discussed in § 11(*a*) of this paper, which gave misleading information about the position of certain mutants. Fortunately the main conclusions of our earlier paper were not upset by these errors in mapping.

In the second place our earlier map gave the impression that the mutants fell into close clusters with wide spaces between them. This appearance was partly due to the abnormally short distances found between adjacent mutants, which can give the appearance of clustering, and partly due to the limited number of mutants then available. Our present map (figure 2) shows that gaps on the map only occur in a few places, and are not of great

extent. However, our mutants are not apparently distributed at random, as some sites have many examples whereas others have only one or two. Moreover the distribution of the signs of our mutants does not appear to be random. All this suggests that phase-shift mutants are not made at all places with equal likelihood (Streisinger *et al.* 1966).

### (*f*) *The metric map*

The map of figure 2 is not metrical. That is, equal distances on the map do not necessarily correspond to equal recombination values.

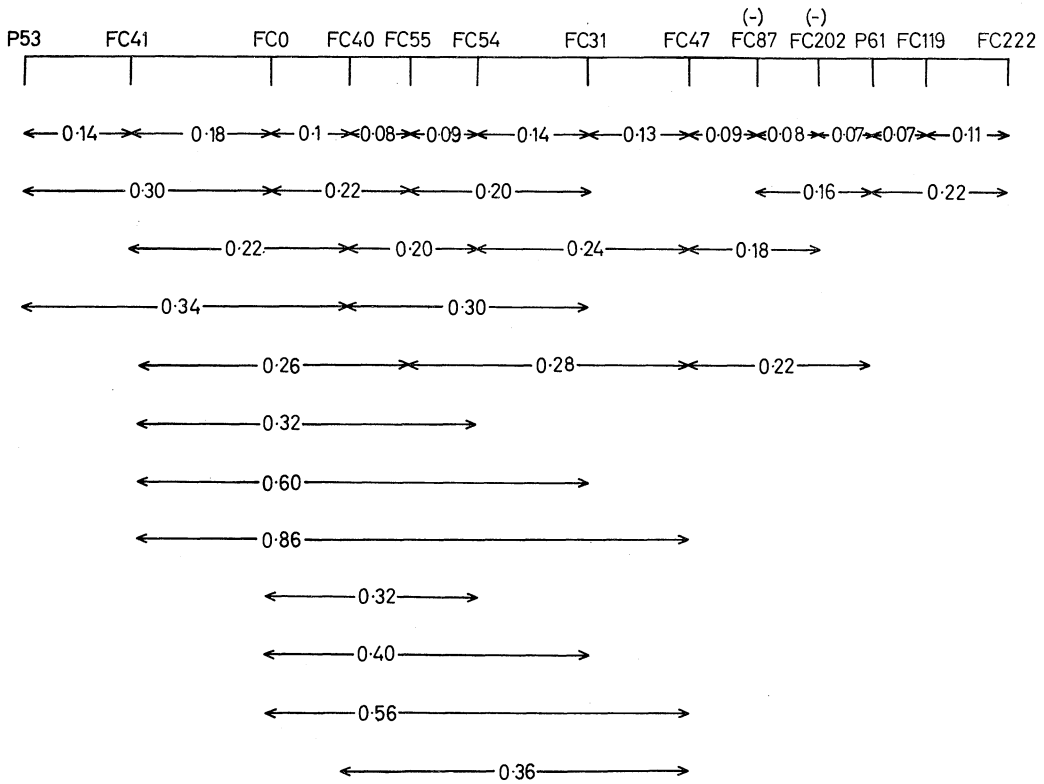


FIGURE 4. Metric map of some of the mutants. Map positions are shown along the top line. Except for P53 and two marked (−), the mutants have sign (+). The numbers underneath, with arrows indicating the distance covered, are the result of standard crosses expressed as the total percentage of recombinants (see § 4(*f*)).

For a selected set of mutants we have measured, using a carefully standardized technique, the recombination distances between pairs of mutants, *without* using u.v. The map distance is defined as the total percentage of recombinants. In many cases this is obtained by scoring the wild recombinants and doubling this figure. For example, when two plus mutants are crossed together the recombinants are likely to be equal numbers of true wilds and (++) doubles. In most cases the latter will not grow on K, and thus will not be scored. Thus to estimate the percentage of total recombinants from any cross it is necessary to know the phenotype of the recombinant which contains both parent mutants.

The results of some of these crosses are shown in figure 4 to illustrate the sort of map distances involved. As can be seen, the distances are reasonably additive, bearing in mind that the reproducibility of the figures is not very high.


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Comparison of the map of figure 2 with the metric map of figure 4 shows that on the left-hand side of the map, up to about FC54, the two maps are reasonably co-metrical. A recombination distance of, say, 0·1 % corresponds approximately to a similar distance on figure 2 in all parts of that region. To the right of FC54 the two maps also correspond approximately, but the scale now differs by a factor of about three in most places. That is, a distance of 0·1 % now corresponds to a much smaller distance on figure 2. Put another way, if figure 2 were made approximately metrical, the right-hand side of it would have to be expanded by a factor of three, except possibly between 370 and FC125.

### (*g*) *The absolute scale of the genetic map*

An examination of figure 2 shows that very roughly the region appears to have a length equivalent to about sixty 'sites', if we assume that mutants closely adjacent on the map occupy adjacent 'sites'. It is reasonable to ask if these sites correspond to adjacent bases on the *DNA*.

Unfortunately it is not easy to give a reliable answer to this question. It should be noted that, under certain circumstances, the position of a phase shift mutation may not be precisely defined. (No problem arises for a mutation of the base-substitution type.) Consider, however, the following case in which the wild-type sequence

...PQRAAAXYZ...

has an A deleted to become ...PQRAAXYZ....

Then clearly it is meaningless to ask which of the three As was deleted, since the result is the same in all three cases. In the same way, if the addition of a base is produced by a repetition of an adjacent base (which may well be a frequent occurrence), as it would be in the above example if the second sequence were the wild type and the first were the plus mutant, then again the precise position of the added base is uncertain. It is, in fact, rather surprising that we have not more mapping anomalies than we do have. However, the exact behaviour in a recombinational event between two sequences which differ because of phase-shift mutants will depend rather intimately on the precise mechanism of recombination, and this is not yet understood at this level.

Naturally if the *r*II B protein were available many interesting questions could be both asked and answered. In its absence we can only make a crude guess about the scale of the map. Stahl, Edgar & Steinberg (1964) have described a useful function for turning map distances in T4 into relative *DNA* distances. This can be put approximately on an absolute scale either by using the known total length of the *DNA* or by comparing the map length of the lysozyme genes with the known size of the polypeptide chain of lysozyme, since the genetic code shows that three bases code one amino acid. In their hands the lysozyme gene has a recombinational distance of 3 %. The corresponding figure for the B cistron of the *r*II locus is 4·3 % (Edgar *et al.* 1962). Phage lysozyme is believed to contain about 150 amino acids, corresponding to 450 base pairs. A detailed consideration of these numbers, using figure 6 of Stahl *et al.* (1964), suggests that the B cistron may contain about 700 base pairs.

From other mapping experiments the distance between FC0 and FC47 appears to be 25 to 30 % of the length of the B cistron, and could thus cover about 200 base pairs. This




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suggests that each of our 'sites' may correspond to about half a dozen base pairs. This is perhaps surprising since our map superficially gives the impression of having many sites rather close together, with relatively few gaps, as if each site corresponded to a single base pair. However, the production of a phase-shift mutant may well not be uniformly likely at all points on the genetic map, especially if it tends to occur (as suggested above) where a base is repeated several times. In addition the difficulties of recombination between phase-shift mutants may appear to spread such a mutant on the map. On the other hand there are many reasons why the calculation given above may not be as reliable as it seems at first sight. It has been shown by Yanofsky that for the A protein of tryptophan synthetase of *E. coli* recombination of base-analogue mutants can take place between adjacent bases (Guest & Yanofsky 1965), and it would not be surprising if this occurred also for T4.

If the proportionality of map distance to *DNA* distance is maintained at this scale a separation of a single base pair would correspond to about 0·003% recombination (without u.v.). We have many examples of mutants separated by this distance. However, Tessman's results (Tessman 1965) for base-analogue mutants suggest that when two mutants are very close together the percentage of recombinants between them is unexpectedly low. It seems unlikely that this issue will be decided for our region until the *r*II B protein is available.

## 5. Establishing signs

### (*a*) *Allocating signs*

As explained earlier the signs of all FC mutants are defined by their method of origin. It was, however, necessary to show that the signs allocated assuming P13 (≡ FC0) as plus were the same as those allocated taking P61 as plus. This was done by constructing the two doubles:

$$(\underset{-}{\text{FC87}}+\underset{+}{\text{P61}}) \quad \text{and} \quad (\underset{+}{\text{FC47}}+\underset{-}{\text{FC205}}),$$

both of which were found to have the $r^+$ phenotype on K. Since FC87 and FC47 were derived ultimately from P13, and since FC205 was derived from P61, either of these doubles establishes that the two sign conventions are consistent.

We have been able to establish the sign of all mutants mapped except for a set of twelve mutants at the extreme left-hand end of the map, close to P53. Three of these mutants are likely to have sign zero. X504 and HE122 are base-analogue revertible and the former is an ochre mutant and the latter an amber. HD263 is a leaky mutant, induced with hydroxylamine and is also base-analogue revertible. The remaining nine mutants are likely to be of the phase-shift type since none is base-analogue revertible and seven of them were induced with acridine dyes. We believe that we cannot determine the sign of any of these mutants because there is a double barrier (i.e. a barrier to shifts in either direction) between this region and the FC6 region. (The concept of barriers is explained in § 7(*c*).) This is plausible *because no FC mutant falls into this region*. In particular, no suppressor of FC1, FC6, FC10 or FC11 maps there, suggesting that there is a barrier for shifts to the left. Nor do any of the suppressors of FC0, FC42 or FC47 fall there, although many of these map in the region covered by FC6, suggesting that there is also a barrier for shifts to the right.

Moreover the 'reversion' of P53, A31 and Ac19 occurs at a very low rate and many of the revertants appear to be produced by a duplication (R. P. Freedman & S. Brenner, manuscript in preparation). This again may be because the postulated barrier prevents the normal method of 'reversion' (by forming suppressors) and unmasks a rarer type of behaviour. The doubles ($\underset{?}{\text{P53}}+\underset{+}{\text{FC41}}$) and ($\underset{?}{\text{P53}}+\underset{-}{\text{FC9}}$) have the *r* phenotype. The double ($\underset{?}{\text{P53}}+\underset{-}{\text{FC6}}$) is very minute on K. We have not attempted to form doubles using the other mutants in this set.

The sign of all other mutants was established by forming doubles with mutants of known sign and observing their phenotype. The wild-type doubles are set out in table 5. By this means a mutant was either shown to be plus or minus, or it appeared to be neither of these. These latter mutants, of which we have about fifteen examples, were given sign zero. All have been shown to revert with base-analogue mutagens. All, except X655, are either ambers or ochres. (See *added in proof*: p. 517).

Thus apart from the mutants at the extreme left-hand end of the region *we have no phase-shift mutants to which we are unable to give either the sign plus or the sign minus.*

TABLE 5. WILD-TYPE DOUBLES WHICH PROVE THE SIGNS OF NON-FC MUTANTS

| double | signs | sign deduced | | double | signs | sign deduced | |
|---|---|---|---|---|---|---|---|
| (X824+FC1) | + − | X824† | + | (FC10+514) | − + | 514 | + |
| (244+$a_2$+FC9) | + − | 244 | + | (FC10+196a) | − + | 196a | + |
| (FC1+997) | − + | 997 | + | (FC90+1018) | + − | 1018 | − |
| (FC10+X763) | − + | X763 | + | (FC90+X146) | + − | X146 | − |
| (X833+FC47) | − + | X833 | − | (FC90+X225) | + − | X225 | − |
| (1074+FC47) | − + | 1074 | − | (D72+FC119) | − + | D72 | − |
| (X707+FC40) | − + | X707 | − | (441+FC119) | − + | 441 | − |
| (FC10+X732) | − + | X732 | + | (FC87+370) | − + | 370 | + |
| (FC10+X806) | − + | X806 | + | (FC87+D10) | − + | D10 | + |
| (FC10+A63) | − + | A63 | + | (FC87+P61) | − + | P61 | + |
| (FC10+NB7017) | − + | NB7017 | + | (FC87+261) | − + | 261 | + |
| (F96+FC47) | − + | F96 | − | (FC87+488) | − + | 488 | + |
| (FC10+1651) | − + | 1651 | + | (FC87+J158) | − + | J158 | + |
| (176+FC38) | − + | 176 | − | | | | |

† These mutants are listed in the order in which they occur on the genetic map.

### (*b*) *Checking signs*

In order to eliminate mistakes and to give us greater confidence in our sign allocation a special spot-test was devised to give some check on the signs of all the mutants in our set. Each mutant was crossed with either the double mutant

$$(\underset{(-)}{\text{FC1}}+\text{deletion PB296})\ldots \quad \text{test } (a)$$

or with

$$(\text{deletion 1231}+\underset{(+)}{\text{FC125}})\ldots \quad \text{test } (b),$$

or in many cases with both of these. The results were scored on K as either 'wild', 'minute' or 'none'. The interpretation of these crosses is in outline quite simple (see figure 5). For example, suppose a mutant of sign plus in the middle of the map is crossed with the double (FC1+deletion PB296). Then the only way that a phage that will grow on K can be produced is by the recombinant double mutant of FC1 (−) with the plus mutant. If the mutant to be tested were of minus sign no recombinant could be produced which would




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grow on K. Because of the complications of barriers and of minutes the implications of the results depend on the region of the map in which the mutant lies. This can only be fully appreciated after these topics have been discussed. The implications of the test are set out in table 6. It suffices to say that with four exceptions no mutant gave a result which contradicted what was expected, and that the signs of all mutants in the middle of the map (that is, all those under both deletion 1231 and deletion PB296) were checked unambiguously. The exceptions were four mutants at the extreme right-hand end of the region, namely 488, FC123, FC215 and FC224. These would have been expected to have given minutes on test (*a*), whereas in fact they gave nothing (see § 7(*b*)).

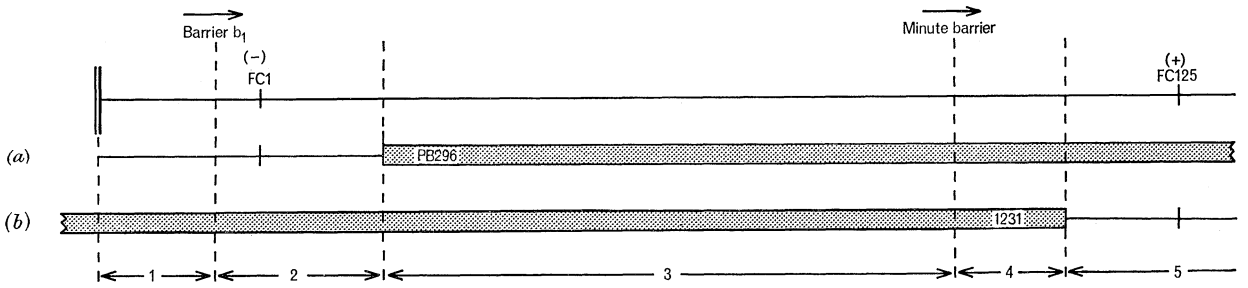


FIGURE 5. Tests used to check the signs of mutants (§ 5(*b*)). The positions of FC1 and FC125 are shown in relation to the two deletions with which they have been combined to form doubles (*a*) and (*b*). Every mapped mutant was tested against one or other of these doubles and against both of them where relevant. The expected results are shown in table 6.

TABLE 6. TEST USED IN CHECKING THE SIGN OF *r* MUTANTS

| region (see figure 5) | recombination test (*a*) | recombination test (*b*) | conclusion (sign) |
|---|---|---|---|
| 1 | . | none | ? |
| 2 | . | none | + or 0 |
| | . | minute | − |
| 3 | wild | none | + |
| | none | none | 0 |
| | none | minute | − |
| | wild | minute | + (m) |
| 4 | minute | none | + |
| | none | none | 0 |
| | none | wild | − |
| 5 | minute | . | + |
| | none | . | ? |

Test (*a*), cross with double mutant ($\overline{\text{FC}}$1+deletion PB296).
Test (*b*), cross with double mutant (deletion 1231+$\overset{+}{\text{FC}}$125).

## 6. THE PRODUCTION OF DOUBLE MUTANTS

### (*a*) *Methods of listing doubles*

We have produced, by various methods, several hundred double mutants. By double mutants we mean phages which contain two mutants which by themselves have the mutant phenotype. We have therefore included as 'doubles' phages such as (FC0+$a_4$ -FC88), which has three changes, one of which is the removal of a barrier. This classification while not strictly logical is convenient. These doubles have been listed in two ways:

(i) In an index (table A 3), in double dictionary order. The dictionary order used has the following conventions for *letters*:

| | |
|---|---|
| | FC comes first |
| then | no letter |
| then | other letters in normal alphabetical order. |

Numbers follow letters, in numerical order. Thus a typical ordering is: FC1, FC6, FC222, 370, N24, X511. Each double is entered *twice*. For example the double (FC6+FC32) is entered as (FC6+FC32) *and* as (FC32+FC6). The index thus shows instantly all the doubles which have been made with any chosen mutant, whether they have been isolated or not. It also lists the sign of each mutant, the direction of phase-shift (where applicable), and the *approximate* phenotype, described as $r^+$, *r* or m.

(ii) In addition, there are separate tables for each class of double, under the following headings:

| | | | |
|---|---|---|---|
| $(+\ -) \leftarrow r^+$ | table A 4(*a*) | $(-\ +) \rightarrow r$ | table A 4(*e*) |
| $(+\ -) \leftarrow r$ | (*b*) | $(+\ +)$ *r* | (*f*) |
| $(-\ +) \rightarrow r^+$ | (*c*) | $(+\ +)$ m | (*g*) |
| $(-\ +) \rightarrow$ m | (*d*) | $(-\ -)$ *r* | (*h*) |

In each of these tables the mutants are listed in *map* dictionary order. That is, a mutant on the left of the map takes precedence over one to its right. Thus each double is listed only once in these tables. Under its entry is shown a rather more precise description of its phenotype. Its method of manufacture is also listed. The mutant which occurs first in each entry in the tables is printed in bold type in the index.

### (*b*) *Methods of obtaining doubles*

The doubles have been manufactured by the following methods.

(i) By the isolation of suppressors: for example, FC1 was isolated as a suppressor of FC0, from the double (FC0+FC1). The method is described in the section on the origin of these mutants (see § 3(*a*)(ii)).

(ii) When two mutants of opposite sign suppress each other they were crossed together. The recombinants were plated on K. Three phenotypes are possible for the recombinant.

(*a*) Wild phenotype on both B and K. In some cases, the recombinants were first screened by orgy crosses (see § 2(*d*)(iv)). More usually a suspected double mutant was crossed against true wild. If it was a double mutant it gave the original *r* parents as recombinants. If it did not, this showed that it was not a double but a true wild. The process was repeated until the double mutant was found.

(*b*) Wild on K, but pseudo-wild on B. This enables one to distinguish the double mutant from the equal number of true wild. The double was checked by backcrossing to wild, and showing that the original *r* parents were produced as recombinants of this cross.

(*c*) Minute on K and *r* on B. In most cases these were assumed to be the double. In a few cases this was checked by backcrossing.

(iii) When two mutants of opposite sign do not suppress each other, so that the double has the *r* phenotype, the method for isolating the double is laborious. The two mutants

were crossed together, using u.v. if necessary, and plated on B. Plaques of *r* phenotype were picked, purified and spot-crossed to the two parents. Most of these were one or other of the parents but eventually a double mutant was found by this method.

If the mutants were very close they were in some cases grown and u.v.-crossed several times to increase recombination before looking for the double.

(iv) (*a*) By crossing together two mutants of like sign and plating the recombinants on K to see whether minute plaques were present. These were isolated as described under (ii) (*c*) above.

(*b*) If the double mutant was not minute but did not grow on K, it was isolated as described under (iii) above.

(v) By segregation from a cross of a triple mutant (in one case a quadruple) against wild, and by spot-crossing to identify the double. This is the preferred way to make doubles of close mutants of like sign.

(vi) By the reversion of a (+ −) double containing a barrier. For example the 'double' (FC73+$a_3$+FC23) was obtained by plating (FC73+FC23) on K, and picking a revertant. In some cases the revertant was spontaneous; in others 2-aminopurine was used to increase the mutation rate.

(vii) By a miscellaneous series of tricks. These were not used very often. They have been noted in every case in the tables. For example, the (+ −) double (FC36+176) was produced by crossing 176 against the (+ +) double (FC36+FC54). FC54 and 176 map very close together so that wild-type recombinants from this cross are likely to be (FC36 +176). The presumed double was checked by backcrossing.

## 7. The character of double mutants

### (*a*) *Combinations of like sign*

We have tested many double mutants of like sign. In no case have we found one with the wild phenotype. All the combinations (− −) we have tried are listed in table A 4(*h*). None of them grows on K. The (+ +) combinations are of two types. Some do not grow on K, and some grow but give minute plaques (tables A 4(*f*) and (*g*)). We shall return to this latter class in § 9.

### (*b*) *Combinations of unlike sign*

We have also constructed many doubles from mutants of *un*like sign. In discussing these we must distinguish clearly between those with the mutant of minus sign on the left of its companion on the map (− +), and those with the minus on the right (+ −). Of the former type it is fair to say that most combinations grow normally on K.

However, no mutant in the group on the extreme left of our map will combine with any other mutant we have tried, to give a double which grows normally on K. None of these mutants was picked up as suppressors. As explained in § 5(*a*), we have thus been unable to allocate a sign to any of these mutants.

All (− +) combinations we have tried, no matter where the mutants are located on the map, will give a normal wild phenotype on K except any combination having its minus mutant to the left of FC151 and its plus mutant to the right of FC151. These give minute plaques on K (table A 4(*d*)), with the exception of four plus mutants (488, FC123,

FC215 and FC224) on the extreme right of the map. These latter do not produce plaques on K if combined with minus mutants on the left of the map. To get an idea of the extent of this phenomenon one of these plus mutants, FC215, was combined with a series of minus mutants along the length of the map. With FC88 the double formed was *r*-type and did not grow on K even at low temperature although it produces lysis when large numbers of plaques are plated. Farther to the right, the double with 176 was also *r*-type but grew on K with minute plaques. All the (− +) doubles tested are listed in table A 4(*c*), (*d*) and (*e*).

With double mutants of type (+ −) the situation is quite different. Apart from those combinations which occurred during the isolation of suppressors it is rather rare to find a double which grows on K, especially if the two mutants are some distance apart.

### (*c*) *The concept of barriers*

Our explanation of this is the same as that outlined in our earlier paper (Crick *et al.* 1961), namely that the shift in reading frame has produced an 'unacceptable' triplet, that is, one which for some reason will not allow the efficient construction of a polypeptide chain. Since the phase of reading depends upon whether the reading frame is shifted to the right (− + combination) or to the left (+ − combination) this explains in a natural manner why this distinction is important. We shall refer to those places where these unacceptable triplets occur as 'barriers' and will denote them by the letter b with suitable prefixes and suffixes. Barriers for shifts to the right will have a prefix, and thus we shall have:

$_1$b the barrier to the left of FC10,

$_m$b the barrier which produces minutes, near FC151,

$_2$b the barrier at the extreme right of the map.

We have made no serious attempt to study the characteristics of $_1$b or $_2$b. The minute barrier $_m$b (see § 8(*h*)) is not strictly a barrier since it produces minutes on K, rather than no growth at all.

Barriers for shifts to the left are denoted by a b with a suffix. Thus the first barrier, to the left of FC1 is denoted by $b_1$, the two near FC0 as $b_2$ and $b_3$, that near FC23 as $b_4$, etc. As explained below in § 8(*a*) we have in all cases been able to decide, with reasonable certainty, whether a barrier is single or multiple. The only clear multiple case, near FC202, we have assumed to be double, and labelled it ($b_7+b_8$). The barriers, together with some of the mutants used to characterize them, are shown in figure 6. They are described in detail in § 8.

### (*d*) *The phenotype of double mutants of unlike sign*

The approximate phenotype on B of double mutants of unlike sign is listed in table A 4(*a*) and (*c*). We consider here only those doubles which give the wild phenotype on K. The phenotypes are described as $r^+$ if they are indistinguishable from the wild phenotype, as '*r*' if they resemble the *r* phenotype, and as '$r^+$' in intermediate cases. Unfortunately the classification is not very precise and in the sequence $r^+$, '$r^+$', '*r*' a phenotype may be mistaken for its neighbour. However, it is unlikely that an $r^+$ phenotype will be scored as '*r*', or *vice versa*. We have examined these results to see if they fall into any significant

pattern, but if they do it is not obvious at first sight. For example, it might be expected that the larger shifts would give a more *r*-like phenotype than the shorter ones. This is certainly not always true. Thus the short shift (FC9+FC40) is scored as '*r*', whereas the long shift (FC9+FC47) is scored as $r^+$. To test more elaborate schemes the data would have to be considerably extended. We have not pursued the matter farther.

## 8. The barriers described in detail

### (*a*) *Introduction*

We have examined each barrier to see whether it is single, and have tested whether or not it is an ochre or an amber. We have also studied its reversion characteristics, and have roughly mapped the revertant to make sure that it has occurred in the expected place. In several cases we appear to have two barriers very close together and this has made characterization more difficult.

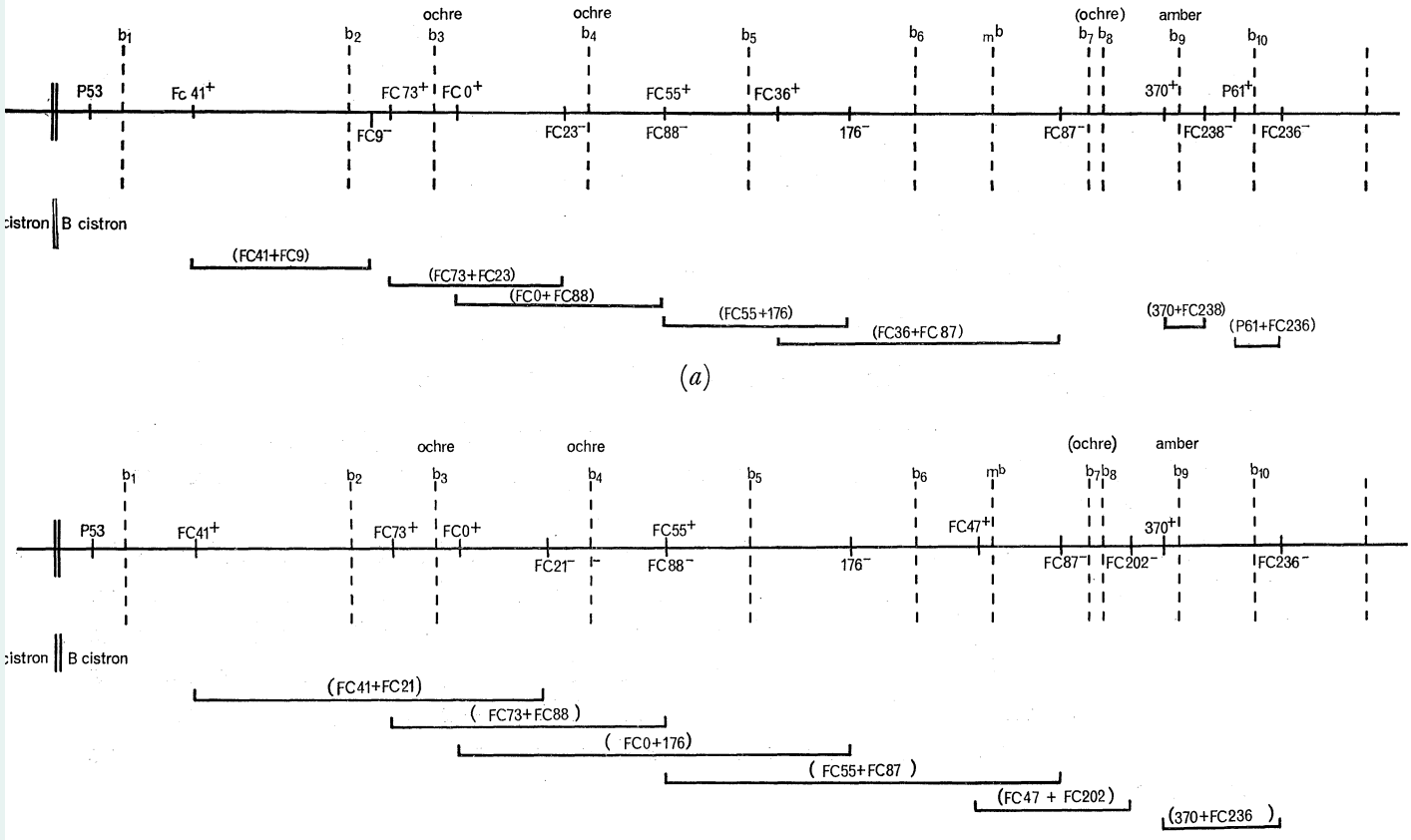


Figure 6. The position of barriers along the genetic map. See § 7 (*c*) for nomenclature. The mutants used to form doubles for barrier reversion studies are shown with their approximate map positions. No double could be made which spanned either $b_7$ or $b_8$ alone because these barriers are very close together. (*a*) Doubles spanning one barrier: $b_2$, (FC41+FC9); $b_3$, (FC73+FC23); $b_4$, (FC0+FC88); $b_5$, (FC55+176); $b_6$, (FC36+FC87); $b_9$, (370+FC238); $b_{10}$, (P61+FC236). (*b*) Doubles spanning two barriers: $b_2$ and $b_3$, (FC41+FC21); $b_3$ and $b_4$, (FC73+FC88); $b_4$ and $b_5$, (FC0+176); $b_5$ and $b_6$, (FC55+FC87); $b_7$ and $b_8$, (FC47+FC202); $b_9$ and $b_{10}$, (370+FC236).

In characterizing a barrier it is important to realize two points:

(i) A mutant very close to a barrier may modify the codons at that point, and thus alter the barrier. Thus to characterize a barrier unambiguously a mutant close to it should not be used.

(ii) A mutant may itself produce an unacceptable triplet in any one of the three reading frames. Thus before it is used to characterize a barrier it must be possible to show that it works (i.e. produces a phage which grows on K) *in the frame in which it is used for testing the barrier.*

These precautions aside, our method of showing that a barrier is single is that it can be reverted to an acceptable codon by a base-analogue mutagen—usually 2-aminopurine—at a reasonable rate. We have checked that a combination spanning *two* (or more) single barriers will *not* revert in this way. The combinations tested are set out in figure 6(*b*).

In the following sections we discuss each barrier or pair of barriers in turn, and give the evidence for the multiplicity of each barrier and its location on the genetic map. The barrier $b_4$ is described first to make the exposition simpler. For the same reason the experimental methods in this subsection are given in more detail. The other barriers follow in numerical order. The main results are displayed in figure 6(*a*). In § 8(*i*) we give what evidence we have on the nature of the triplets which produce the various barriers.

### (*b*) *Barrier* $b_4$

No mutant was picked up as a revertant of FC0 to the right of FC23. This suggests that there is (+ −) barrier at or near FC23. The double (FC0+FC88) will not grow on K, though it does grow somewhat on one or two $su^+$ strains, showing that it contains an ochre (see table 10, § 8(*i*)). The double (FC42+FC88) has the wild phenotype, proving that FC88 itself works satisfactorily in a ← frame. FC0 is known to work in the zero frame. All these facts suggest that there is at least one barrier somewhere between FC23 and FC88.

The double (FC0+FC88) was shown to revert well with both 2-aminopurine and 5-bromodeoxyuridine. This shows that only a single barrier is present. A revertant was picked and purified. This was called (FC0+$a_4$+FC88), the $a_4$ designating the presumed 'altered barrier' derived from barrier $b_4$. To prove this description it was crossed to wild, and the progeny was plated on B. A total of 23 *r* plaques was picked and purified. Some of these were FC0 or FC88, but others were (FC0+$a_4$) and ($a_4$+FC88). They could be identified by backcrossing each of them with FC0, FC88 and (FC0+FC88). The expected behaviour of the segregants and the number of each one isolated is shown in table 7(*a*). We deduce from this that $a_4$ is not very close to either FC0 or FC88, but rather nearer, if anything, to FC88. This is where we expected $b_4$ to be.

In this case (but not in other cases) the identification of the mutants was doubly checked by resynthesizing (FC0+$a_4$+FC88) by crossing together (FC0+$a_4$) and ($a_4$+FC88). Some of the wild type arising from this cross were shown to be (FC0+$a_4$ +FC88) by backcrossing to true wild and isolating the *r* parents. Approximately half the wild type arising, however, when backcrossed to true wild gave no *r* plaques at all. As both the parents in this cross contained the reverted barrier $a_4$ this proves that $a_4$ is wild in the unshifted frame.




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TABLE 7

(*a*) *Behaviour of segregants and number isolated, from the cross* (*FC*0+$a_4$+*FC*88) *versus wild*

| | FC0 | FC88 | (FC0+FC88) | no. | |
|---|---|---|---|---|---|
| (FC0+$a_4$) | 0 | + | + | 4 | 6 |
| FC88 | + | 0 | 0 | 2 | |
| ($a_4$+FC88) | + | 0 | + | 8 | 17 |
| FC0 | 0 | + | 0 | 9 | |

The + denotes that wild or pseudo-wild recombinants are produced

(*b*) *Segregants from the cross* (*FC*73+$a_3$+*FC*23) *versus wild*

| type | no. | |
|---|---|---|
| (FC73+$a_3$) | 2 | 13 |
| FC23 | 11 | |
| ($a_3$+FC23) | 0 | 0 |
| FC73 | 0 | |

(*c*) *Segregants from the cross* (*FC*41+$a_2$+*FC*9) *versus wild*

| type | no. | |
|---|---|---|
| (FC41+$a_2$) | 0 | 0 |
| FC9 | 0 | |
| ($a_2$+FC9) | 13 | 19 |
| FC41 | 6 | |

### (*c*) *Barriers* $b_2$ *and* $b_3$

The double (FC41+FC21) does not grow on K, whereas (FC0+FC21) is wild. This suggests that there may be a (+ −) barrier between FC41 and FC0. The reversion of the double (FC41+FC21) is scarcely increased, if at all, by 5-bromodeoxyuridine or 2-aminopurine, but the latter has been shown to revert the double to an ochre. Thus one suspects that the barrier may be double. Unfortunately, the map distance is too small to make a rigorous proof possible. We proceed as follows. The double (FC73+FC23) will not grow on K, though it does grow on several *su*$^+$ strains, which shows that it contains an ochre (see table 10, § 8(*i*)). Its reversion to wild is greatly increased by 2-aminopurine. We call this revertant (FC73+$a_3$+FC23), and characterize it by crossing to wild, picking *r*s and backcrossing. The results are shown in table 7 (*b*) and indicate that $a_3$ is very close to FC73. Thus from this evidence alone we are unable to show whether the barrier exists when FC73 is not there, or whether it is created (or modified) by FC73.

The double (FC41+FC9) does not grow on K. Its reversion is increased by 2-aminopurine. The revertant is called (FC41+$a_2$+FC9). Crossing to wild and backcrossing the progeny gave the result in table 7(*c*), showing that $a_2$ is very close to FC9. Again we cannot say from this whether the barrier $b_2$ exists when FC9 is not there.

Thus there appears to be a double barrier near FC9 and FC73. We cannot characterize it with complete certainty, since the two barriers are too close to be separated unambiguously. However, the reversion of (FC41+FC21) to an ochre by 2-aminopurine supports this interpretation.

### (*d*) *Barrier* $b_5$

(FC55+176) does not grow on K, but (FC57+176) does. This shows that 176 works in the ← frame. That FC55 works in the zero frame is proved by the triple (FC55+FC57 +FC47) which grows on K. This triple and several others show that FC57 cannot be to the right of the $b_5$ barrier.

The triple (FC57+FC54+FC47)—and several others of this type starting with FC57—also grows on K. This shows that FC57 cannot be to the left of $b_5$. The most likely explanation of these two facts is that FC57 coincides with $b_5$, and abolishes the barrier in doing so.

The mapping of the base-analogue revertant of (FC55+176) (not detailed here) shows that the barrier is not very close to either FC55 or 176, and is roughly equidistant from both of them. This is what one would expect if $b_5$ coincided with FC57.

### (*e*) *Barrier* $b_6$

This is quite straightforward. (FC57+FC87) does not grow on K, whereas (FC57+176) and (FC47+FC87) do. This shows that there is a barrier somewhere between 176 and FC47.

(FC90+FC87) grows on K, as does the triple (FC36+FC31+FC47), whereas (FC31+FC87) does not, suggesting that the barrier is close to FC31 and FC90.

(FC57+FC87) reverts with base analogues, and mapping the revertant $a_6$ shows it to be not very near either FC57 or FC87, but nearer to the latter than the former, as it should be if it were close to FC31 and FC90.

A cross made with (FC36+$a_6$) and ($a_6$+FC87), isolated from the backcross of (FC36+$a_6$+FC87), gave wild-type recombinants. Some of these were shown to be the double mutant with barrier $b_6$ removed but approximately half of them gave no *r* at all when backcrossed to wild. Since both the parent *rs* contained the reverted barrier $a_6$ this shows $a_6$ to be wild in the unshifted frame.

### (*f*) *Barriers* $b_7$ *and* $b_8$

The double (FC47+FC202) will not grow on K. It is not reverted appreciably by 2-aminopurine if the induced stocks are plated on the standard K strain (KB). On the other hand (FC47+FC87) does grow on K, as does (FC38+FC151). Thus we presume that there are two or more barriers between FC87 and FC202. Unfortunately, we have no suitable mutants available in this region so that we have been unable to split this multiple barrier into single barriers. However, reversion studies, using special host strains, show that two barriers are present and we have therefore called them $b_7$ and $b_8$.

The existence of two barriers was shown as follows. Stocks of (FC47+FC202), induced to revert with 2-aminopurine, were plated on strain KB1, or CA165, and in both cases revertants were obtained (table 8(*a*)). KB1 is a strain which permits the growth of a class of leaky mutants, while CA165 contains an ochre suppressor; table 8(*b*) shows that these two revertants are different. From each of these revertants, further revertants capable of growing on strain KB may be obtained and in each case the reversion is induced by 2-aminopurine. It therefore appears that (FC47+FC202) can be altered by two successive base-analogue induced mutations to give a phage capable of growing on strain KB, and that two different paths can be followed, through an ochre or through a *leaky* revertant.

To see whether this implies the existence of two separable barriers, the *leaky* revertant (FC47+FC202/3) and the ochre (FC47+FC202/1) were crossed and the progeny plated on strain KB. No increase in wild-type phages was observed, but the results were difficult




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to record because of the leakiness of one of the parents. If there are two separate barriers they are very close to each other.

Another complication is that the barriers are very close to FC202. This was shown as follows. When the double revertant of (FC47+FC202), capable of growing on KB, was crossed with wild, *r*-type segregants were obtained. All were either FC47 or ($a_7+a_8$ +FC202).

TABLE 8. REVERSION OF (FC47+FC202)

(*a*) Mutagenesis

| host | control | 2-aminopurine |
|---|---|---|
| KB | $<10^{-8}$ | $<10^{-8}$ |
| KB1 | $4\times10^{-8}$ | $3{\cdot}7\times10^{-5}$ |
| CA165 | $1\times10^{-7}$ | $1{\cdot}3\times10^{-5}$ |
| host | control | hydroxylamine |
| KB1 | $1\times10^{-7}$ | $2\times10^{-6}$ |
| CA165 | $1{\cdot}6\times10^{-7}$ | $1{\cdot}2\times10^{-7}$ |

(*b*) Characteristics

| double | KB | KB1 | CA165 |
|---|---|---|---|
| (FC47+FC202) | 0 | 0 | 0 |
| (FC47+FC202/3) | leaky | + | leaky |
| (FC47+FC202/1) | 0 | 0 | + |

### (*g*) *Barriers $b_9$ and $b_{10}$*

This is somewhat similar to the case of $b_2$ and $b_3$. The double (370+FC236) does not grow on K. Its reversion is not increased by 2-aminopurine. This makes one suspect there may be two barriers there. The distance is too small to make a rigorous proof possible. However, the doubles (370+FC238) and (P61+FC236), both of which fail to grow on K, can be reverted at least 100-fold by 2-aminopurine. This suggests that $b_9$ is between 370 and FC238 and $b_{10}$ between P61 and FC236. The double (370+FC238) grows on several *su*$^+$ strains, showing that $b_9$ is an amber (see table 10, § 8(*i*)).

It has not been possible to map the revertant $a_9$ because (370+$a_9$+FC238) cannot be split by backcrossing to wild. Mapping shows, however, that the revertant $a_{10}$ is roughly equidistant from P61 and FC236.

The two barriers have also been characterized by reversion studies. The double (370+FC236), containing the two barriers, can be reverted in two distinct ways.

(i) It reverts to grow *on K* at a low frequency, about $10^{-8}$. This frequency is not increased by base-analogues. The plaque morphology of the revertants cannot be distinguished from true wild. Three such independently arising revertants were backcrossed to wild using u.v.; no *r*s were found amongst 3000 plaques in each case. This shows that neither of the original mutants is present in the revertant, and we suggest that the revertant is due to a deletion which removes both mutants and the two barriers between them.

(ii) If (370+FC236) is plated on CR63 it reverts to an amber and this reversion is induced by 2-aminopurine. This is likely to be due to the removal of barrier $b_{10}$ (see § 8(*i*)). The amber barrier $b_9$ can then be removed from this phage by a further mutation; again, this is induced by 2-aminopurine. When this doubly mutated phage (370+$a_9$+$a_{10}$ +FC236) was crossed with wild the following segregants were obtained: 370, (370+$a_9$), (370+$a_9$+$a_{10}$), ($a_9$+$a_{10}$+FC236), ($a_{10}$+FC236) and FC236, proving the constitution of the original phage.


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### (*h*) *The minute barrier* $_{m}b$

This barrier has been reverted, but it has not been studied in great detail.

(FC1+FC123) was treated with 2-aminopurine and wild-type plaques selected on strain D0 at 42° C to cut down the leakiness of this double (see § 9 (*b*)). One of these was backcrossed to true wild and shown to contain only FC1 and FC123. Several of the FC1 isolated from this cross gave $r^+$ recombinants with (FC1+FC123), showing them to have lost the barrier and to be (FC1+$_{m}$a). One of these was crossed with (1231+FC125), a sign-tester double discussed in § 5 (*b*). Only wild-type recombinants were observed in this cross, whereas a control cross of FC1 with (1231+FC125) gave only minute recombinants. Since true wild cannot be formed, owing to the presence of the deletion 1231, this shows that the minute barrier has been removed and the double of FC1 with FC125 is now wild type.

TABLE 9. CHARACTERISTICS OF BASE-ANALOGUE MUTANTS

| | hydroxylamine† | characteristics | comments |
|---|---|---|---|
| HD263 | induced | temperature-sensitive | |
| HE122 | . | amber | |
| X504 | . | ochre | converted to amber by 2AP‡ |
| UV375 | . | ochre | converted to amber by 2AP |
| 360 | induced | ochre | converted to amber by 2AP |
| X511 | . | ochre | converted to amber by 2AP |
| 2074 | . | amber | |
| UV357 | . | ochre | converted to amber by 2AP |
| X27 | . | ochre | converted to amber by 2AP |
| 375 | induced | ochre | converted to amber by 2AP |
| EM84 | induced | amber | |
| HB74 | induced | amber | |
| N24 | induced | ochre | converted to amber by 2AP |
| X655 | induced | non-suppressible | (see *added in proof:* p. 517) |
| NT332 | . | amber | |

† This is a forward induction by hydroxylamine.
‡ 2AP, 2-aminopurine.

### (*i*) *The nature of the barriers*

The two close barriers, $b_7$ and $b_8$, have not been studied further. As already stated, one appears to be leaky and the other an ochre. Nor have we tried to characterize further barrier $b_1$ or the minute barrier, $_{m}b$, for shifts to the right. The remaining seven barriers have been studied to try to characterize the triplets responsible for their unacceptability.

A good deal of evidence suggests that the amino acid sequence controlled by the first part of the B cistron is not critical for the function of the protein. The deletion, *r*1589, which removes segments B1 to B3 and substitutes the first part of the A cistron still preserves B activity (Champe & Benzer 1962*b*) and a phase-shift to the right over most of the region as in (FC1+FC47) is without effect on the function. A further striking property is that all but two of the base-analogue mutants in this region are either amber or ochre mutants (table 9). One of the exceptions, HD263, is temperature-sensitive. It therefore appears likely that most, if not all, amino acid substitutions in the region do not effectively alter the function of the protein and that the only mutants which can be detected are those with drastic effects. This would explain the strong representation of chain-terminating mutants in the base-analogue revertible spectrum. We might therefore expect that barriers would also be mainly of this type. Table 10 shows that of the seven barriers

studied only one, $b_9$, is suppressed by amber suppressors, while two others are suppressed by ochre suppressors although the suppression of $b_4$ is poor. The amber and ochre barriers have the same properties as amber and ochre mutants in the unshifted frame. They are

TABLE 10. GROWTH ON SUPPRESSOR STRAINS

| | | | amber suppressors | | | ochre suppressors | | |
|---|---|---|---|---|---|---|---|---|
| barrier | mutant | *su*⁻ CA244 | $su_I^+$ CA266 | $su_{II}^+$ CA180 | $su_{III}^+$ CA265 | $su_B^+$ CA165 | $su_C^+$ CA167 | $su_D^+$ CA248 |
| $b_2$ | (FC41+FC9) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $b_3$ | (FC73+FC23) | 0 | 0 | 0 | 0 | + | poor | + |
| $b_4$ | (FC0+FC88) | 0 | 0 | 0 | 0 | v. poor | 0 | + |
| $b_5$ | (FC55+176) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $b_6$ | (FC36+FC87) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $b_9$ | (370+FC238) | 0 | + | + | + | poor | poor | + |
| $b_{10}$ | (P61+FC236) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Stocks containing about $2 \times 10^9$ phages/ml. were streaked on the strains plated in top agar. The plates were incubated at 30 °C and scored for appearance of plaques after 24 h.

TABLE 11

(*a*) *Reversion of ochre and amber barriers to* $r^+$

| | | frequency $\times 10^{-7}$ | | frequency $\times 10^{-7}$ | |
|---|---|---|---|---|---|
| barrier | mutant | control | 2-aminopurine | control | hydroxylamine |
| $b_3$ | (FC73+FC23) | 0·04 | 55 | 0·69 | 1·1 |
| $b_4$ | (FC0+FC88) | 0·06 | 750 | 0·69 | 0·69 |
| $b_9$ | (370+FC238) | 0·04 | 1000 | 2·2 | 10·0 |

(*b*) *Reversion of ochre barriers to amber by 2-aminopurine*

| | | control | 2-aminopurine | |
|---|---|---|---|---|
| barrier | mutant | $r^+$ and amber | $r^+$ | amber |
| $b_3$ | (FC73+FC23) | 0·08 | 106 | 14 |
| $b_4$ | (FC0+FC88) | 0·07 | 880 | 2·6 |

Stocks grown in the presence of 2-aminopurine were assayed on K and B to measure induction to $r^+$. The same stocks were then plated with CA266 ($su_I^+$) in a top layer over CA244 ($su^-$), and turbid plaques scored. Results are recorded as frequency $\times 10^{-7}$. See § 2 for details of this and other methods.

TABLE 12. REVERSION OF BARRIERS AND X655 WITH 2-AMINOPURINE

| barrier | mutant | control, $r^+$ frequency | 2-aminopurine, $r^+$ frequency |
|---|---|---|---|
| $b_2$ | (FC41+FC9) | 0·27 | 245 |
| $b_5$ | (FC55+176) | 0·10 | 184 |
| $b_6$ | (FC36+FC87) | 0·31 | 100 |
| $b_{10}$ | (P61+FC236) | 0·14 | 310 |
| | X655 | 0·34 | 350 |

Frequency is entered as $10^{-7}$. See § 2 (*e*) for methods.

induced to revert with 2-aminopurine and are not reverted by hydroxylamine (Brenner, Stretton & Kaplan 1965). In particular the two ochre barriers can be converted into amber barriers by 2-aminopurine (table 11 (*a*) and (*b*)).

The remaining four barriers appear to be different. We are confident that they are not ambers, since they fail to respond to three different strong amber suppressors. However,


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ochre suppressors are weak (Brenner & Beckwith 1965) and it is just possible that the barriers might be poorly suppressed ochres.

The only point mutant in the unshifted frame with similar properties is X655 (tables 9, 12). A further study of this mutant and the four barriers shows that they all have the following properties: (i) they revert spontaneously to wild type. This reversion is strongly increased by 2-aminopurine (table 12); (ii) they are not induced to revert to wild type with hydroxylamine (table 13).

TABLE 13. REVERSION OF BARRIERS AND X655 WITH HYDROXYLAMINE

| | | frequency $\times 10^{-7}$ | |
|---|---|---|---|
| barrier | mutant | control | hydroxylamine |
| $b_2$ | (FC41+FC9) | 4·3 | 4·1 |
| | | 0·9 | 1·0 |
| | | 1·2 | 1·4 |
| $b_5$ | (FC55+176) | 1·4 | 2·1 |
| | | 2·2 | 3·3 |
| | | 1·0 | 6·1 |
| $b_6$ | (FC31+FC87) | 4·5 | 5·2 |
| | | 2·7 | 2·8 |
| | (FC36+FC87) | 1·7 | 3·9 |
| $b_{10}$ | (P61+FC236) | 3·1 | 3·9 |
| | | 2·5 | 4·7 |
| | X655 | 2·3 | 5·2 |

See § 2 (*e*) for method of hydroxylamine mutagenesis

The first result shows that the mutants can be corrected by single base substitutions and, in particular, by transitions. Their insensitivity to hydroxylamine shows that the transitions are unlikely to be of the G — C → A — T type. The effects of these mutants must be expressed during protein synthesis because the barriers are produced by a phase-shift, and X655 is abolished by one (table A 6). This shows that they cannot be one of the signals controlling mRNA synthesis. The triplets responsible for these mutants either contain no G — C pairs, or if they do they are connected to other unacceptable triplets by G — C → A — T transitions.

As pointed out previously, we think it unlikely that the unacceptability of these mutants is due to an amino acid substitution. One amino acid substitution which might have a drastic effect is cysteine. However, the codons for cysteine are UGU and UGC, and these should be susceptible to hydroxylamine. We are inclined to believe that the barriers and X655 are due to a nonsense codon. Further work now in progress (not reported here) suggests that this codon may be UGA. *Added in proof:* Brenner, Barnett, Katz & Crick (1967) have shown that $b_2$, $b_5$, $b_6$, and X655 are all UGA.

## 9. MINUTES

### (*a*) *The description of minutes*

Minutes are phages which while growing on both B and K have unusually small plaques on the latter. There is a whole range of sizes, down to plaques which are so small as to be almost invisible. The minuteness of the plaques is also often influenced by temperature. They are usually larger at 30 than at 37 °C. The phenotype on B is *r*.

Although minutes have a range of plaque size on K it is fortunate that we have never had any difficulty in distinguishing the wild-type phenotype from the various grades of minutes, even when they are on the large side, since all minutes on K give plaques with sharp edges which are quite different from the turbid edges of a wild-type plaque.

### (*b*) *Types of minutes*

Our minutes fall into three classes.

(i) Minutes of the type $-$ (+) which have the minus to the left of FC151 and the plus to the right of it, as listed in table A 4(*d*). All such doubles are minute except those made with certain plus mutants on the extreme right (488, FC123, FC215 and FC224), as already stated in § 7(*b*). Doubles of FC1 with any of these four mutants, while not producing plaques, will produce lysis of K when large numbers of phages are plated.

We can say little about the mechanism. It may be that the shift produces an unacceptable amino acid, such as perhaps a cysteine, or alternatively the chain may be terminated but starts again at a low rate. We have not investigated this further.

(ii) Minutes of the type (++++). These are listed in table A 5(*c*), together with other quadruples which have the *r* phenotype. These have only been found to be minute when FC57 is included. Thus the (++++) mutant FC(0+40+57+31) is minute on K whereas FC(0+40+36+31) does not grow on K, in spite of the fact that FC57 and FC36 are both the same sign (+) and map at the same point. In addition, the ($-$++) triple (FC88+FC57+FC47) is minute on K (table A 5(*d*)). It will be recalled that FC57 shows mapping anomalies. We have no explanation of these minutes.

(iii) Minutes of the type (++). These are listed in table A 4(*g*). With a few exceptions such doubles are minute on K if they span barrier $b_6$ or the double barrier ($b_7$ and $b_8$). The detailed rules are discussed in § 9(*d*) below. In almost all other cases (++) doubles do not grow on K. As already stated, we have *no* case of a (++) double which has the wild-type phenotype on K. In view of the association of minutes with doubles spanning barriers we have tested all barriers for this property. The results are summarized in the next section.

### (*c*) *The association of barriers with minutes*

(i) *Barriers $b_2$ and $b_3$*

These cannot be tested rigorously because the only plus mutant between them, FC73, is rather close to both of them. However, neither FC41 nor 244 give minutes (at either 30 or 37 °C) when either is combined with FC0, FC28, FC40 or FC32. This suggests that $b_2$ does not help to produce minutes. All these mutants have been shown, in other doubles, to work in the relevant frame.

Even though FC73 is rather close to $b_2$ and $b_3$ one can still use it to see what happens when it is present. It does not produce minutes, either at 30 or 37 °C, when combined with either FC41 or 244, or again with FC28, FC40 and FC32. Thus all this evidence suggests that neither $b_2$ nor $b_3$ produces minutes.

(ii) *Barrier $b_4$*

Most (++) doubles straddling this barrier do not give minutes. Examples include either FC0 or FC40 combined with FC55, FC33 or FC92, tested at both 30 and 37 °C.


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However, FC91, combined with any one of these last three mutants, does give minutes. These minutes were only discovered recently and we have not studied them further.

(iii) *Barrier* $b_5$

This does not appear to help in the production of minutes. No minutes are found with FC55, FC96 or FC42 combined with either FC54 or FC31, either at 30 or 37 °C.

(iv) *Barrier* $b_6$

Many (++) double mutants straddling this barrier give minutes. These are discussed in more detail in § 9(*d*) and (*e*).

(v) *Barriers* ($b_7$ *and* $b_8$)

Many combinations of FC47 with plus mutants to the right of it are minute. This suggests that either $b_7$ or $b_8$ can help produce minutes. The likelihood of $b_7$ or $b_8$ producing minutes is supported by the fact that FC47 combined with either 370 or FC58 is minute.

(vi) *Barriers* $b_9$ *and* $b_{10}$

These two barriers do not appear to give minutes. No double of 370 with either P61, FC217, FC125, FC211, FC119 or FC222 is minute. Nor is either FC58 or P61 with FC125.

### (*d*) *Conditions necessary to produce minutes*

We tentatively explain the (++) minutes by assuming that when the reading mechanism encounters certain barriers it behaves, at a low rate, as if it had encountered a plus mutant. The low rate explains why the plaques on K are minute. Not all barriers appear to have this property, which is mainly associated with barrier $b_6$ and barriers ($b_7$ + $b_8$).

It is important to notice that our hypothesis implies that after passing the barrier there is a shift in reading frame. Consequently any subsequent barriers in the same frame which may be encountered are not registered as such, because the frame has changed. Conversely any barriers for the → shift of reading frame must be allowed for. The only barrier of this latter type is the barrier to → shifts near FC151 which, as we have seen, produces minutes. Thus a double (++) which spans both $b_6$ and the → barrier is likely to be *very* minute on K, or even not grow at all, in which case it is of course classed as *r*.

For minutes associated with barrier $b_6$, the first plus cannot be to the left of $b_5$, or this barrier will itself produce the *r* phenotype. The possible combinations of plus mutants are shown in table 14, which lists the expected phenotypes predicted by our hypothesis.

This table shows that good minutes are likely to be of two types only:

(i) Where the first plus lies on $b_5$ or between $b_5$ and $b_6$, and the second between $b_6$ and the → (minute) barrier.

(ii) Where the first plus lies between $b_6$ and the → (minute) barrier and the second anywhere to the right of ($b_7$ and $b_8$). The phenotype of all the (++) mutants to the right of $b_4$ is shown in figure 7. It will be seen that almost all our results fit these predictions. The only important exception is that no double containing either FC54 or 514 at the left is minute.

It is natural to ask if the failure of FC54 to give minutes is due to an unfavourable codon produced by FC54. However, the (+−) double (FC54+FC87), which includes the




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barrier $b_6$, reverts 100-fold with 2-aminopurine which suggests that FC54 is normal in the zero frame. In addition the triple (FC54+FC31+FC47) has the $r^+$ phenotype, which suggests the same thing.

The (++) double (FC54+FC90) which was expected to be minute on K, but actually does not grow on it, was plated on K and a minute spontaneous revertant picked. This was named (FC54*+FC90). From this FC54* was segregated and various other doubles constructed. Those with FC90, 514 or FC47 were minute, or very minute, on K, whereas (FC54*+FC31) did not grow at all on K. Thus FC54* behaves as FC54 was expected to behave. We have not explored this further.

TABLE 14. PHENOTYPE EXPECTED FROM (++) DOUBLE MUTANTS

| $b_5$ | $b_6$ | $_mb$ | $b_7$ $b_8$ | phenotype |
|---|---|---|---|---|
| ++ | | | | *r* |
| + | + | | | *r* |
| + | | + | | *r* |
| + | | (+)† | | *r* |
| + | | | +‡ | *r* |
| | ++ | | | *r* |
| | + | + | | minute |
| | + | (+) | | (very minute or *r*) |
| | + | | + | very minute or *r* |
| | | ++ | | *r* |
| | | + (+) | | (*r*) |
| | | + | + | minute |
| | | (+) | + | (minute) |
| | | | ++ | *r* |

† No plus mutants found here.
‡ Plus mutants in this column can be anywhere to the right of barriers $b_7$ and $b_8$.

### (e) *The effect of barrier removal*

If the presence of a barrier is a necessary condition for the production of a (++) minute, then removal of the barrier by mutation should abolish the minute phenotype. This appears to be the case for minutes produced by the barrier $b_6$. The evidence is documented below. Attempts to carry out similar experiments for plus mutants spanning the ($b_7$+$b_8$) barrier have failed because of our inability to separate ($a_7$ and $a_8$) from FC202.

The phage (FC31+$a_6$) was isolated as a segregant of (FC31+$a_6$+FC87), which was a spontaneous revertant of (FC31+FC87). This was u.v.-crossed with FC47 and a phage of presumed constitution (FC31+$a_6$+FC47) was isolated. This phage does not make

minutes on K and has the *r* phenotype. No recombinants were obtained with either (FC31 + $a_6$) or FC47. A cross with FC31 yielded minutes, thus proving that the double contains FC47. The phage also gave pseudo-wild recombinants with X146 and also with

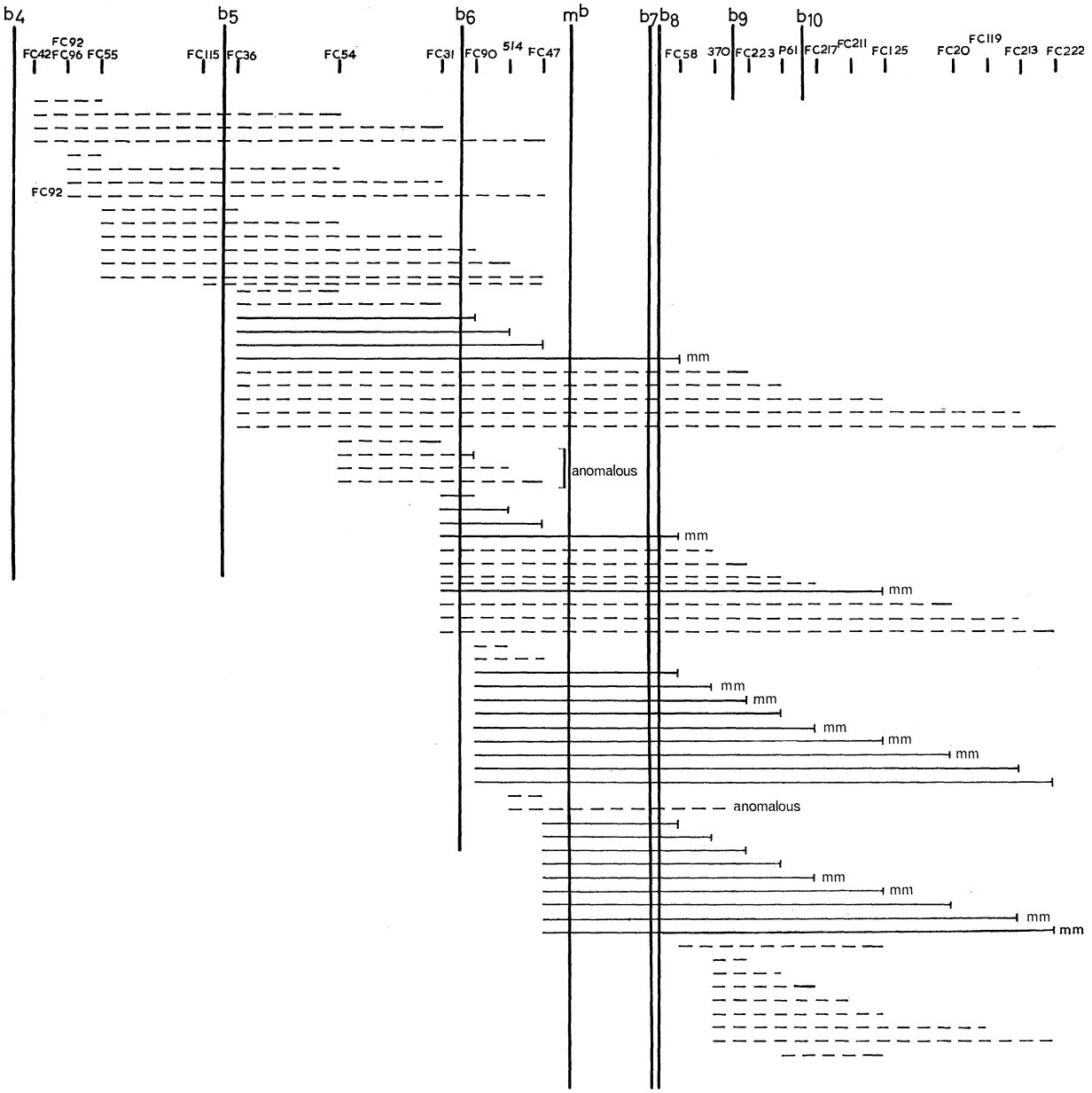


FIGURE 7. Schematic diagram of (+ +) doubles. Selected mutants are shown on the top line in their approximate map positions. Lines are drawn between these points to show which doubles have been made; – – –, *r*-type; ——, minute or very minute (mm). Heavy vertical lines represent the positions of some of the barriers (see § 9(*d*)).

(FC31 + X146) which showed that it contains FC31 and also $a_6$. When (FC31 + $a_6$ + FC47) was crossed with FC36 both wild type and *minute* recombinants were found. The wild-type recombinants included true wilds and triples with the constitution (FC36 + FC31 + $a_6$ + FC47).




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In a separate experiment (FC36+$a_6$) was isolated as a segregant of (FC36+$a_6$+FC87), itself an *independent* spontaneous revertant of (FC36+FC87). When this was crossed with (FC31+$a_6$+FC47) *no* minutes were found, only wild-type recombinants and triples.

These experiments show that at least two independent spontaneous revertants of the barrier $b_6$ remove the ability of plus mutants spanning it to make minutes. Other experiments have shown that base-analogue-induced revertants of $b_6$ behave in the same way.

### (*f*) *The explanation of minutes*

The experiments just described suggest that for doubles spanning the barrier $b_6$ the presence of the barrier is necessary for the production of minutes. The evidence previously discussed makes it likely that the barrier is due to nonsense rather than to an unacceptable amino acid, though the nonsense appears to be different from the amber and ochre chain-terminating codons. The barrier must be associated with a phase error in order to generate the minutes.

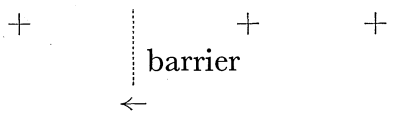
One possibility is that the reading halts at the barrier and a plus phase error occurs with low frequency at the site. This might be the case if there were no sRNA corresponding to the barrier triplet in the phage-infected cell. However, we note that production of minutes is not a universal property of the non-suppressible barriers since (++) mutants straddling $b_2$, $b_5$ and $b_{10}$ are not minute. If reading halts at $b_6$ zero errors should be possible as well and (+−) doubles straddling the barrier should be minute, which is not the case. The phase-error hypothesis requires that barrier $b_6$ has very special properties.

The other possibility is that the barrier is chain-terminating nonsense and that reading can begin to the right of it at a low frequency. The initiation would have to require a special configuration which may not be present with the other barriers. Furthermore, the initiation has to be in a fixed frame, in this case minus, to compensate the plus to the right of the barrier. A similar explanation could apply to the minutes straddling the double barrier ($b_7$+$b_8$) since it is possible that one of these barriers is due to a chain-terminating ochre codon.

## 10. Combinations of three or more mutants

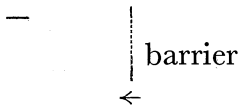
### (*a*) *Triples*

Wild-type triples can be constructed from three plus mutants provided there is no barrier between the first and second mutant sites. Two plus sites are equivalent to a minus site and a barrier in this position would cause a forbidden shift.




A barrier in any other position will have no effect, although if the second and third plus straddle the minute barrier $_m$b the triple would be expected to be minute. We have one example of this (FC0+FC40+FC58).

Similarly with minus mutants a wild-type triple can be formed from any three mutants provided there is no barrier between the second and third mutant sites.



Again, if the first and second minus straddle $_{m}$b we should expect the triple to be minute. We have not constructed any triple of this type.

If forbidden shifts are made the triple will be mutant *r*-type. Many of these barriers have, however, been removed (see § 8) and successful wild-type triples made which cross these points. We somewhat arbitrarily describe these phages as triples even though they may contain in addition to three phase-shift mutants one or more changes which remove the barriers. A list of triples is given in table A 5(*a*) and (*b*) with details of their phenotype and isolation.

*Methods of isolation.* (i) If two *r* doubles (a+b) and (b+c) which have one site in common are crossed together, no true wild recombinants can form and so the triple (a+b+c) if wild type, can be easily isolated. (If no wild-type plaques are seen in such a cross the triple can reasonably be assumed to be *r*.) Many of our triples were isolated in this way but very often the necessary double mutants were not available.

(ii) The main alternative method used was to cross a double mutant (a+b) with a single mutant c lying outside the double. In this cross recombination will give true wild as well as the wild-type triple and each isolate has to be verified.

With both methods of isolation the resultant triple was usually backcrossed to true wild to show that all three *r* mutants were present. From this cross doubles can also be picked up, and the frequency with which all recombinants occur is an indication of the distance apart of the three mutants used.

TABLE 15. SEGREGANTS FROM THE CROSS OF (FC0+FC40+FC38) WITH WILD

| segregant | no. | segregant | no. |
|---|---|---|---|
| FC0 | 4 | (FC40+FC38) | 4 |
| FC40 | 1 | (FC0+FC38) | 1 |
| FC38 | 20 | (FC0+FC40) | 18 |

Several other methods were used in specific cases to isolate triples and these are listed in footnotes to table A 5(*a*) and (*b*). It was often possible by careful examination of the phenotypes to pick out the wild-type triple. This was very often larger than the true wild and sometimes almost *r* on B.

As an example we shall describe in detail one of the first plus triples made, (FC0+FC40+FC38), which was rather thoroughly checked. It was constructed by crossing (FC0+FC40) with (FC0+FC38), both of which had been made by isolating an *r* double from a cross of the *r* parents. These doubles had been identified by spot crosses to the parents and were checked by u.v. crossing them to each of their parents. (FC0+FC40) was further checked by crossing with FC21. Wild-type recombinants were picked from this cross and shown by backcross to true wild to be the double (FC0+FC21). FC21 is a minus mutant at the FC40 site and suppresses FC0, which is plus, forming a wild-type double. True wild was not formed.

The triple (FC0+FC40+FC38) was backcrossed to true wild and the progeny obtained are listed in table 15. This is the sort of result one would expect from their position on the map.

One of the FC0 segregants arising from this cross was checked by u.v. crossing it with the original FC0 (P13). No recombination took place. The same FC0 was crossed with

FC9, which is a minus mutant, and the wild-type suppressed pair (FC0+FC9) isolated. This was backcrossed to true wild and by spot crosses shown to contain only FC0 and FC9. An exactly analogous check was made on one of the FC40 and one of the FC38 segregants. A special check was also made with one of the doubles arising from the triple backcross; (FC0+FC40) gave no recombination on a u.v. cross with FC0, FC40 or with the original isolate of (FC0+FC40).

### (*b*) *Quadruples*

Our theory predicts that no quadruple composed of mutants of like sign will have the wild-type phenotype. We have no exception to this rule. However, some of the quadruple (+) mutants isolated were *r*-type on B but gave minute plaques on K. These exceptional cases have already been mentioned in § 9(*b*).

Two wild-type triples, containing mutants of the same sign, can be crossed together and recombinant *r* types selected. If triples have two mutants in common, the only *r*-type recombinants will be the quadruple and a double. By spot-crossing to the constituent *r* singles the double is easily identified. It only remains therefore to make sure that the suspected quadruple contains all four *r* singles. If the quadruple is crossed in turn with each single which it contains the only wild-type recombinant from the crosses will be a triple, because true wild cannot be made. Each of these triples can therefore in their turn be isolated and backcrossed to true wild for a simple identification by spot crosses of the single and double *r* mutants segregated. Sometimes this technique can be short-circuited by tricks of one sort or another. Some examples of these will be given in the detailed isolation of a sextuple given below. Table A 5(*c*) lists all quadruples made from triples.

### (*c*) *Sextuple*

#### (i) *Introduction*

Our theory predicts that a sextuple containing six mutants of like sign will be wild type provided no forbidden shifts are made. The map (figure 2) shows a very even distribution of plus mutants and many wild-type triple mutants can be constructed. The possibilities for making triples from minus mutants are more restricted and often require the removal of one or more barriers (table A 5(*a*)). A sextuple can therefore be more easily constructed from plus mutants.

#### (ii) *Details of construction*

The steps followed in the construction of the sextuple are outlined in table 16. The strategy is to alternate the phenotype of the phages in each stage so that the progeny can be distinguished from the parents. This can be done except for the first step of constructing doubles from singles. Thus wild-type triples are constructed from *r*-type doubles, and *r*-type quadruples from the wild-type triples. The same strategy is used in the decomposition of multiple mutants.

Six single sign-plus mutants were chosen which span most of the region. These are FC0, FC40, FC55, FC36, FC31 and FC47. (FC0+FC40) was constructed by crossing FC0 and FC40, isolating *r* progeny and spot-test crossing these against both parents. The double was found after 342 plaques were tested. The triple (FC0+FC40+FC55) was derived from a cross of (FC0+FC40) and FC55, by isolating wild-type recombinants and back-

crossing these to true wild. The backcross also generated the doubles (FC40+FC55) and (FC0+FC55).

(FC36+FC31) was derived by crossing FC36 and FC31 and testing *r* progeny by crossing them against both parents. (FC90+FC47) was constructed in a similar way. FC90 was chosen because it maps very close to FC31 but unlike FC31 does not produce minutes when combined with FC47.

TABLE 16. CONSTRUCTION OF THE SEXTUPLE

| | mutants | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| stage | + | + | + | + | + | + | + | type |
| 1 | FC0 *v.* FC40 | | | FC36 *v.* FC31 | | | | |
| | | | | | | | FC90 *v.* FC47 | *r* |
| 2 | (FC0+FC40) *v.* FC55 | | | (FC36+FC31) *v.* (FC90+FC47) | | | | *r* |
| 3 | (FC0+FC40+FC55) *v.* (FC36+FC31+FC47) | | | | | | | $r^+$ |
| 4 | FC(0+40+55+47) *v.* FC(0+36+31+47) | | | | | | | *r* |
| 5 | FC(0+40+55+36+31+47) | | | | | | | $r^+$ |

TABLE 17. (FC0+FC40+FC55) VERSUS (FC36+FC31+FC47): RECOMBINANTS ON SINGLE CROSSOVER

| mutant | type | no. found |
|---|---|---|
| FC(0+36+31+47) | *r* | 2 |
| FC(0+40+36+31+47) | *r* but minute on K | (6)† |
| sextuple‡ | $r^+$ | . |
| FC(0+40+55+31+47) | *r* but minute on K | (6)† |
| FC(0+40+55+47) | *r* | 1 |
| (FC40+FC55) | *r* | 4 |
| FC55 | *r* | 6 |
| true wild | $r^+$ | . |
| FC36 | *r* | 4 |
| (FC36+FC31) | *r* | 1 |

† This is a shared number as these mutants were not differentiated.
‡ The sextuple, though formed in this cross, was not isolated because of the difficulty of separating it from the true wild recombinant and the wild-type triple parents.

(FC36+FC31) and (FC90+FC47) were then crossed together and wild-type plaques picked. As FC31 and FC90 are very close together it was unlikely that true wild would be formed in this cross. In fact none was found among ten wild-type plaques picked. These were then triples and must be (FC36+FC31+FC47) because the barrier $b_6$ lies between FC31 and FC90 and the triple (FC36+FC90+FC47) would therefore be an *r*. One of these triples was backcrossed to true wild and shown to contain FC36, FC31 and FC47; the segregants included (FC36+FC31) and a number of *r*s which were minute on K. These were shown to be (FC31+FC47) and (FC36+FC47). To make sure that the triple by some miscalculation, did not contain FC90, one of the FC31 isolates was u.v.-crossed with our original FC31. No recombination took place. A control cross of the isolate with FC90 gave a small recombination figure compatible with their separation on the map.

The two wild-type triples, (FC0+FC40+FC55) and (FC36+FC31+FC47) were next crossed. From this cross *r* plaques were picked on B (see table 17). Among the recombinants we looked for the quadruples FC(0+40+55+47) and FC(0+36+31+47). These were identified by spot-test crosses against their constituent singles as follows.

*FC(0+40+55+47)* gave recombinants with FC0, FC40, FC55 and FC47 because in each case a wild-type triple can be generated. In two cases the wild-type triples were isolated and checked. First the triple obtained by crossing the quadruple against FC55 was backcrossed to true wild. *r* plaques were picked and by spot crossing shown to be FC0, FC40, FC55 and the recombinant doubles of these three. The triple is therefore (FC0+FC40+FC55). Note that the triple (FC40+FC55+FC47) contains barrier $b_4$ and will be an *r*. Secondly, the quadruple was crossed with FC47, a wild-type plaque was isolated and by backcrossing to true wild was shown to be (FC0+FC40+FC47). Here again no other wild-type triple can be formed because of barriers. This check of the quadruple is summarized in table 18(*a*).

TABLE 18

(*a*) *Crosses of quadruples versus singles*

| crossed | $r^+$ segregant |
|---|---|
| I. FC(0+40+55+47) | |
| *v.* FC55 | (FC0+FC40+FC55) |
| *v.* FC47 | (FC0+FC40+FC47) |
| II. FC(0+36+31+47) | |
| *v.* FC47 | (FC36+FC31+FC47) |
| *v.* FC0 | none |

(*b*) *Crosses of quadruples versus doubles*

| crossed | $r^+$ segregant |
|---|---|
| I. FC(0+40+55+47) | |
| 1 *v.* (FC0+FC40) | (FC0+FC40+FC47) |
| | (FC0+FC40+FC55) |
| 2 *v.* (FC0+FC55) | (FC0+FC40+FC55) |
| II. FC(0+36+31+47) | |
| 3 *v.* (FC0+FC40) | (FC0+FC40+FC47) |
| 4 *v.* (FC0+FC55) | none |

*FC(0+36+31+47)* gave wild-type recombinants with FC36, FC31 and FC47, which were triples, but gave no recombinants in a spot cross with FC0. This is because the barrier $b_4$ lies between FC0 and FC36 and no wild-type triples can be formed. A u.v. cross of this quadruple with FC0 yielded wild-type plaques at a frequency of $1{\cdot}7 \times 10^{-6}$ which is the level of the spontaneous reversion of FC0. The cross with FC47 was also repeated, a wild-type plaque isolated and, by backcrossing to true wild, shown to be the triple (FC36+FC31+FC47). This check of the quadruple is also summarized in table 18(*a*).

There are no other *r*s isolated from the cross of (FC0+FC40+FC55) and (FC36+FC31+FC47) which give the same pattern on spot crosses to the constituent *r* mutants. However, the quadruples were checked further by crossing with two doubles. The expected results are shown in table 18(*b*). No true wild can come out of these crosses and so only one wild-type plaque was picked from cross 2, purified and backcrossed to true wild. All *r*s isolated from this cross were shown to be either FC0, FC40, FC55, or their recombinant doubles by spot crosses to the constituent *r* mutants. Similarly a wild-type plaque was picked from cross 3 and backcrossed to true wild. All the *r*s arising were shown to be either FC0, FC40, FC47, or their recombinant doubles.

The sextuple was constructed by crossing the two quadruples FC(0+40+55+47) and FC(0+36+31+47). Wild-type recombinants were obtained; these cannot be true wild, and are either rare wild-type triples or the presumed sextuple (see table 19). One of these was selected; its phenotype was indistinguishable from true wild. It was shown to be a sextuple by decomposing it into a single and a quintuple and proving the structure of each as described below.

TABLE 19. SCHEME OF CROSS, SET OUT TO SHOW POSITIONS ON THE GENETIC MAP

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| quadruple | FC0 | FC40 | FC55 | | | | FC47 |
| *v.* | | | $b_4$ | | | | $b_6$ |
| quadruple | FC0 | | | FC36 | FC31 | | FC47 |

Recombinants on single crossover will be:

| mutant | type |
|---|---|
| FC(0+40+36+31+47) | *r* but minute on K |
| FC(0+40+55+36+31+47) | $r^+$ sextuple |
| FC(0+40+55+31+47) | *r* but minute on K |
| (FC0+FC36+FC47) | *r* triple with barrier |
| (FC0+FC47) | *r* |
| (FC0+FC55+FC47) | *r* triple with barrier |

NOTE. The only wild-type triple which can be formed is (FC0+FC40+FC47) which requires a *double* crossover.

(iii) *Breakdown of sextuple*

The presumed sextuple was backcrossed to true wild, *r*-type recombinants isolated and identified by spot-crossing against the constituent single mutants. Table 20 lists the *r*-type recombinants expected on a single crossover. Those actually isolated contained one or more of the mutants in the sextuple and each single mutant was represented in some combination or another. Other *r*-type recombinants were isolated as well, some of which required double and triple crossovers.

TABLE 20. CROSS OF FC(0+40+55+36+31+47) VERSUS WILD
*r*-TYPE RECOMBINANTS ON SINGLE CROSSOVER

| mutant | type | no. identified |
|---|---|---|
| FC0 | *r* | 6 |
| (FC0+FC40) | *r* | 7 |
| FC(0+40+55+36) | *r* | .† |
| FC(0+40+55+36+31) | *r* | . |
| FC47 | *r* | 2 |
| (FC31+FC47) | *r* minute on K | 4 |
| FC(55+36+31+47) | *r* | . |
| FC(40+55+36+31+47) | *r* | 3 |

† The point implies that no attempt was made to identify these recombinants as it is technically difficult to do so.

As a further proof, those recombinants thought to be FC0 on spot crosses were crossed against the deletion PB296. As FC0 lies outside this deletion true wild recombinants will arise but nothing else. One such recombinant was picked therefore and backcrossed to true wild. In this cross 0·3% *r* plaque was seen. Nine of these were tested; eight were *r*I mutants and one was an A cistron mutant. Allowing for multiple crossovers there are two *r*

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triples and three *r* quadruples which might be mistaken for FC0 on spot crosses. (These have not been listed here.) In four of these cases minute recombinants will arise with FC36, FC31 and FC47 and can be seen, especially when a more accurate cross is done. In the fifth case, FC(0+55+36+31) formed by three crossovers, no wild-type recombinants will arise when it is crossed with PB296. True wild cannot be formed because FC55, FC36 and FC31 are under the deletion and the triple (FC55+FC36 +FC31) will not be wild type because of barriers. This identifies the FC0 segregant.

The presumed quintuple FC(40+55+36+31+47), containing all mutants except FC0, gave recombinants with neither FC40 nor with FC55, proving the presence of these two mutants. Its structure was further analysed as follows. From figure 2 it will be seen that of the five single mutants in this phage, FC47 is the only one mapping under the deletion NB3157. By crossing the quintuple with this deletion the only wild-type recombinant possible is the triple (FC36+FC31+FC47). This cross was made, a wild-type recombinant picked and backcrossed to true wild. The *r* plaques arising were identified by spot crosses and shown to be either FC36, FC31, FC47 or their recombinant doubles. Crossing the quintuple with (FC40+FC55) gave no recombinants, as would be expected, and was further proof of the presence of these two single mutants.

### (*d*) *Miscellaneous combinations*

Most of these are triples which have various combinations of mutants of unlike sign. One quadruple having two plus mutants and two minus mutants has been made. In all cases the combinations have the phenotype expected from our theory, except the triple (FC88+FC57+FC47) which is minute on K (see § 9(*b*)(ii)). They are listed in table A 5(*d*) which also shows how they were made.

## 11. Special topics

### (*a*) *The splitting of deletion r*196

One of the standard deletions originally used for mapping was *r*196. Unlike other deletions, *r*196 when crossed with *r* mutants often gives minute recombinants when no wild-type recombinants appear. This indicates that *r*196 is in some sense not a simple deletion.

One of our mutants which gives minute recombinants when crossed with *r*196 is FC20. This cross was performed, plated on K and a minute picked. This minute was then crossed with wild type and plated on B: 128 *r* plaques were picked, of which three failed to grow on K. Backcrossing showed that two of these mapped as FC20. The third, which did not, was surmised to be a part of *r*196, and was called 196a. It was shown to have sign plus. The (++) double (196a+FC20) is minute on K. The recombination frequency of 196a with FC20 is about 2·7 times that of *r*196 with FC20.

It was more laborious to obtain the other part of deletion *r*196. *r*196 was u.v.-crossed with wild type, and the lysate was regrown and recrossed four times: 64 *r* plaques were picked from the lysate of the final cross. These were purified and crossed with 196a. Nine showed significant recombination. They were completely non-reverting and appeared to be true deletions. One of these strains was taken as 196b. This is the deletion used for

mapping the mutants in our map (figure 2). It will be seen that 196a lies next to the left-hand end of 196b.

Mutant FC87 gives recombinants with *r*196, since the (+ −) double of (196a + FC87) is wild type. Mutants FC34 and FC58 give minutes with *r*196, for the same reason that FC20 does.

On the other hand FC47 coincides with 196a, so no mapping anomaly occurs when FC47 is mapped with 196b. These results account for our mis-mapping of these mutants in our original paper (Crick *et al.* 1961) since at that time the anomalous behaviour of *r*196 was not understood.

It is interesting to speculate on the origin of this behaviour. If we assume that a deletion is often formed by the omission of a region between two points having identical or very similar base sequences an easy explanation suggests itself. Consider the sequence

W X Y Z A B C D E F G P Q R . . . A B C D E E F G R S T

this could easily delete to give the sequence

. . . W X Y Z A B C D E E F G R S T.

Thus the sequence D E F has become D E E F, as if an extra base had been added at the point just before the deletion of (P Q R . . . E F G). This would thus behave as if it had the structure which we postulate for *r*196.

Two other independently isolated deletions, *r*1236 and NB7182, have the same properties as *r*196, suggesting that mutants of this type arise as a single event and not as a very rare double event.

### (*b*) *P 61/13*

This spontaneous revertant of P61 is anomalous. When originally backcrossed to wild, all of the seventeen *r* plaques which were found gave no recombination with P61 and no suppressor could be isolated. Since we had previously observed asymmetrical segregation with other doubles, a thorough search was made for the suppressor: 125 *r* segregants were isolated from a u.v. cross of P61/13 with true wild. Of these, 116 were identical to P61. 6 grew on K and were therefore *r*I or *r*III mutants of the parents, while the remaining 3 appeared to be *r*II mutants of the parents since they were all unlike each other and mapped either in the A cistron or in a distant part of the B cistron. In this cross, a further 22 *r* plaques were picked from mottled plaques; again all gave no recombination with P61. Six of the *r* segregants isolated were spot-crossed with neighbouring mutants; their behaviour was similar to that of P61.

These results showed that the only *r* which can be segregated from P61/13 is P61. The suppressor may be either wild or lethal, both of which are unlikely, or suppression may be achieved by some special mechanism. Without specifying how P61 is suppressed we can map the site of the suppressor by 3-factor crosses. This was done by backcrossing P61/13 with amber mutants NT332 and X237 on either side of P61 and comparing the ratio of P61 to (P61 + amber) segregants. NT332 maps to the left of P61 (see figure 2) and X237 to the right of P61, just beyond the limit of our present map. When P61/13 was crossed with NT332, of 32 *r*s, 9 were (NT332 + P61) while 23 were P61. If the suppressor maps to the *right* of P61, almost all the segregants should have been P61. However, in the cross P61/13 with X237, 33 segregants were P61 while 9 were (P61 + X237). If the




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suppressor maps to the *left* of P61 almost all the segregants should be P61. These results suggest that the suppressor does not map in a simple way. All of our results on P61/13 can be explained by assuming that this phage contains two P61 mutants; that is, it contains a reduplication of a small region of the phage. On this hypothesis there should have been approximately equal numbers of the two types of recombinants in each of the above crosses.

A reduplication alone would not be enough to restore the correct phase but a total sign of zero can be obtained if the origin of the duplication has a plus frame shift. The reduplication must not generate any barriers and its right end must therefore be between P61 and $b_{10}$ (see figure 8).

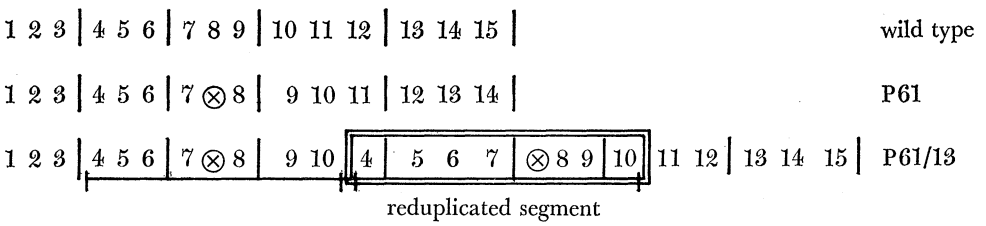


FIGURE 8. This figure shows the presumed structure of P61/13. ⊗ represents an addition of a nucleotide producing the (+) phase shift in P61.

One consequence of this hypothesis is that P61/13 should segregate P61 by incestuous recombination. We have not observed any great increase in the frequency of *r*s in P61/13. We explain this by saying that the pairing region in recombination must be greater than the length of the reduplication, preventing the shift that would be necessary to generate P61 in a recombination act. Naturally when P61/13 is crossed with wild this condition no longer applies, since the two parents are of unequal length in this region, and segregants can be obtained.

### (*c*) *Frame shift of base-substitution mutants*

In a separate paper (Brenner & Stretton 1965) it has been reported that when a nonsense base-substitution mutant, which by itself gives the *r*-phenotype, has been phase-shifted it can no longer express itself, since the combination will grow on K. This is, of course, just what would be expected. Since the publication of that paper several more examples have been constructed. For convenience these are all listed in table A6.

### (*d*) *FC238 and FC237*

One of the revertants of FC222, when backcrossed with true wild, gave *r*-type recombinants, all of which were FC222. It was then noticed that, in addition to these recombinants, there were others which gave a *small* *r*-type plaque on B and did not grow on K. Six of these were picked and were found to be identical by recombination tests, and one was kept as FC238. Another revertant of FC222 segregated FC237, which also gives a smaller *r* plaque on B than other *r*II mutants but the reduction in plaque size is not as extreme as in FC238. However, FC236, segregated from another revertant of FC222, is normal. The small-plaque phenotype of FC238 on B must be distinguished from the minute phenotypes on K; FC238 does not grow at all on K. This property is unique to FC238 and FC237.


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We first checked whether this phenotype was directly due to the frame-shift mutant itself. FC238 was u.v.-crossed with wild type, and no large *r* segregants were found amongst 2000 plaques examined. FC238 was then crossed with FC222 and the wild-type double isolated. On backcrossing to true wild, two segregants were again found: large *r* plaques which were FC222 and the characteristic small *r* plaques of FC238. These experiments show that the special phenotype is a property of the mutant itself.

We have considered two kinds of hypotheses to explain this property. The first is that the smallness of the plaques is due to a polarity effect exerted by the mutant on the expression of a neighbouring gene. We consider this to be most unlikely, mainly because this phenotype is not shown by any other phase-shift mutant (with the exception of FC237), nor by any amber or ochre mutant in the region. The other hypothesis is that a peculiar polypeptide fragment is synthesized by FC238 which interferes in some way with phage growth. For example, it could inhibit a bacterial or a phage-controlled enzyme. In support of this we have found that combination of FC238 with amber and ochre mutants to its left restores the large plaque size on B. Doubles of FC238 with 2074, EM84, HB74, N24 and NT332 were constructed. These have standard *r* plaques on B, but do not grow on strains of K carrying amber and ochre suppressors, showing that FC238 retains the *r*II phenotype of a phase-shift mutant. On the other hand, the double of FC238 with an A cistron amber mutant, N11, gives a *small r* plaque on B, indistinguishable from that of FC238 itself.

Stocks of FC238 when plated on B contain a small number of large *r* plaques. The frequency is increased by 2-aminopurine to about 1%. Twenty-five of these mutants were picked and, as expected, all failed to grow on K. Many of these mutants are double mutants containing in addition to FC238 an amber or ochre mutant to its left. Thus mutants identical with HB74, N24 and 375 were recovered from doubles. One of these doubles was of interest. On crossing with the deletion 1231, it segregated a phage which made a wild-type plaque on K but a large fuzzy-edged plaque on B, in fact, a typical strongly leaking *r*III mutant. The method of recovery of the segregant places it to the left of FC238. This leaky mutant when again combined with FC238 restores the large plaque phenotype. The cross shows, in addition to the fuzzy-edged small *r*s of the parents, the double with a large *r* plaque and wild-type recombinants. This result shows that the restoration of large plaque size to FC238 can be accomplished by what appears to be a missense mutant and not only by a nonsense mutant. Indeed there may be mutations with this property which may have no effect on *r*II function by themselves.

The hypothesis that FC238 makes a specific toxic polypeptide fragment demands that chain termination must occur somewhere to its right. Since there are no barriers between FC238 and FC222, termination of the chain must occur to the right of this mutant, and may be accomplished by the barrier presumed to bound the region we have studied. Fragments of the same length would be made by the four minus mutants FC201, FC237, FC238 and FC236 since there are no $(-) \rightarrow (+)$ barriers in the region covered by these mutants but the extents of the frame-shifted sequences would be different. Of these mutants FC238 and possibly FC237 have the reduced plaque size on B, which suggests that the postulated inhibitory effect requires a special sequence.


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*Note added in proof;* 30 *May* 1967

It can be seen from table A 2 (*a*), group 3 B, that FC88 maps near FC96, and is thus presumably to the right of it, since it is some distance from FC42. In the same way EM84 is near FC88, but some distance from FC96. It is thus presumably to the right of EM84. This establishes the order

FC96 FC88 EM84

Unfortunately the position of 375 is unclear. It is near FC88, but not near either FC96 or EM84. Thus neither a position to the left of FC88 nor to the right of FC88 is satisfactory. Similar difficulties arise if FC55 (a plus mutant at the FC88 site) is used instead of FC88. We have not pursued these anomalies further.



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## 12. Appendices

### Table A 1 (*a*). Non-FC mutants

| mutant* | sign† | source‡ | origin§ | suppressible¶ | sign check | remarks |
|---|---|---|---|---|---|---|
| Ac19 | ? | . | AA | – | ? | reverts by duplication |
| X819 | ? | . | AY | – | ? | . |
| X504 | ? | . | PD | ochre | ? | sign presumably zero |
| HD263 | ? | . | HA | – | ? | sign presumably zero |
| 326 | ? | Benzer | S | – | ? | . |
| HE122 | ? | . | HA | amber | ? | sign presumably zero |
| P53 | ? | . | P | . | ? | reverts by duplication |
| X726 | ? | . | AY | . | ? | . |
| X744 | ? | . | AY | . | ? | . |
| X759 | ? | . | AY | . | ? | . |
| X799 | ? | . | AY | . | ? | . |
| A31 | ? | Benzer | S | – | + or 0 | reverts by duplication |
| X824 | + | . | AY | . | + or 0 | . |
| 244 | + | Benzer | S | . | + or 0 | . |
| UV375 | 0 | Drake | u.v. | ochre | + or 0 | . |
| 360 | 0 | Benzer | S | ochre | + or 0 | . |
| 739 | 0 | Benzer | S | ochre | + or 0 | like 360 |
| 997 | + | Benzer | S | . | + or 0 | . |
| X511 | 0 | . | PD | ochre | + or 0 | . |
| X763 | + | . | AY | . | + or 0 | . |
| X833 | – | . | AY | . | – | . |
| P13 | + | . | P | . | + or 0 | renamed FCO |
| 1074 | – | Benzer | S | . | – | . |
| X707 | – | . | AY | . | – | . |
| X732 | + | . | AY | . | + or 0 | . |
| X806 | + | . | AY | . | + or 0 | . |
| 2074 | 0 | Benzer | S | amber | 0 | . |
| UV357 | 0 | Drake | u.v. | ochre | 0 | . |
| A63 | + | Benzer | S | . | + | . |
| NB7017 | + | Benzer | NA | . | + | . |
| X27 | 0 | . | PD | ochre | 0 | . |
| 375 | 0 | Benzer | S | ochre | 0 | . |
| EM84 | 0 | Benzer | EMS | amber | 0 | . |
| F96 | – | Benzer | S | . | – | was a double mutant |
| 1651 | + | Benzer | S | . | + | . |
| HB74 | 0 | Benzer | HA | amber | 0 | . |
| N24 | 0 | Benzer | NA | ochre | 0 | . |
| X655 | 0 | . | 2AP | – | 0 | . |
| 176 | – | Benzer | S | . | – | . |
| NT332 | 0 | Benzer | NA | amber | 0 | . |
| 514 | + | Benzer | S | . | + | . |
| 196a | + | . | S | . | + | split off deletion *r*196 |
| 1018 | – | Benzer | S | . | – | . |
| X146 | – | . | H | . | – | . |
| X225 | – | . | H | . | – | . |
| D72 | – | Benzer | S | . | – | . |
| 441 | – | Benzer | S | . | – | . |
| 370 | + | Benzer | S | . | + | . |
| D10 | + | Benzer | S | . | + | . |
| P61 | + | . | P | . | + | . |
| 261 | + | Benzer | S | . | + | . |
| 488 | + | Benzer | S | . | ? | . |
| J158 | + | Benzer | S | . | + | . |
| (556) | . | Benzer | S | . | . | high reverter |

* These are listed in map order (see figure 2). HD263 is a leaky temperature-sensitive mutant which maps near X504.

† ?, in this region it is not possible to determine signs.

‡ Mutants are from the Cambridge collection unless another source is indicated.

§ Abbreviations used: AA, aminoacridine; AY, acridine yellow; 2AP, 2-aminopurine; EMS, ethyl methane sulphonate; H, hydrazine; HA, hydroxylamine; NA, nitrous acid; P, proflavine; PD, photodynamic oxidation with toluidine blue; u.v., ultraviolet light; S, spontaneous origin.

¶ A point in this column indicates that the mutant has not been tested for extragenic suppression. A minus indicates that it has been tested on amber and ochre suppressors and given a negative result.


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TABLE A 1 (*b*). FC MUTANTS

| mutant | sign | parent | p* | s* | checked† | sign check‡ | map segment | remarks§ |
|---|---|---|---|---|---|---|---|---|
| FC1 | – | P13 | 13 | 4 | ✓ | – | 2 | . |
| FC6 | – | P13 | 9 | 3 | . | – | 2 | . |
| FC7 | – | P13 | 6 | 7 | . | – | 3B | . |
| FC8 | – | P13 | 2 | 8 | . | – | 3B | . |
| FC9 | – | P13 | 2 | 5 | . | – | 3A | . |
| FC10 | – | P13 | 3 | 10 | . | – | 2 | . |
| FC11 | – | P13 | 2 | 10 | . | – | 2 | . |
| FC12 | – | P13 | 5 | 8 | . | – | 2 | . |
| (FC13) | – | P13 | 6 | 4 | . | . | . | discarded, because probably another copy of FC10 |
| FC14 | – | P13 | 6 | 3 | . | – | 3B | . |
| FC15 | – | P13 | 6 | 4 | . | – | 3B | . |
| (FC16) | – | P13 | 0 | 10 | ✓ | – | . | discarded, because probably another copy of FC8 |
| (FC17) | – | P13 | 2 | 6 | . | – | . | discarded, because probably another copy of FC8 |
| FC18 | – | P13 | 1 | 9 | ✓ | – | 3B | . |
| FC19 | – | P13 | 4 | 5 | . | – | 2 | . |
| FC20 | + | FC1 | 5 | 3 | . | + | 6 | (FC1+FC20) is minute on K |
| FC21 | – | P13 | 2 | 8 | . | – | 3B | . |
| FC22 | – | P13 | 2 | 7 | . | – | 2 | . |
| FC23 | – | P13 | 3 | 7 | . | – | 3B | . |
| (FC24) | – | P13 | 2 | 6 | . | . | . | discarded, because probably another copy of FC11 |
| (FC25) | – | P13 | 4 | 6 | . | . | . | discarded, because probably another copy of FC10 |
| (FC26) | – | P13 | 7 | 3 | . | – | . | discarded, because probably another copy of FC6 |
| FC27 | – | P13 | 2 | 8 | . | – | 2 | . |
| FC28 | + | FC6 | 4 | 6 | . | + | 3B | . |
| FC29 | + | FC6 | 2 | 8 | . | + | 4 | . |
| FC30 | + | FC6 | 2 | 6 | ✓ | + (m) | 5 | . |
| FC31 | + | FC6 | 4 | 6 | . | + | 5 | . |
| FC32 | + | FC6 | 4 | 6 | . | + | 3B | . |
| FC33 | + | FC6 | 3 | 7 | . | + | 3B | . |
| FC34 | + | FC6 | 4 | 6 | . | + | 6 | (FC34+FC6) is minute on K |
| FC35 | + | FC6 | 0 | 10 | ✓ | + | 3B | . |
| FC36 | + | FC6 | 4 | 6 | . | + | 4 | . |
| FC38 | + | FC6 | 3 | 6 | . | + (m) | 5 | . |
| FC39 | + | FC6 | 4 | 6 | . | + (m) | 5 | . |
| FC40 | + | FC1 | 6 | 12 | ✓ | + | 3B | . |
| FC41 | + | FC1 | 0 | 2 | ✓ | + or 0 | 2 | . |
| FC42 | + | FC7 | 4 | 6 | . | + | 3B | . |
| FC43 | + | FC7 | 4 | 6 | . | + | 4 | . |
| FC44 | + | FC7 | 5 | 5 | . | + | 4 | . |
| FC45 | + | FC7 | 2 | 6 | . | + | 3B | . |
| FC46 | + | FC7 | 4 | 6 | . | + or 0 | 3A | . |
| FC47 | + | FC7 | 6 | 4 | . | + (m) | 5 | . |

* 'p' and 's', segregants of backcross: p = parent, s = suppressor, see §3 (*b*).
† ✓ implies that it has been checked directly that the mutant and the parent suppress each other.
‡ See §5 (*b*).
§ FC mutants were spontaneous in origin unless a mutagen is indicated in this column.


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 1 (*b*) (*cont.*)

| mutant | sign | parent | p* | s* | checked† | sign check‡ | map segment | remarks§ |
|---|---|---|---|---|---|---|---|---|
| FC48 | + | FC7 | 7 | 3 | . | + or 0 | 3A | . |
| FC49 | + | FC9 | 5 | 2 | ✓ | + | 3B | . |
| FC50 | + | FC9 | 14 | 3 | ✓ | + | 3B | . |
| FC51 | + | FC9 | 5 | 2 | ✓ | + | 3B | . |
| FC52 | + | FC9 | 7 | 3 | . | + | 4 | . |
| FC53 | + | FC9 | 6 | 4 | . | + | 4 | . |
| FC54 | + | FC9 | 6 | 4 | . | + | 4 | . |
| FC55 | + | FC9 | 4 | 5 | . | + | 3B | . |
| FC56 | + | FC9 | 6 | 9 | ✓ | + | 3B | . |
| FC57 | + | FC9 | 5 | 5 | . | + | 4 | . |
| FC58 | + | FC9 | 5 | 4 | . | + | 6 | (FC58+FC9) is minute on K |
| FC62 | + | FC10 | 5 | 5 | ✓ | + | 4 | . |
| FC63 | + | FC10 | 3 | 6 | . | + (m) | 5 | . |
| FC64 | + | FC10 | 4 | 6 | . | + | 3B | . |
| FC66 | + | FC11 | 4 | 5 | . | + | 4 | . |
| FC67 | + | FC11 | 6 | 4 | . | + | 3B | . |
| FC68 | + | FC11 | 4 | 5 | . | + or 0 | 3A | . |
| FC69 | + | FC11 | 6 | 3 | . | + | 4 | . |
| FC71 | + | FC11 | 5 | 5 | . | + | 4 | . |
| FC72 | + | FC11 | 7 | 3 | . | + or 0 | 3A | . |
| FC73 | + | FC11 | 7 | 2 | ✓ | + or 0 | 3A | . |
| FC74 | − | FC42 | 2 | 7 | . | − | 2 | . |
| FC75 | − | FC42 | 2 | 7 | . | − | 2 | . |
| FC76 | − | FC42 | 1 | 9 | ✓ | − | 2 | . |
| FC77 | − | FC42 | 4 | 5 | . | − | 2 | . |
| FC78 | − | FC42 | 2 | 8 | . | − | 2 | . |
| FC80 | − | FC42 | 1 | 9 | ✓ | − | 2 | . |
| FC81 | − | FC47 | 5 | 5 | . | − | 2 | . |
| FC82 | − | FC47 | 4 | 6 | . | − | 3A | . |
| FC83 | − | FC47 | 2 | 7 | . | − | 3B | . |
| FC84 | − | FC47 | 6 | 4 | . | − | 3B | . |
| FC85 | − | FC47 | 4 | 6 | . | − | 2 | . |
| FC86 | − | FC47 | 3 | 6 | . | − | 2 | . |
| FC87 | − | FC47 | 7 | 3 | . | − | 5 | . |
| FC88 | − | FC47 | 4 | 6 | . | − | 3B | . |
| FC89 | + | FC7 | 5 | 5 | . | + (m) | 5 | acridine yellow |
| FC90 | + | FC7 | 3 | 7 | . | + (m) | 5 | acridine yellow |
| FC91 | + | FC7 | 4 | 6 | . | + or 0 | 3B | acridine yellow |
| FC92 | + | FC7 | 4 | 5 | . | + | 3B | acridine yellow |
| FC94 | + | FC7 | 2 | 8 | . | + or 0 | 3A | acridine yellow |
| FC95 | + | FC7 | 4 | 3 | . | + or 0 | 3A | acridine yellow |
| FC96 | + | FC7 | 2 | 7 | . | + | 3B | acridine yellow |
| FC98 | + | FC7 | 5 | 4 | . | + or 0 | 3A | acridine yellow |
| (FC100) | − | P13 | 3 | 5 | . | − | . | discarded, because probably another copy of FC11 |
| (FC101) | − | P13 | 3 | 6 | . | − | . | discarded, because probably another copy of FC21 |
| FC102 | + | FC7 | 2 | 8 | . | + | 4 | . |
| FC103 | + | FC7 | 5 | 3 | . | + | 3B | . |
| FC104 | + | FC9 | 22 | 5 | . | + | 3B | . |
| FC105 | + | FC10 | 5 | 3 | . | + or 0 | 3A | . |
| FC106 | + | FC10 | 5 | 3 | . | + or 0 | 2 | . |
| FC108 | + | FC88 | 3 | 5 | . | + (m) | 5 | . |
| FC109 | + | FC88 | 25 | 5 | . | + (m) | 5 | . |
| FC110 | + | FC88 | 6 | 3 | . | + (m) | 5 | . |
| FC111 | + | FC88 | 5 | 3 | . | + (m) | 5 | . |
| FC112 | + | FC88 | 14 | 4 | . | + | 4 | . |
| FC113 | + | FC88 | 8 | 4 | . | + | 4 | . |




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 1 (*b*) (*cont.*)

| mutant | sign | parent | p* | s* | checked† | sign check‡ | map segment | remarks§ |
|---|---|---|---|---|---|---|---|---|
| FC114 | + | FC88 | 6 | 3 | . | + | 4 | . |
| FC115 | + | FC88 | 11 | 6 | ✓ | + | 4 | . |
| FC116 | + | FC88 | 16 | 4 | . | + | 4 | . |
| FC117 | + | FC87 | 4 | 5 | . | + | 6 | . |
| FC118 | + | FC87 | 5 | 5 | . | + | 6 | . |
| FC119 | + | FC87 | 6 | 3 | . | + | 6 | . |
| FC120 | + | FC87 | 5 | 5 | . | + | 6 | . |
| FC121 | + | FC87 | 4 | 4 | . | + | 6 | . |
| FC122 | + | FC87 | 2 | 7 | . | + | 6 | . |
| FC123 | + | FC87 | 4 | 5 | . | ? | 6 | . |
| FC124 | + | FC87 | 5 | 5 | . | + | 6 | . |
| FC125 | + | FC87 | 5 | 6 | . | + | 6 | . |
| FC126 | – | FC42 | 6 | 6 | . | – | 3B | . |
| FC127 | – | FC42 | 6 | 9 | . | – | 3B | . |
| FC128 | + | FC11 | 12 | 2 | ✓ | + or 0 | 3A | . |
| FC129 | – | FC47 | 4 | 12 | . | – | 3A | proflavine |
| (FC130) | – | FC47 | . | . | . | . | . | proflavine, high reverter |
| FC131 | – | FC47 | 7 | 3 | . | – | 3A | proflavine |
| FC132 | – | FC47 | 4 | 6 | . | – | 3A | proflavine |
| FC133 | – | FC47 | 6 | 3 | . | – | 3A | proflavine |
| FC134 | – | FC47 | 4 | 6 | . | – | 3B | proflavine |
| FC135 | – | FC47 | 22 | 5 | . | – | 3A | proflavine |
| FC136 | – | FC47 | 3 | 6 | . | – | 3A | proflavine |
| FC137 | – | FC47 | 6 | 4 | . | – | 3B | proflavine |
| (FC138) | – | FC47 | . | . | . | . | . | proflavine, high reverter |
| FC139 | – | FC47 | 2 | 17 | . | – | 3A | aminoacridine |
| FC140 | – | FC47 | 5 | 5 | . | – | 3A | aminoacridine |
| FC141 | – | FC47 | 11 | 6 | . | – | 3A | aminoacridine |
| FC142 | – | FC47 | 7 | 3 | . | – | 3A | aminoacridine |
| FC143 | – | FC47 | 4 | 4 | . | – | 3B | aminoacridine |
| FC144 | – | FC47 | 6 | 4 | . | – | 3A | aminoacridine |
| FC145 | – | FC47 | 15 | 4 | . | – | 3A | aminoacridine |
| FC146 | – | FC47 | 13 | 6 | . | – | 3A | aminoacridine |
| FC147 | – | FC47 | 15 | 4 | . | – | 3A | aminoacridine |
| FC148 | – | FC47 | 7 | 3 | . | – | 3A | aminoacridine |
| (FC149) | – | FC38 | . | . | . | . | . | high reverter |
| FC150 | – | FC38 | 3 | 4 | . | – | 3B | . |
| FC151 | – | FC38 | 2 | 11 | . | – | 5 | . |
| FC152 | – | FC38 | 3 | 6 | . | – | 3B | . |
| FC153 | – | FC38 | 3 | 6 | . | – | 3B | . |
| FC201 | – | P61 | 4 | 4 | . | – | 6 | . |
| FC202 | – | P61 | 6 | 6 | . | – | 6 | . |
| FC203 | – | P61 | 7 | 5 | . | – | 6 | . |
| FC204 | – | P61 | 2 | 3 | . | – | 6 | . |
| FC205 | – | P61 | 3 | 6 | . | – | 5 | . |
| FC206 | – | P61 | 6 | 8 | . | – | 5 | . |
| FC207 | – | P61 | 3 | 5 | . | – | 5 | . |
| FC208 | – | P61 | 6 | 5 | . | – | 6 | . |
| FC209 | – | P61 | 7 | 6 | . | – | 6 | . |
| FC210 | – | P61 | 12 | 9 | . | – | 5 | . |
| FC211 | + | FC205 | 3 | 5 | . | + | 6 | . |
| FC212 | + | FC205 | 12 | 6 | . | + | 6 | . |
| FC213 | + | FC205 | 4 | 6 | . | + | 6 | . |
| FC214 | + | FC205 | 6 | 3 | . | + | 6 | . |
| FC215 | + | FC205 | 6 | 4 | . | ? | 6 | . |
| FC216 | + | FC205 | 4 | 3 | . | + | 6 | . |
| FC217 | + | FC205 | 4 | 5 | . | + | 6 | . |
| FC218 | + | FC205 | 4 | 15 | . | + | 6 | . |
| FC219 | + | FC205 | 24 | 8 | . | + | 6 | . |



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 1 (*b*) (*cont.*)

| mutant | sign | parent | p* | s* | checked† | sign check‡ | map segment | remarks§ |
|---|---|---|---|---|---|---|---|---|
| FC220 | + | FC205 | 6 | 11 | . | + | 6 | . |
| FC221 | + | FC202 | 6 | 8 | . | + | 6 | . |
| FC222 | + | FC202 | 6 | 6 | . | + | 6 | . |
| FC223 | + | FC202 | 8 | 4 | . | + | 6 | . |
| FC224 | + | FC202 | 2 | 7 | . | ? | 6 | . |
| FC225 | + | FC202 | 3 | 6 | . | + | 6 | . |
| FC226 | + | FC202 | 3 | 7 | . | + | 6 | . |
| FC227 | + | FC202 | 2 | 7 | . | + | 6 | . |
| FC228 | + | FC202 | 4 | 4 | . | + | 6 | . |
| FC229 | + | FC202 | 5 | 5 | . | + | 6 | . |
| FC230 | + | FC202 | 6 | 29 | . | + | 6 | . |
| FC231 | – | FC222 | 2 | 23 | . | – | 6 | . |
| FC232 | – | FC222 | 3 | 18 | . | – | 5 | . |
| FC233 | – | FC222 | 3 | 15 | . | – | 6 | . |
| FC234 | – | FC222 | 2 | 9 | . | – | 5 | . |
| FC235 | – | FC222 | 4 | 6 | . | – | 6 | . |
| FC236 | – | FC222 | 2 | 7 | . | – or 0 | 6 | identical to sign tester phage |
| FC237 | – | FC222 | 6 | 4 | . | – | 6 | see §11 (*d*) |
| FC238 | – | FC222 | 7 | 6 | . | – | 6 | see §11 (*d*) |
| FC239 | – | FC222 | 3 | 9 | . | – | 5 | |





PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 2(*a*). MAPPING OF THE CANONICAL SET (SEE § 4(*b*))

group 1

| mutant | Ac19 | 326 | X504 | P53 |
|---|---|---|---|---|
| Ac19 | 0·04 | . | . | . |
| 326 | 0·04 | 0·04 | . | . |
| X504 | 46 | 0·04 | 0·02 | . |
| P53 | + | 0·03 | 28 | 0·007 |
| A31 | + | + | + | + |

group 2

| mutant | A31 | FC10 | FC11 | FC41 | FC1 | FC6 | UV375 | 739 |
|---|---|---|---|---|---|---|---|---|
| A31 | 0·01 | . | . | . | . | . | . | . |
| FC10 | 0·2 | 0·2 | . | . | . | . | . | . |
| FC11 | 100 | 11 | 0·11 | . | . | . | . | . |
| FC41 | 220 | 225 | 0·7 | 0·3 | . | . | . | . |
| FC1 | + | + | 50 | 10 | 0·5 | . | . | . |
| FC6 | 20 | 0·1 | 0·05 | 0·4 | 0·3 | 0·06 | . | . |
| UV375 | + | + | + | 74 | 68 | 0·2 | 0·2 | . |
| 739 | + | + | + | + | + | + | 8 | 0·006 |
| P53 | + | + | + | + | + | + | + | + |
| FC105 | + | + | + | + | + | + | + | + |

group 3A

| mutant | 997 | FC105 | X511 | FC82 | FC9 | FC73 | FC148 | FC0 |
|---|---|---|---|---|---|---|---|---|
| 997 | 0·04 | . | . | . | . | . | . | . |
| FC105 | 12 | 0·05 | . | . | . | . | . | . |
| X511 | + | 3·0 | 0·006 | . | . | . | . | . |
| FC82 | + | 5·0 | 0·2 | 0·06 | . | . | . | . |
| FC9 | + | + | 4·0 | 0·1 | 0·2 | . | . | . |
| FC73 | + | + | + | 24 | 0·1 | 0·04 | . | . |
| FC148 | + | + | + | + | 58 | 0·3 | 0·2 | . |
| FC0 | + | + | + | + | + | 100 | 0·2 | 0·08 |
| 739 | + | + | + | + | + | + | + | + |
| FC152 | + | + | + | + | + | + | + | + |

group 3B

| nutant | FC152 | FC28 | FC18 | FC35 | FC7 | NB7017 | FC8 | FC32 | FC23 | FC104 | FC42 | FC96 | FC88 | 375 | EM84 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 'C152 | 0·11 | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 'C28 | 0·1 | 0·06 | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 'C18 | + | 14 | 0·1 | . | . | . | . | . | . | . | . | . | . | . | . |
| 'C35 | + | 20 | 0·15 | 0·07 | . | . | . | . | . | . | . | . | . | . | . |
| 'C7 | + | + | 1·0 | 0·2 | 0·06 | . | . | . | . | . | . | . | . | . | . |
| NB7017 | + | + | 0·08 | 0·19 | 0·09 | 0·16 | . | . | . | . | . | . | . | . | . |
| 'C8 | + | + | 3·0 | 0·27 | 0·95 | 0·12 | 0·05 | . | . | . | . | . | . | . | . |
| 'C32 | + | + | + | 7·0 | 18 | 0·11 | 0·3 | 0·04 | . | . | . | . | . | . | . |
| 'C23 | + | + | + | + | + | 0·4 | 0·05 | 0·77 | 0·04 | . | . | . | . | . | . |
| 'C104 | + | + | + | + | + | 100 | 5·0 | 9·2 | 0·06 | 0·04 | . | . | . | . | . |
| 'C42 | + | + | + | + | + | + | + | + | 2·0 | 0·07 | 0·29 | . | . | . | . |
| 'C96 | + | + | + | + | + | + | + | + | + | 100 | 0·7 | 0·04 | . | . | . |
| 'C88 | + | + | + | + | + | + | + | + | + | + | + | 20 | 0·04 | . | . |
| '75 | + | + | + | + | + | + | + | + | + | + | + | + | 4·5 | 0·006 | . |
| EM84 | + | + | + | + | + | + | + | + | + | + | + | + | 47 | 270 | 0·07 |
| 'C0 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 'C115 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 2(*a*) (*cont.*)

group 4

| mutant | FC115 | FC36 | HB74 | N24 | 176 | FC54 |
|---|---|---|---|---|---|---|
| FC115 | 0·68 | . | . | . | . | . |
| FC36 | 1·5 | 0·06 | . | . | . | . |
| HB74 | + | 46 | 0·003 | . | . | . |
| N24 | + | + | 12 | 0·07 | . | . |
| 176 | + | + | + | 24 | 0·003 | . |
| FC54 | + | + | + | 70 | 0·25 | 0·03 |
| EM84 | + | + | + | + | + | + |
| NT332 | + | + | + | + | + | + |

group 5

| mutant | NT332 | FC31 | FC90 | 514 | FC47 | FC151 | FC87 |
|---|---|---|---|---|---|---|---|
| NT332 | 0·43 | . | . | . | . | . | . |
| FC31 | 108 | 0·3 | . | . | . | . | . |
| FC90 | + | 15 | 0·57 | . | . | . | . |
| 514 | + | + | 198 | 0·22 | . | . | . |
| FC47 | + | + | + | 55 | 0·23 | . | . |
| FC151 | + | + | + | + | + | 1·35 | . |
| FC87 | + | + | + | + | + | 194 | 1·0 |
| FC54 | + | + | + | + | + | + | + |
| FC201 | + | + | + | + | + | + | + |

group 6

| tant | FC201 | 370 | FC34 | FC223 | P61 | FC217 | FC125 | FC211 | 488 | FC213 | FC215 | FC119 | FC222 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 201 | 0·2 | . | . | . | . | . | . | . | . | . | . | . | . |
| ) | + | 0·15 | . | . | . | . | . | . | . | . | . | . | . |
| 34 | + | 0·4 | 0·6 | . | . | . | . | . | . | . | . | . | . |
| 223 | + | 20 | 0·4 | 0·25 | . | . | . | . | . | . | . | . | . |
| 1 | + | + | 0·3 | 0·5 | 0·32 | . | . | . | . | . | . | . | . |
| 217 | + | + | + | + | 20 | 0·6 | . | . | . | . | . | . | . |
| 125 | + | + | + | + | + | + | 0·58 | . | . | . | . | . | . |
| 211 | + | + | 1 | 40 | 0·3 | 0·7 | 0·4 | 0·3 | . | . | . | . | . |
| 3 | + | + | + | + | + | + | 0·66 | 0·4 | 0·14 | . | . | . | . |
| 213 | + | + | + | + | + | + | 0·5 | 0·2 | 0·43 | 0·2 | . | . | . |
| 215 | + | + | + | + | + | + | + | 0·5 | 0·22 | 0·2 | 0·3 | . | . |
| 119 | + | + | + | + | + | + | + | 5 | 0·49 | 0·4 | 0·8 | 0·4 | . |
| 222 | + | + | + | + | + | + | + | + | + | 0·2 | 0·3 | + | 0·3 |
| 87 | + | + | + | + | + | + | + | + | + | + | + | + | + |


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

## TABLE A 2(*b*). MAPPING OF THE NON-CANONICAL SET (SEE § 4(*c*))

Of the three columns labelled u.v., the central column shows the canonical mutants with which no recombination was found. The other four columns show mutants which gave recombinants, the inner two using u.v., the outer two using spot-test crosses. The mutants are in map order.

| | | ← u.v. → | | | |
|---|---|---|---|---|---|
| mutant | ++ | + | 0 | + | ++ |
| X819 | . | . | Ac19 | X504 | P53 |
| 326 | . | . | Ac19, P53, X504 | . | FC10 |
| HE122 | Ac19 | . | X504 | P53 | FC10 |
| X726 | . | Ac19, X504 | P53 | . | FC10 |
| X744 | . | Ac19, X504 | P53 | . | FC10 |
| X759 | Ac19 | X504 | P53 | . | FC10 |
| X799 | . | Ac19, X504 | P53 | . | FC10 |
| X824 | P53 | . | FC10 | FC11 | FC1 |
| FC12 | P53 | FC10 | FC11 | FC41, FC1 | UV375 |
| FC19 | P53 | FC10 | FC11 | FC41, FC1 | UV375 |
| FC22 | P53 | . | FC11 | . | UV375 |
| FC27 | P53 | . | FC11 | . | UV375 |
| FC74 | P53 | . | FC11 | . | UV375 |
| FC75 | P53 | . | FC11 | . | UV375 |
| FC76 | P53 | . | FC11 | . | UV375 |
| FC77 | P53 | . | FC11 | . | UV375 |
| FC78 | P53 | . | FC11 | . | UV375 |
| FC80 | P53 | . | FC11 | . | UV375 |
| FC81 | P53 | . | FC11 | . | UV375 |
| FC85 | P53 | . | FC11 | . | UV375 |
| FC106 | FC10 | . | FC41 | . | UV375 |
| 244 | FC10 | FC11 | FC41 | FC1 | UV375 |
| FC86 | P53 | . | FC10, UV375, FC6 | . | 739 |
| 360 | FC1 | . | 739 | . | FC105 |
| FC129 | FC105 | X511, FC82 | FC9 | FC73 | FC148 |
| FC132 | FC105 | . | FC9 | . | FC148 |
| FC136 | FC105 | . | FC9 | . | FC148 |
| FC140 | FC105 | . | FC9 | . | FC148 |
| FC142 | FC105 | . | FC9 | . | FC148 |
| FC131 | FC82 | FC9 | FC73 | FC148 | FC0 |
| FC133 | FC82 | . | FC73 | . | FC0 |
| FC135 | FC82 | . | FC73 | . | FC0 |
| FC141 | FC82 | . | FC73 | . | FC0 |
| FC144 | FC82 | . | FC73 | . | FC0 |
| FC145 | FC82 | . | FC73 | . | FC0 |
| FC146 | FC82 | . | FC73 | . | FC0 |
| X763 | FC82 | . | FC73 | . | FC0 |
| X833 | FC82 | . | FC73 | . | FC0 |
| FC139 | FC82 | FC73 | FC0 | . | FC28 |
| X707 | FC82 | . | FC0 | . | FC28 |
| FC46 | FC73 | . | FC0 | . | FC28 |
| FC48 | FC73 | . | FC0 | . | FC28 |
| FC68 | FC73 | . | FC0 | . | FC28 |
| FC72 | FC73 | . | FC0 | . | FC28 |
| FC94 | FC73 | . | FC0 | . | FC28 |
| FC95 | FC73 | . | FC0 | . | FC28 |
| FC98 | FC73 | . | FC0 | . | FC28 |
| FC128 | FC73 | . | FC0 | . | FC28 |
| FC147 | FC73 | . | FC0 | . | FC28 |
| 1074 | FC73 | . | FC0 | . | FC28 |
| X732 | FC73 | . | FC0 | . | FC28 |
| X806 | FC73 | . | FC0 | . | FC28 |
| FC91 | FC0 | . | FC152 | . | FC18, FC35 |
| 2074 | FC0 | . | FC28 | . | FC18, FC35 |

| | | ← u.v. → | | | |
|---|---|---|---|---|---|
| mutant | ++ | + | 0 | + | ++ |
| UV357 | FC0 | . | FC28 | . | FC7 |
| FC103 | FC0 | . | A63 | . | FC28 |
| FC21 | FC0 | FC28 | FC18 | FC7 | FC32, FC23 |
| FC40 | FC0 | FC28 | FC18 | FC7 | FC23 |
| FC49 | FC0 | . | FC18 | FC32 | FC23 |
| FC64 | FC0 | . | FC18 | . | FC23 |
| FC14 | FC28 | FC18 | FC7, FC35, FC8 | FC32 | FC23 |
| FC15 | FC28 | FC18 | FC7, FC35, FC8 | FC32 | FC23 |
| FC137 | FC28 | . | FC7, FC35, FC8 | . | FC23 |
| FC134 | FC28 | FC18 | FC7, FC8, FC23 | . | FC42 |
| FC67 | FC28 | . | FC7 | . | FC42 |
| FC51 | FC18 | . | FC8, FC32 | . | FC42 |
| FC92 | FC104 | . | FC96 | . | FC88 |
| X27 | FC42 | . | FC96 | . | FC88 |
| FC33 | FC42 | . | FC88 | . | EM84 |
| FC45 | FC42 | . | FC88 | . | EM84 |
| FC50 | FC42 | . | FC88 | . | EM84 |
| FC55 | FC42 | FC96 | FC88 | . | EM84 |
| FC56 | FC42 | . | FC88 | . | EM84 |
| FC83 | FC42 | . | FC88 | . | EM84 |
| FC84 | FC42 | . | FC88 | . | EM84 |
| FC126 | FC42 | . | FC88 | . | EM84 |
| FC127 | FC42 | FC96 | FC88 | . | EM84 |
| FC143 | FC42 | . | FC88 | . | EM84 |
| FC150 | FC42 | . | FC88 | . | EM84 |
| FC153 | FC42 | . | FC88 | . | EM84 |
| F96 | EM84 | . | FC115 | FC36 | HB74 |
| FC29 | EM84 | . | FC36 | . | HB74 |
| FC43 | EM84 | FC115 | FC36 | . | HB74 |
| FC44 | EM84 | . | FC36 | . | HB74 |
| FC52 | EM84 | . | FC36 | . | HB74 |
| FC53 | EM84 | . | FC36 | . | HB74 |
| FC57 | EM84 | . | FC36 | . | HB74 |
| FC62 | EM84 | FC115 | FC36 | . | HB74 |
| FC66 | EM84 | FC115 | FC36 | . | HB74 |
| FC69 | EM84 | . | FC36 | . | HB74 |
| FC71 | EM84 | FC115 | FC36 | . | HB74 |
| FC112 | EM84 | FC115 | FC36 | . | HB74 |
| FC113 | EM84 | FC115 | FC36 | . | HB74 |
| FC114 | EM84 | FC115 | FC36 | . | HB74 |
| FC116 | EM84 | FC115 | FC36 | . | HB74 |
| 1651 | EM84 | FC115 | FC36 | . | HB74 |
| X655 | FC36 | . | N24 | . | FC54 |
| FC102 | N24 | . | FC54 | . | NT332 |
| FC30 | 514 | . | FC47 | . | FC151 |
| FC38 | 514 | . | FC47 | . | FC151 |
| FC39 | 514 | . | FC47 | . | FC151 |
| FC63 | 514 | . | FC47 | . | FC151 |
| FC89 | 514 | . | FC47 | . | FC151 |
| FC108 | 514 | . | FC47 | . | FC151 |
| FC109 | 514 | . | FC47 | . | FC151 |
| FC110 | 514 | . | FC47 | . | FC151 |
| FC111 | 514 | . | FC47 | . | FC151 |
| 196a | 514 | . | FC47 | . | FC151 |
| 1018 | 514 | . | FC47 | . | FC151 |
| X146 | 514 | . | FC47 | . | FC151 |
| X225 | 514 | . | FC47 | . | FC151 |
| FC205 | FC47 | . | FC151 | . | FC87 |


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 2(*b*) (*cont.*)

| mutant | ++ | ← u.v. → + | 0 | + | ++ |
|---|---|---|---|---|---|
| FC206 | FC47 | . | FC151 | . | FC87 |
| FC207 | FC47 | . | FC151 | . | FC87 |
| FC210 | FC47 | . | FC151 | . | FC87 |
| FC232 | FC47 | . | FC151 | . | FC87 |
| FC234 | FC47 | . | FC151 | . | FC87 |
| FC239 | FC47 | . | FC151 | . | FC87 |
| D72 | FC47 | . | FC151 | . | FC87 |
| FC202 | FC87 | . | FC201 | . | FC34, 370 |
| FC203 | FC87 | . | FC201 | . | FC34, 370 |
| FC204 | FC87 | . | FC201 | . | FC34, 370 |
| FC208 | FC87 | . | FC201 | . | FC34, 370 |
| FC209 | FC87 | . | FC201 | . | FC34, 370 |
| FC231 | FC87 | . | FC201 | . | FC34, 370 |
| FC233 | FC87 | . | FC201 | . | FC34, 370 |
| FC235 | FC87 | . | FC201 | . | FC34, 370 |
| 441 | FC87 | . | FC201 | . | FC34, 370 |
| FC237 | FC201 | . | 370 | FC223 | P61 |
| D10 | FC201 | . | 370 | FC223 | P61 |
| FC58 | FC201 | . | FC34, 370, P61 | FC211, FC217 | FC125 |
| FC238 | 370 | . | FC223, P61 | FC211, FC217 | FC125 |
| FC212 | 370 | . | P61 | . | FC125 |
| FC214 | 370 | . | P61 | . | FC125 |
| FC216 | 370 | . | P61 | . | FC125 |

| mutant | ++ | ← u.v. → + | 0 | + | ++ |
|---|---|---|---|---|---|
| FC218 | 370 | . | P61 | . | FC125 |
| FC219 | 370 | . | P61 | . | FC125 |
| FC220 | 370 | . | P61 | . | FC125 |
| FC225 | 370 | . | P61 | . | FC125 |
| FC226 | 370 | . | P61 | . | FC125 |
| FC228 | 370 | . | P61 | . | FC125 |
| FC230 | 370 | . | P61 | . | FC125 |
| 261 | 370 | . | P61 | . | FC125 |
| FC227 | 370 | FC34, P61 | FC217 | . | FC125 |
| FC229 | FC217 | . | FC125 | . | FC119 |
| FC236 | FC217 | . | FC125 | . | FC119 |
| FC117 | 370, FC223 | . | P61, FC215 | . | FC222 |
| FC118 | 370, FC223 | . | P61, FC215 | . | FC222 |
| FC120 | 370, FC223 | . | P61, FC215 | . | FC222 |
| FC122 | 370, FC223 | . | P61, FC215 | . | FC222 |
| FC20 | 370, FC223 | P61 | FC215 | . | FC222 |
| FC124 | 370, FC223 | P61 | FC215 | . | FC222 |
| FC221 | 370, FC223 | P61 | FC215 | . | FC222 |
| J158 | 370, FC223 | P61 | FC125, FC215 | FC119 | FC222 |
| FC121 | FC217 | . | FC125, FC119, FC222 | . | . |
| FC123 | FC217 | FC125 | FC119, FC222 | . | . |
| FC224 | FC125 | FC119 | FC222 | . | . |

TABLE A 3. INDEX OF DOUBLES

| mutant | sign | mutant | sign | direction | type |
|---|---|---|---|---|---|
| FC0 | + | **FC1** | − | → | *r*+ |
| FC0 | + | **FC6** | − | → | *r*+ |
| **FC0** | + | FC7 | − | ← | *r*+ |
| **FC0** | + | FC8 | − | ← | *r*+ |
| FC0 | + | **FC9** | − | → | *r*+ |
| FC0 | + | **FC10** | − | → | *r*+ |
| FC0 | + | **FC11** | − | → | *r*+ |
| FC0 | + | **FC12** | − | → | *r*+ |
| FC0 | + | **FC13** | − | → | *r*+ |
| **FC0** | + | FC14 | − | ← | *r*+ |
| **FC0** | + | FC15 | − | ← | *r*+ |
| **FC0** | + | FC16 | − | ← | *r*+ |
| **FC0** | + | FC17 | − | ← | *r*+ |
| **FC0** | + | FC18 | − | ← | *r*+ |
| FC0 | + | **FC19** | − | → | *r*+ |
| **FC0** | + | FC21 | − | ← | *r*+ |
| FC0 | + | **FC22** | − | → | *r*+ |
| **FC0** | + | FC23 | − | ← | *r*+ |
| FC0 | + | **FC24** | − | → | *r*+ |
| FC0 | + | **FC25** | − | → | *r*+ |
| FC0 | + | **FC26** | − | → | *r*+ |
| FC0 | + | **FC27** | − | → | *r*+ |
| **FC0** | + | FC31 | + | . | *r* |
| **FC0** | + | FC33 | + | . | *r* n.i. |
| **FC0** | + | FC36 | + | . | *r* n.i. |
| **FC0** | + | $a_4$ FC36 | + | . | *r* n.i. |
| **FC0** | + | FC38 | + | . | *r* |
| **FC0** | + | FC40 | + | . | *r* |
| FC0 | + | **FC41** | + | . | *r* |

| mutant | sign | mutant | sign | direction | type |
|---|---|---|---|---|---|
| **FC0** | + | FC47 | + | . | *r* |
| **FC0** | + | FC54 | + | . | *r* |
| **FC0** | + | FC55 | + | . | *r* |
| **FC0** | + | FC57 | + | . | *r* |
| **FC0** | + | FC58 | + | . | *r* |
| FC0 | + | **FC86** | − | → | *r*+ |
| **FC0** | + | FC87 | − | ← | *r* |
| **FC0** | + | FC88 | − | ← | *r* |
| **FC0** | + | $a_4$ FC88 | − | ← | *r*+ |
| **FC0** | + | FC91 | + | . | *r* |
| **FC0** | + | FC92 | + | . | *r* n.i. |
| FC0 | + | **FC100** | − | → | *r*+ |
| **FC0** | + | FC101 | − | ← | *r*+ |
| FC0 | + | **FC106** | + | . | *r* |
| **FC0** | + | 176 | − | ← | *r* |
| FC0 | + | **244** | + | . | *r* n.i. |
| FC0 | + | **360** | 0 | . | *r* |
| FC0 | + | **739** | 0 | . | *r* |
| **FC0** | + | 2074 | 0 | . | *r* |
| FC0 | + | **UV375** | 0 | . | *r* |
| **FC1** | − | FC0 | + | → | *r*+ |
| **FC1** | − | FC9 | − | . | *r* |
| **FC1** | − | FC20 | + | → | m |
| **FC1** | − | FC21 | − | . | *r* |
| **FC1** | − | FC23 | − | . | *r* |
| **FC1** | − | FC38 | + | → | *r*+ |
| **FC1** | − | FC40 | + | → | *r*+ |
| FC1 | − | **FC41** | + | ← | *r*+ |
| **FC1** | − | FC42 | + | → | *r*+ |


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 3 (*cont.*)

| mutant | sign | mutant | sign | direction | type | mutant | sign | mutant | sign | direction | type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **FC1** | – | FC58 | + | → | m | **FC9** | – | FC49 | + | → | $r^+$ |
| **FC1** | – | FC119 | + | → | m | **FC9** | – | FC50 | + | → | $r^+$ |
| **FC1** | – | FC123 | + | → | *r* | **FC9** | – | FC51 | + | → | $r^+$ |
| **FC1** | – | $_{m}$a FC123 | + | → | $r^+$ | **FC9** | – | FC52 | + | → | $r^+$ |
| **FC1** | – | FC125 | + | → | m | **FC9** | – | FC53 | + | → | $r^+$ |
| **FC1** | – | FC211 | + | → | m | **FC9** | – | FC54 | + | → | $r^+$ |
| **FC1** | – | FC213 | + | → | m | **FC9** | – | FC55 | + | → | $r^+$ |
| **FC1** | – | FC215 | + | → | *r* n.i. | **FC9** | – | FC56 | + | → | $r^+$ |
| **FC1** | – | FC217 | + | → | m | **FC9** | – | FC57 | + | → | $r^+$ |
| **FC1** | – | FC222 | + | → | m | **FC9** | – | FC58 | + | → | m |
| **FC1** | – | 370 | + | → | m | **FC9** | – | FC104 | + | → | $r^+$ |
| **FC1** | – | 514 | + | → | $r^+$ | FC9 | – | **FC106** $a_2$ | + | ← | $r^+$ |
| **FC1** | – | 556 | + | → | m | FC9 | – | **244** $a_2$ | + | ← | $r^+$ |
| **FC1** | – | 997 | + | → | $r^+$ | FC9 | – | **360** $a_2$ | 0 | . | *r* |
| **FC1** | – | EM84 | 0 | . | *r* | **FC9** | – | 2074 | 0 | . | *r* |
| **FC1** | – | N24 | 0 | . | *r* | FC9 | – | **P53** | ? | . | *r* |
| **FC1** | – | P61 | + | → | m | **FC10** | – | FC0 | + | → | $r^+$ |
| **FC1** | – | UV104 | 0 | . | *r* | **FC10** | – | FC62 | + | → | $r^+$ |
| **FC1** | – | UV357 | 0 | . | *r* | **FC10** | – | FC63 | + | → | $r^+$ |
| **FC1** | – | X655 | 0 | . | *r* | **FC10** | – | FC64 | + | → | $r^+$ |
| FC1 | – | **X824** | + | ← | $r^+$ | **FC10** | – | FC105 | + | → | $r^+$ |
| **FC6** | – | FC0 | + | → | $r^+$ | **FC10** | – | FC106 | + | → | $r^+$ |
| **FC6** | – | FC28 | + | → | $r^+$ | **FC10** | – | 196a | + | → | $r^+$ |
| **FC6** | – | FC29 | + | → | $r^+$ | **FC10** | – | 360 | 0 | . | *r* |
| **FC6** | – | FC30 | + | → | $r^+$ | **FC10** | – | 514 | + | → | $r^+$ |
| **FC6** | – | FC31 | + | → | $r^+$ | **FC10** | – | 739 | 0 | . | *r* |
| **FC6** | – | FC32 | + | → | $r^+$ | **FC10** | – | 1651 | + | → | $r^+$ |
| **FC6** | – | FC33 | + | → | $r^+$ | **FC10** | – | A63 | + | → | $r^+$ |
| **FC6** | – | FC34 | + | → | m | **FC10** | – | NB7017 | + | → | $r^+$ |
| **FC6** | – | FC35 | + | → | $r^+$ | **FC10** | – | UV375 | 0 | . | *r* |
| **FC6** | – | FC36 | + | → | $r^+$ | **FC10** | – | X511 | 0 | . | *r* |
| **FC6** | – | FC38 | + | → | $r^+$ | **FC10** | – | X732 | + | → | $r^+$ |
| **FC6** | – | FC39 | + | → | $r^+$ | **FC10** | – | X763 | + | → | $r^+$ |
| **FC6** | – | 375 | 0 | . | *r* | **FC10** | – | X806 | + | → | $r^+$ |
| **FC6** | – | 2074 | 0 | . | *r* | **FC11** | – | FC0 | + | → | $r^+$ |
| **FC6** | – | EM84 | 0 | . | *r* | **FC11** | – | FC66 | + | → | $r^+$ |
| **FC6** | – | HB74 | 0 | . | *r* | **FC11** | – | FC67 | + | → | $r^+$ |
| **FC6** | – | NT332 | 0 | . | *r* | **FC11** | – | FC68 | + | → | $r^+$ |
| FC6 | – | **P53** | ? | . | m | **FC11** | – | FC69 | + | → | $r^+$ |
| **FC6** | – | X27 | 0 | . | *r* | **FC11** | – | FC71 | + | → | $r^+$ |
| FC7 | – | **FC0** | + | ← | $r^+$ | **FC11** | – | FC72 | + | → | $r^+$ |
| **FC7** | – | FC42 | + | → | $r^+$ | **FC11** | – | FC73 | + | → | $r^+$ |
| **FC7** | – | FC43 | + | → | $r^+$ | **FC11** | – | FC128 | + | → | $r^+$ |
| **FC7** | – | FC44 | + | → | $r^+$ | **FC11** | – | X732 | + | → | $r^+$ |
| **FC7** | – | FC45 | + | → | $r^+$ | **FC12** | – | FC0 | + | → | $r^+$ |
| FC7 | – | **FC46** | + | ← | $r^+$ | **FC13** | – | FC0 | + | → | $r^+$ |
| **FC7** | – | FC47 | + | → | $r^+$ | FC14 | – | **FC0** | + | ← | $r^+$ |
| FC7 | – | **FC48** | + | ← | $r^+$ | FC15 | – | **FC0** | + | ← | $r^+$ |
| **FC7** | – | FC89 | + | → | $r^+$ | FC16 | – | **FC0** | + | ← | $r^+$ |
| **FC7** | – | FC90 | + | → | $r^+$ | FC17 | – | **FC0** | + | ← | $r^+$ |
| FC7 | – | **FC91** | + | ← | $r^+$ | FC18 | – | **FC0** | + | ← | $r^+$ |
| **FC7** | – | FC92 | + | → | $r^+$ | **FC19** | – | FC0 | + | → | $r^+$ |
| FC7 | – | **FC94** | + | ← | $r^+$ | FC20 | + | **FC1** | – | → | m |
| FC7 | – | **FC95** | + | ← | $r^+$ | FC20 | + | **FC31** | + | . | *r* n.i. |
| **FC7** | – | FC96 | + | → | $r^+$ | FC20 | + | **FC47** | + | . | m |
| FC7 | – | **FC98** | + | ← | $r^+$ | FC20 | + | **FC90** | + | . | m |
| **FC7** | – | FC102 | + | → | $r^+$ | FC20 | + | **FC202** | – | → | $r^+$ |
| FC7 | – | **FC103** | + | ← | $r^+$ | FC20 | + | **FC205** | – | → | $r^+$ |
| FC8 | – | **FC0** | + | ← | $r^+$ | FC20 | + | **196a** | + | . | m |
| **FC9** | – | FC0 | + | → | $r^+$ | FC21 | – | **FC0** | + | ← | $r^+$ |
| FC9 | – | **FC1** | – | . | *r* | FC21 | – | **FC1** | – | . | *r* |
| **FC9** | – | FC21 | – | . | *r* | FC21 | – | **FC9** | – | . | *r* |
| **FC9** | – | FC23 | – | . | *r* | **FC21** | – | FC23 | – | . | *r* |
| **FC9** | – | FC38 | + | → | $r^+$ | **FC21** | – | FC38 | + | → | $r^+$ |
| **FC9** | – | FC40 | + | → | $r^+$ | FC21 | – | **FC41** | + | ← | *r* |
| FC9 | – | **FC41** | + | ← | *r* | **FC21** | – | FC58 | + | → | m |
| FC9 | – | **FC41** $a_2$ | + | ← | $r^+$ | FC21 | – | **FC73** | + | ← | *r* |
| **FC9** | – | FC42 | + | → | $r^+$ | **FC22** | – | FC0 | + | → | $r^+$ |
| **FC9** | – | FC47 | + | → | $r^+$ | FC23 | – | **FC0** | + | ← | $r^+$ |



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 3 (*cont.*)

| mutant | sign | mutant | sign | direction | type | mutant | sign | mutant | sign | direction | type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FC23 | − | **FC1** | − | . | *r* | FC33 | + | **FC6** | − | → | $r^+$ |
| FC23 | − | **FC9** | − | . | *r* | **FC33** | + | FC31 | + | . | *r* n.i. |
| FC23 | − | **FC21** | − | . | *r* | FC33 | + | **FC40** | + | . | *r* n.i. |
| FC23 | − | **FC40** | + | ← | $r^+$ | **FC33** | + | FC47 | + | . | *r* n.i. |
| FC23 | − | **FC73** | + | ← | *r* | FC33 | + | **FC91** | + | . | m |
| FC23 | − | **FC73** $a_3$ | + | ← | $r^+$ | FC34 | + | **FC6** | − | → | m |
| **FC23** | − | FC88 | − | . | *r* | FC34 | + | **X146** | − | → | m |
| **FC23** | − | $a_5$ 176 | − | . | *r* | FC34 | + | **X225** | − | → | m |
| FC23 | − | **2074** | 0 | . | *r* | FC35 | + | **FC6** | − | → | $r^+$ |
| **FC24** | − | FC0 | + | → | $r^+$ | **FC35** | + | FC47 | + | . | *r* n.i. |
| **FC25** | − | FC0 | + | → | $r^+$ | FC36 | + | **FC0** | + | . | *r* n.i. |
| **FC26** | − | FC0 | + | → | $r^+$ | FC36 | + | **FC0** $a_4$ | + | . | *r* n.i. |
| **FC27** | − | FC0 | + | → | $r^+$ | FC36 | + | **FC6** | − | → | $r^+$ |
| FC28 | + | **FC6** | − | → | $r^+$ | **FC36** | + | FC30 | + | . | m n.i. |
| **FC28** | + | FC31 | + | . | *r* n.i. | **FC36** | + | FC31 | + | . | *r* |
| FC28 | + | **FC41** | + | . | *r* n.i. | **FC36** | + | FC38 | + | . | m n.i. |
| **FC28** | + | FC47 | + | . | *r* n.i. | **FC36** | + | FC39 | + | . | m n.i. |
| FC28 | + | **FC73** | + | . | *r* n.i. | FC36 | + | **FC40** | + | . | *r* |
| FC28 | + | **244** | + | . | *r* n.i. | **FC36** | + | FC47 | + | . | m |
| FC29 | + | **FC6** | − | → | $r^+$ | **FC36** | + | FC54 | + | . | *r* |
| **FC29** | + | FC47 | + | . | m | FC36 | + | **FC55** | + | . | *r* |
| **FC29** | + | FC58 | + | . | *r* n.i. | **FC36** | + | FC58 | + | . | m |
| FC29 | + | **FC88** | − | → | $r^+$ | **FC36** | + | FC63 | + | . | m n.i. |
| **FC29** | + | FC90 | + | . | m | **FC36** | + | FC87 | − | ← | *r* |
| **FC29** | + | FC125 | + | . | *r* n.i. | **FC36** | + | $a_6$ FC87 | − | ← | $r^+$ |
| **FC29** | + | 176 | − | ← | $r^+$ | FC36 | + | **FC88** | − | → | $r^+$ |
| FC30 | + | **FC6** | − | → | $r^+$ | **FC36** | + | FC89 | + | . | m n.i. |
| FC30 | + | **FC36** | + | . | m n.i. | **FC36** | + | FC90 | + | . | m n.i. |
| FC31 | + | **FC0** | + | . | *r* | **FC36** | + | FC108 | + | . | m n.i. |
| FC31 | + | **FC6** | − | → | $r^+$ | **FC36** | + | FC109 | + | . | m n.i. |
| **FC31** | + | FC20 | + | . | *r* n.i. | **FC36** | + | FC110 | + | . | m n.i. |
| FC31 | + | **FC28** | + | . | *r* n.i. | **FC36** | + | FC111 | + | . | m n.i. |
| FC31 | + | **FC32** | + | . | *r* n.i. | **FC36** | + | FC125 | + | . | *r* |
| FC31 | + | **FC33** | + | . | *r* n.i. | **FC36** | + | FC213 | + | . | *r* n.i. |
| FC31 | + | **FC36** | + | . | *r* | **FC36** | + | FC222 | + | . | *r* n.i. |
| **FC31** | + | FC38 | + | . | m | **FC36** | + | FC223 | + | . | *r* n.i. |
| FC31 | + | **FC40** | + | . | *r* | **FC36** | + | 176 | − | ← | $r^+$ |
| FC31 | + | **FC41** | + | . | *r* n.i. | **FC36** | + | 196a | + | . | m n.i. |
| FC31 | + | **FC42** | + | . | *r* n.i. | **FC36** | + | 514 | + | . | m n.i. |
| **FC31** | + | FC47 | + | . | m | **FC36** | + | P61 | + | . | *r* |
| **FC31** | + | $a_6$ FC47 | + | . | *r* | **FC36** | + | X146 | − | ← | *r* |
| FC31 | + | **FC54** | + | . | *r* | **FC36** | + | $a_6$ X146 | − | ← | $r^+$ |
| FC31 | + | **FC54*** | + | . | *r* n.i. | FC38 | + | **FC0** | + | . | *r* |
| FC31 | + | **FC55** | + | . | *r* | FC38 | + | **FC1** | − | → | $r^+$ |
| FC31 | + | **FC55** $a_5$ | + | . | *r* | FC38 | + | **FC6** | − | → | $r^+$ |
| FC31 | + | **FC57** | + | . | *r* | FC38 | + | **FC9** | − | → | $r^+$ |
| **FC31** | + | FC58 | + | . | m | FC38 | + | **FC21** | − | → | $r^+$ |
| **FC31** | + | FC87 | − | ← | *r* | FC38 | + | **FC31** | + | . | m |
| **FC31** | + | $a_6$ FC87 | − | ← | $r^+$ | FC38 | + | **FC36** | + | . | m n.i. |
| **FC31** | + | FC90 | + | . | m | FC38 | + | **FC40** | + | . | *r* |
| FC31 | + | **FC96** | + | . | *r* n.i. | **FC38** | + | FC58 | + | . | m |
| **FC31** | + | FC125 | + | . | m | **FC38** | + | FC87 | − | ← | $r^+$ |
| **FC31** | + | FC213 | + | . | *r* n.i. | FC38 | + | FC149 | − | . | $r^+$ |
| **FC31** | + | FC217 | + | . | *r* n.i. | FC38 | + | **FC150** | − | → | $r^+$ |
| **FC31** | + | FC222 | + | . | *r* n.i. | **FC38** | + | FC151 | − | ← | $r^+$ |
| **FC31** | + | FC223 | + | . | *r* n.i. | FC38 | + | **FC152** | − | → | $r^+$ |
| FC31 | + | **176** | − | → | $r^+$ | FC38 | + | **FC153** | − | → | $r^+$ |
| **FC31** | + | 196a | + | . | m n.i. | FC38 | + | **176** | − | → | $r^+$ |
| **FC31** | + | 370 | + | . | *r* n.i. | FC38 | + | **375** | 0 | . | *r* |
| **FC31** | + | 514 | + | . | m | FC38 | + | **2074** | 0 | . | *r* |
| **FC31** | + | P61 | + | . | *r* | FC38 | + | **EM84** | 0 | . | *r* |
| **FC31** | + | $a_6$ X146 | − | ← | $r^+$ | FC38 | + | **F96** | − | → | $r^+$ |
| FC32 | + | **FC6** | − | → | $r^+$ | FC38 | + | **HB74** | 0 | . | *r* |
| **FC32** | + | FC31 | + | . | *r* n.i. | FC38 | + | **N24** | 0 | . | *r* |
| FC32 | + | **FC41** | + | . | *r* n.i. | FC38 | + | **NT332** | 0 | . | *r* |
| **FC32** | + | FC47 | + | . | *r* n.i. | FC38 | + | **X27** | 0 | . | *r* |
| FC32 | + | **FC73** | + | . | *r* n.i. | FC38 | + | **X655** | 0 | . | *r* |
| FC32 | + | **244** | + | . | *r* n.i. | FC39 | + | **FC6** | − | → | $r^+$ |
| FC33 | + | **FC0** | + | . | *r* n.i. | FC39 | + | **FC36** | + | . | m n.i. |




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 3 (*cont.*)

| mutant | sign | mutant | sign | direction | type | mutant | sign | mutant | sign | direction | type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FC40 | + | **FC0** | + | . | *r* | **FC47** | + | FC20 | + | . | m |
| FC40 | + | **FC1** | − | → | $r^+$ | FC47 | + | **FC28** | + | . | *r* n.i. |
| FC40 | + | **FC9** | − | → | $r^+$ | FC47 | + | **FC29** | + | . | m |
| **FC40** | + | FC23 | − | ← | $r^+$ | FC47 | + | **FC31** | + | . | m |
| **FC40** | + | FC31 | + | . | *r* | FC47 | + | **FC31** $a_6$ | + | . | *r* |
| **FC40** | + | FC33 | + | . | *r* n.i. | FC47 | + | **FC32** | + | . | *r* n.i. |
| **FC40** | + | FC36 | + | . | *r* | FC47 | + | **FC33** | + | . | *r* n.i. |
| **FC40** | + | FC38 | + | . | *r* | FC47 | + | **FC35** | + | . | *r* n.i. |
| FC40 | + | **FC41** | + | . | *r* n.i. | FC47 | + | **FC36** | + | . | m |
| **FC40** | + | FC47 | + | . | *r* | FC47 | + | **FC40** | + | . | *r* |
| **FC40** | + | FC54 | + | . | *r* | FC47 | + | **FC41** | + | . | *r* n.i. |
| **FC40** | + | FC55 | + | . | *r* | FC47 | + | **FC42** | + | . | *r* n.i. |
| **FC40** | + | FC57 | + | . | *r* | FC47 | + | **FC43** | + | . | m n.i. |
| **FC40** | + | FC58 | + | . | *r* | FC47 | + | **FC44** | + | . | *r* n.i. |
| FC40 | + | **FC73** | + | . | *r* n.i. | FC47 | + | **FC45** | + | . | *r* n.i. |
| FC40 | + | **FC82** | − | → | $r^+$ | FC47 | + | **FC48** | + | . | *r* n.i. |
| FC40 | + | **FC86** | − | → | $r^+$ | FC47 | + | **FC51** | + | . | *r* n.i. |
| **FC40** | + | FC87 | − | ← | *r* | FC47 | + | **FC52** | + | . | m n.i. |
| **FC40** | + | FC90 | + | . | *r* | FC47 | + | **FC53** | + | . | m n.i. |
| **FC40** | + | FC92 | + | . | *r* n.i. | FC47 | + | **FC54** | + | . | *r* |
| **FC40** | + | 176 | − | ← | *r* | FC47 | + | **FC54*** | + | . | m |
| FC40 | + | **244** | + | . | *r* n.i. | FC47 | + | **FC55** | + | . | *r* |
| FC40 | + | **X707** | − | → | $r^+$ | FC47 | + | **FC55** $a_5$ | + | . | *r* n.i. |
| **FC41** | + | FC0 | + | . | *r* | FC47 | + | **FC56** | + | . | *r* n.i. |
| **FC41** | + | FC1 | − | ← | $r^+$ | FC47 | + | **FC57** | + | . | m |
| **FC41** | + | FC9 | − | ← | *r* | FC47 | + | **FC57** $a_6$ | + | . | *r* n.i. |
| **FC41** | + | $a_2$ FC9 | − | ← | $r^+$ | **FC47** | + | FC58 | + | . | m |
| **FC41** | + | FC21 | − | ← | *r* | FC47 | + | **FC62** | + | . | m n.i. |
| **FC41** | + | FC28 | + | . | *r* n.i. | FC47 | + | **FC64** | + | . | *r* n.i. |
| **FC41** | + | FC31 | + | . | *r* n.i. | FC47 | + | **FC66** | + | . | m n.i. |
| **FC41** | + | FC32 | + | . | *r* n.i. | FC47 | + | **FC67** | + | . | *r* n.i. |
| **FC41** | + | FC40 | + | . | *r* n.i. | FC47 | + | **FC69** | + | . | *r* n.i. |
| **FC41** | + | FC47 | + | . | *r* n.i. | FC47 | + | **FC71** | + | . | m n.i. |
| **FC41** | + | FC55 | + | . | *r* n.i. | FC47 | + | **FC72** | + | . | *r* n.i. |
| **FC41** | + | FC82 | − | ← | *r* | FC47 | + | **FC73** | + | . | *r* n.i. |
| **FC41** | + | FC87 | − | ← | *r* | FC47 | + | **FC81** | − | → | $r^+$ |
| **FC41** | + | FC88 | − | ← | *r* | FC47 | + | **FC82** | − | → | $r^+$ |
| **FC41** | + | 360 | 0 | . | *r* | FC47 | + | **FC83** | − | → | $r^+$ |
| FC41 | + | **P53** | ? | . | *r* | FC47 | + | **FC84** | − | → | $r^+$ |
| FC42 | + | **FC1** | − | → | $r^+$ | FC47 | + | **FC85** | − | → | $r^+$ |
| FC42 | + | **FC7** | − | → | $r^+$ | FC47 | + | **FC86** | − | → | $r^+$ |
| FC42 | + | **FC9** | − | → | $r^+$ | **FC47** | + | FC87 | − | ← | $r^+$ |
| **FC42** | + | FC31 | + | . | *r* n.i. | FC47 | + | **FC88** | − | → | $r^+$ |
| **FC42** | + | FC47 | + | . | *r* n.i. | FC47 | + | **FC90** | + | . | *r* |
| **FC42** | + | FC54 | + | . | *r* n.i. | FC47 | + | **FC91** | + | . | *r* n.i. |
| **FC42** | + | FC55 | + | . | *r* | FC47 | + | **FC92** | + | . | *r* n.i. |
| FC42 | + | **FC74** | − | → | $r^+$ | FC47 | + | **FC98** | + | . | *r* n.i. |
| FC42 | + | **FC75** | − | → | $r^+$ | FC47 | + | **FC102** | + | . | *r* n.i. |
| FC42 | + | **FC76** | − | → | $r^+$ | FC47 | + | **FC104** | + | . | *r* n.i. |
| FC42 | + | **FC77** | − | → | $r^+$ | FC47 | + | **FC112** | + | . | m n.i. |
| FC42 | + | **FC78** | − | → | $r^+$ | FC47 | + | **FC113** | + | . | m n.i. |
| FC42 | + | **FC80** | − | → | $r^+$ | FC47 | + | **FC114** | + | . | m n.i. |
| FC42 | + | **FC82** | − | → | $r^+$ | FC47 | + | **FC115** | + | . | *r* n.i. |
| FC42 | + | **FC86** | − | → | $r^+$ | FC47 | + | **FC116** | + | . | m n.i. |
| **FC42** | + | FC87 | − | ← | *r* | **FC47** | + | FC125 | + | . | m |
| **FC42** | + | FC126 | − | ← | $r^+$ | FC47 | + | **FC129** | − | → | $r^+$ |
| **FC42** | + | FC127 | − | ← | $r^+$ | FC47 | + | **FC130** | − | → | $r^+$ |
| FC43 | + | **FC7** | − | → | $r^+$ | FC47 | + | **FC131** | − | → | $r^+$ |
| **FC43** | + | FC47 | + | . | m n.i. | FC47 | + | **FC132** | − | → | $r^+$ |
| FC44 | + | **FC7** | − | → | $r^+$ | FC47 | + | **FC133** | − | → | $r^+$ |
| **FC44** | + | FC47 | + | . | *r* n.i. | FC47 | + | **FC134** | − | → | $r^+$ |
| FC44 | + | **FC88** | − | → | $r^+$ | FC47 | + | **FC135** | − | → | $r^+$ |
| **FC44** | + | 176 | − | ← | $r^+$ | FC47 | + | **FC136** | − | → | $r^+$ |
| FC45 | + | **FC7** | − | → | $r^+$ | FC47 | + | **FC137** | − | → | $r^+$ |
| **FC45** | + | FC47 | + | . | *r* n.i. | FC47 | + | **FC138** | − | → | $r^+$ |
| **FC46** | + | FC7 | − | ← | $r^+$ | FC47 | + | **FC139** | − | → | $r^+$ |
| FC47 | + | **FC0** | + | . | *r* | FC47 | + | **FC140** | − | → | $r^+$ |
| FC47 | + | **FC7** | − | → | $r^+$ | FC47 | + | **FC141** | − | → | $r^+$ |
| FC47 | + | **FC9** | − | → | $r^+$ | FC47 | + | **FC142** | − | → | $r^+$ |



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 3 (*cont.*)

| mutant | sign | mutant | sign | direction | type | mutant | sign | mutant | sign | direction | type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FC47 | + | **FC143** | − | → | $r^+$ | **FC55** | + | FC87 | − | ← | *r* |
| FC47 | + | **FC144** | − | → | $r^+$ | **FC55** | + | $a_5 a_6$ FC87† | − | ← | $r^+$ |
| FC47 | + | **FC145** | − | → | $r^+$ | **FC55** | + | FC90 | + | . | *r* |
| FC47 | + | **FC146** | − | → | $r^+$ | **FC55** | + | $a_5$ FC90 | + | . | *r* n.i. |
| FC47 | + | **FC147** | − | → | $r^+$ | FC55 | + | **FC91** | + | . | m |
| FC47 | + | **FC148** | − | → | $r^+$ | FC55 | + | **FC96** | + | . | *r* |
| **FC47** | + | FC202 | − | ← | *r* | FC55 | + | **FC104** | + | . | *r* |
| **FC47** | + | $a_7 a_8$ FC202 | − | ← | $r^+$ | **FC55** | + | 176 | − | ← | *r* |
| **FC47** | + | FC205 | − | ← | $r^+$ | **FC55** | + | $a_5$ 176 | − | ← | $r^+$ |
| **FC47** | + | FC213 | + | . | m | **FC55** | + | $a_5$ 514 | + | . | *r* n.i. |
| **FC47** | + | FC215 | + | . | m | FC56 | + | **FC9** | − | → | $r^+$ |
| **FC47** | + | FC217 | + | . | m | **FC56** | + | FC47 | + | . | *r* n.i. |
| **FC47** | + | FC222 | + | . | m | FC57 | + | **FC0** | + | . | *r* |
| **FC47** | + | FC223 | + | . | m | FC57 | + | **FC9** | − | → | $r^+$ |
| **FC47** | + | FC236 | − | ← | *r* | **FC57** | + | FC31 | + | . | *r* |
| **FC47** | + | FC238 | − | ← | *r* | FC57 | + | **FC40** | + | . | *r* |
| FC47 | + | **176** | − | → | $r^+$ | **FC57** | + | FC47 | + | . | m |
| **FC47** | + | 370 | + | . | m | **FC57** | + | $a_6$ FC47 | + | . | *r* n.i. |
| FC47 | + | **514** | + | . | *r* n.i. | **FC57** | + | FC54 | + | . | *r* |
| FC47 | + | 556 | + | . | m | FC57 | + | **FC55** | + | . | *r* |
| FC47 | + | **1074** | − | → | $r^+$ | **FC57** | + | FC87 | − | ← | *r* |
| FC47 | + | **F96** | − | → | $r^+$ | **FC57** | + | $a_6$ FC87 | − | ← | $r^+$ |
| **FC47** | + | P61 | + | . | m | FC57 | + | **FC88** | − | → | $r^+$ |
| FC47 | + | **UV104** | 0 | . | *r* | **FC57** | + | FC90 | + | . | m |
| FC47 | + | **UV357** | 0 | . | *r* | **FC57** | + | 176 | − | ← | $r^+$ |
| FC47 | + | **X511** | 0 | . | *r* | FC58 | + | **FC0** | + | . | *r* |
| FC47 | + | **X833** | − | → | $r^+$ | FC58 | + | **FC1** | − | → | m |
| **FC48** | + | FC7 | − | ← | $r^+$ | FC58 | + | **FC9** | − | → | m |
| **FC48** | + | FC47 | + | . | *r* n.i. | FC58 | + | **FC21** | − | → | m |
| FC49 | + | **FC9** | − | → | $r^+$ | FC58 | + | **FC29** | + | . | *r* n.i. |
| FC50 | + | **FC9** | − | → | $r^+$ | FC58 | + | **FC31** | + | . | m |
| FC51 | + | **FC9** | − | → | $r^+$ | FC58 | + | **FC36** | + | . | m |
| **FC51** | + | FC47 | + | . | *r* n.i. | FC58 | + | **FC38** | + | . | m |
| FC52 | + | **FC9** | − | → | $r^+$ | FC58 | + | **FC40** | + | . | *r* |
| **FC52** | + | FC47 | + | . | m n.i. | FC58 | + | **FC47** | + | . | m |
| FC53 | + | **FC9** | − | → | $r^+$ | FC58 | + | **FC82** | − | → | m |
| **FC53** | + | FC47 | + | . | m n.i. | FC58 | + | **FC86** | − | → | m |
| FC54 | + | **FC0** | + | . | *r* | FC58 | + | **FC87** | − | → | $r^+$ n.i. |
| FC54 | + | **FC9** | − | → | $r^+$ | FC58 | + | **FC88** | − | → | m |
| **FC54** | + | FC31 | + | . | *r* | FC58 | + | **FC90** | + | . | m |
| **FC 54*** | + | FC31 | + | . | *r* n.i. | **FC58** | + | FC125 | + | . | *r* n.i. |
| FC54 | + | **FC36** | + | . | *r* | FC58 | + | **X707** | − | → | m |
| FC54 | + | **FC40** | + | . | *r* | FC58 | + | **X833** | − | → | m |
| FC54 | + | **FC42** | + | . | *r* n.i. | FC62 | + | **FC10** | − | → | $r^+$ |
| **FC54** | + | FC47 | + | . | *r* | **FC62** | + | FC47 | + | . | m n.i. |
| **FC54*** | + | FC47 | + | . | m | FC63 | + | **FC10** | − | → | $r^+$ |
| FC54 | + | **FC55** | + | . | *r* | FC63 | + | **FC36** | + | . | m n.i. |
| FC54 | + | **FC55** $a_5$ | + | . | *r* | FC63 | + | **FC82** | − | → | $r^+$ |
| FC54 | + | **FC57** | + | . | *r* | FC63 | + | **FC86** | − | → | $r^+$ |
| **FC54** | + | FC87 | − | ← | *r* | FC63 | + | **FC88** | − | → | $r^+$ |
| **FC54** | + | $a_6$ FC87 | − | ← | $r^+$ | FC64 | + | **FC10** | − | → | $r^+$ |
| **FC54** | + | FC90 | + | . | *r* | **FC64** | + | FC47 | + | . | *r* n.i. |
| **FC54*** | + | FC90 | + | . | m | FC66 | + | **FC11** | + | → | $r^+$ |
| FC54 | + | **FC96** | + | . | *r* n.i. | **FC66** | + | FC47 | + | . | m n.i. |
| **FC54** | + | 514 | + | . | *r* | FC67 | + | **FC11** | − | → | $r^+$ |
| **FC54*** | + | 514 | + | . | m | **FC67** | + | FC47 | + | . | *r* n.i. |
| FC55 | + | **FC0** | + | . | *r* | FC68 | + | **FC11** | − | → | $r^+$ |
| FC55 | + | **FC9** | − | → | $r^+$ | FC69 | + | **FC11** | − | → | $r^+$ |
| **FC55** | + | FC31 | + | . | *r* | **FC69** | + | FC47 | + | . | *r* n.i. |
| **FC55** | + | $a_5$ FC31 | + | . | *r* | FC71 | + | **FC11** | − | → | $r^+$ |
| **FC55** | + | FC36 | + | . | *r* | **FC71** | + | FC47 | + | . | m n.i. |
| FC55 | + | **FC40** | + | . | *r* | FC72 | + | **FC11** | − | → | $r^+$ |
| FC55 | + | **FC41** | + | . | *r* n.i. | **FC72** | + | FC47 | + | . | *r* n.i. |
| FC55 | + | **FC42** | + | . | *r* | FC73 | + | **FC11** | − | → | $r^+$ |
| **FC55** | + | FC47 | + | . | *r* | **FC73** | + | FC21 | − | ← | *r* |
| **FC55** | + | $a_5$ FC47 | + | . | *r* n.i. | **FC73** | + | FC23 | − | ← | *r* |
| **FC55** | + | FC54 | + | . | *r* | **FC73** | + | $a_3$ FC23 | − | ← | $r^+$ |
| **FC55** | + | $a_5$ FC54 | + | . | *r* | **FC73** | + | FC28 | + | . | *r* n.i. |
| **FC55** | + | FC57 | + | . | *r* | **FC73** | + | FC32 | + | . | *r* n.i. |

† Not characterized.




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 3 (*cont.*)

| mutant | sign | mutant | sign | direction | type | mutant | sign | mutant | sign | direction | type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **FC73** | + | FC40 | + | . | *r* n.i. | **FC88** | – | FC57 | + | → | $r^+$ |
| **FC73** | + | FC47 | + | . | *r* n.i. | **FC88** | – | FC58 | + | → | m |
| **FC73** | + | FC88 | – | ← | *r* | **FC88** | – | FC63 | + | → | $r^+$ |
| **FC74** | – | FC42 | + | → | $r^+$ | FC88 | – | **FC73** | + | ← | *r* |
| **FC75** | – | FC42 | + | → | $r^+$ | **FC88** | – | FC108 | + | → | $r^+$ |
| **FC76** | – | FC42 | + | → | $r^+$ | **FC88** | – | FC109 | + | → | $r^+$ |
| **FC77** | – | FC42 | + | → | $r^+$ | **FC88** | – | FC110 | + | → | $r^+$ |
| **FC78** | – | FC42 | + | → | $r^+$ | **FC88** | – | FC111 | + | → | $r^+$ |
| **FC80** | – | FC42 | + | → | $r^+$ | **FC88** | – | FC112 | + | → | $r^+$ |
| **FC81** | – | FC47 | + | → | $r^+$ | **FC88** | – | FC113 | + | → | $r^+$ |
| **FC82** | – | FC40 | + | → | $r^+$ | **FC88** | – | FC114 | + | → | $r^+$ |
| FC82 | – | **FC41** | + | ← | *r* | **FC88** | – | FC115 | + | → | $r^+$ |
| **FC82** | – | FC42 | + | → | $r^+$ | **FC88** | – | FC116 | + | → | $r^+$ |
| **FC82** | – | FC47 | + | → | $r^+$ | **FC88** | – | FC125 | + | → | m |
| **FC82** | – | FC58 | + | → | m | **FC88** | – | FC215 | + | → | *r* |
| **FC82** | – | FC63 | + | → | $r^+$ | **FC88** | – | $a_5$ 176 | – | . | *r* |
| **FC83** | – | FC47 | + | → | $r^+$ | FC89 | + | **FC7** | – | → | $r^+$ |
| **FC84** | – | FC47 | + | → | $r^+$ | FC89 | + | **FC36** | + | . | m n.i. |
| **FC85** | – | FC47 | + | → | $r^+$ | FC90 | + | **FC7** | – | → | $r^+$ |
| **FC86** | – | FC0 | + | → | $r^+$ | **FC90** | + | FC20 | + | . | m |
| **FC86** | – | FC40 | + | → | $r^+$ | FC90 | + | **FC29** | + | . | m |
| **FC86** | – | FC42 | + | → | $r^+$ | FC90 | + | **FC31** | + | . | m |
| **FC86** | – | FC47 | + | → | $r^+$ | FC90 | + | **FC36** | + | . | m n.i. |
| **FC86** | – | FC58 | + | → | m | FC90 | + | **FC40** | + | . | *r* |
| **FC86** | – | FC63 | + | → | $r^+$ | **FC90** | + | FC47 | + | . | *r* |
| FC87 | – | **FC0** | + | ← | *r* | FC90 | + | **FC54** | + | . | *r* |
| FC87 | – | **FC31** | + | ← | *r* | FC90 | + | **FC54*** | + | . | m |
| FC87 | – | **FC31** $a_6$ | + | ← | $r^+$ | FC90 | + | **FC55** | + | . | *r* |
| FC87 | – | **FC36** | + | ← | *r* | FC90 | + | **FC55** $a_5$ | + | . | *r* n.i. |
| FC87 | – | **FC36** $a_6$ | + | ← | $r^+$ | FC90 | + | **FC57** | + | . | m |
| FC87 | – | **FC38** | + | ← | $r^+$ | **FC90** | + | FC58 | + | . | m |
| FC87 | – | **FC40** | + | ← | *r* | **FC90** | + | FC87 | – | ← | $r^+$ |
| FC87 | – | **FC41** | + | ← | *r* | FC90 | + | **FC102** | + | . | *r* n.i. |
| FC87 | – | **FC42** | + | ← | *r* | **FC90** | + | FC125 | + | . | m |
| FC87 | – | **FC47** | + | ← | $r^+$ | **FC90** | + | FC202 | – | ← | *r* |
| FC87 | – | **FC54** | + | ← | *r* | **FC90** | + | FC213 | + | . | m |
| FC87 | – | **FC54** $a_6$ | + | ← | $r^+$ | **FC90** | + | FC215 | + | . | m |
| FC87 | – | **FC55** | + | ← | *r* | **FC90** | + | FC217 | + | . | m |
| FC87 | – | **FC55** $a_5$ $a_6$† | + | ← | $r^+$ | **FC90** | + | FC222 | + | . | m |
| FC87 | – | **FC57** | + | ← | *r* | **FC90** | + | FC223 | + | . | m |
| FC87 | – | **FC57** $a_6$ | + | ← | $r^+$ | **FC90** | + | 370 | + | . | m |
| **FC87** | – | FC58 | + | → | $r^+$ n.i. | **FC90** | + | 514 | + | . | *r* n.i. |
| FC87 | – | **FC90** | + | ← | $r^+$ | **FC90** | + | 1018 | – | ← | $r^+$ |
| **FC87** | – | FC117 | + | → | $r^+$ | **FC90** | + | P61 | + | . | m |
| **FC87** | – | FC118 | + | → | $r^+$ | **FC90** | + | X146 | – | ← | $r^+$ |
| **FC87** | – | FC119 | + | → | $r^+$ | **FC90** | + | X225 | – | ← | $r^+$ |
| **FC87** | – | FC120 | + | → | $r^+$ | FC91 | + | **FC0** | + | . | *r* |
| **FC87** | – | FC121 | + | → | $r^+$ | **FC91** | + | FC7 | – | ← | $r^+$ |
| **FC87** | – | FC122 | + | → | $r^+$ | **FC91** | + | FC33 | + | . | m |
| **FC87** | – | FC123 | + | → | $r^+$ | **FC91** | + | FC47 | + | . | *r* n.i. |
| **FC87** | – | FC124 | + | → | $r^+$ | **FC91** | + | FC55 | + | . | m |
| **FC87** | – | FC125 | + | → | $r^+$ | **FC91** | + | FC92 | + | . | m |
| **FC87** | – | FC215 | + | → | $r^+$ | FC92 | + | **FC0** | + | . | *r* n.i. |
| FC87 | – | **176** $a_6$ | – | . | *r* | FC92 | + | **FC7** | – | → | $r^+$ |
| **FC87** | – | 261 | + | → | $r^+$ | FC92 | + | **FC40** | + | . | *r* n.i. |
| **FC87** | – | 370 | + | → | $r^+$ | **FC92** | + | FC47 | + | . | *r* n.i. |
| **FC87** | – | 488 | + | → | $r^+$ | FC92 | + | **FC91** | + | . | m |
| FC87 | – | **514** | + | ← | $r^+$ | **FC94** | + | FC7 | – | ← | $r^+$ |
| **FC87** | – | D10 | + | → | $r^+$ | **FC95** | + | FC7 | – | ← | $r^+$ |
| **FC87** | – | J158 | + | → | $r^+$ | FC96 | + | **FC7** | – | → | $r^+$ |
| **FC87** | – | P61 | + | → | $r^+$ | **FC96** | + | FC31 | + | . | *r* n.i. |
| FC88 | – | **FC0** | + | ← | *r* | **FC96** | + | FC54 | + | . | *r* n.i. |
| FC88 | – | **FC0** $a_4$ | + | ← | $r^+$ | **FC96** | + | FC55 | + | . | *r* |
| FC88 | – | **FC23** | – | . | *r* | **FC98** | + | FC7 | – | ← | $r^+$ |
| **FC88** | – | FC29 | + | → | $r^+$ | **FC98** | + | FC47 | + | . | *r* n.i. |
| **FC88** | – | FC36 | + | → | $r^+$ | **FC100** | – | FC0 | + | → | $r^+$ |
| FC88 | – | **FC41** | + | ← | *r* | FC101 | – | **FC0** | + | ← | $r^+$ |
| **FC88** | – | FC44 | + | → | $r^+$ | FC102 | + | **FC7** | – | → | $r^+$ |
| **FC88** | – | FC47 | + | → | $r^+$ | **FC102** | + | FC47 | + | . | *r* n.i. |

† Not characterized.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 3 (*cont.*)

| mutant | sign | mutant | sign | direction | type | mutant | sign | mutant | sign | direction | type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **FC102** | + | FC90 | + | . | *r* n.i. | **FC139** | – | FC47 | + | → | $r^+$ |
| **FC103** | + | FC7 | – | ← | $r^+$ | **FC140** | – | FC47 | + | → | $r^+$ |
| FC104 | + | **FC9** | – | → | $r^+$ | **FC141** | – | FC47 | + | → | $r^+$ |
| **FC104** | + | FC47 | + | . | *r* n.i. | **FC142** | – | FC47 | + | → | $r^+$ |
| **FC104** | + | FC55 | + | . | *r* | **FC143** | – | FC47 | + | → | $r^+$ |
| FC104 | + | **2074** | 0 | . | *r* | **FC144** | – | FC47 | + | → | $r^+$ |
| FC105 | + | **FC10** | – | → | $r^+$ | **FC145** | – | FC47 | + | → | $r^+$ |
| **FC105** | + | X511 | 0 | . | *r* | **FC146** | – | FC47 | + | → | $r^+$ |
| **FC106** | + | FC0 | + | . | *r* | **FC147** | – | FC47 | + | → | $r^+$ |
| **FC106** | + | $a_2$ FC9 | – | ← | $r^+$ | **FC148** | – | FC47 | + | → | $r^+$ |
| FC106 | + | **FC10** | – | → | $r^+$ | FC149 | – | FC38 | + | . | $r^+$ |
| FC108 | + | **FC36** | + | . | m n.i. | **FC150** | – | FC38 | + | → | $r^+$ |
| FC108 | + | **FC88** | – | → | $r^+$ | FC151 | – | **FC38** | + | ← | $r^+$ |
| FC109 | + | **FC36** | + | . | m n.i. | **FC151** | – | FC215 | + | → | $r^+$ |
| FC109 | + | **FC88** | – | → | $r^+$ | **FC151** | – | 556 | + | → | $r^+$ |
| FC110 | + | **FC36** | + | . | m n.i. | **FC152** | – | FC38 | + | → | $r^+$ |
| FC110 | + | **FC88** | – | → | $r^+$ | **FC153** | – | FC38 | + | → | $r^+$ |
| FC111 | + | **FC36** | + | . | m n.i. | **FC201** | – | FC215 | + | → | $r^+$ |
| FC111 | + | **FC88** | – | → | $r^+$ | **FC201** | – | P61 | + | → | $r^+$ |
| **FC112** | + | FC47 | + | . | m n.i. | **FC202** | – | FC20 | + | → | $r^+$ |
| FC112 | + | **FC88** | – | → | $r^+$ | FC202 | – | **FC47** | + | ← | *r* |
| **FC113** | + | FC47 | + | . | m n.i. | FC202 | – | **FC47** $a_7 a_8$ | + | ← | $r^+$ |
| FC113 | + | **FC88** | – | → | $r^+$ | FC202 | – | **FC90** | + | ← | *r* |
| **FC114** | + | FC47 | + | . | m n.i. | **FC202** | – | FC221 | + | → | $r^+$ |
| FC114 | + | **FC88** | – | → | $r^+$ | **FC202** | – | FC222 | + | → | $r^+$ |
| **FC115** | + | FC47 | + | . | *r* n.i. | **FC202** | – | FC223 | + | → | $r^+$ |
| FC115 | + | **FC88** | – | → | $r^+$ | **FC202** | – | FC224 | + | → | $r^+$ |
| **FC115** | + | $a_5$ 176 | – | ← | $r^+$ | **FC202** | – | FC225 | + | → | $r^+$ |
| **FC116** | + | FC47 | + | . | m n.i. | **FC202** | – | FC226 | + | → | $r^+$ |
| FC116 | + | **FC88** | – | → | $r^+$ | **FC202** | – | FC227 | + | → | $r^+$ |
| FC117 | + | **FC87** | – | → | $r^+$ | **FC202** | – | FC228 | + | → | $r^+$ |
| FC118 | + | **FC87** | – | → | $r^+$ | **FC202** | – | FC229 | + | → | $r^+$ |
| FC119 | + | **FC1** | – | → | m | **FC202** | – | FC230 | + | → | $r^+$ |
| FC119 | + | **FC87** | – | → | $r^+$ | **FC202** | – | P61 | + | → | $r^+$ |
| FC119 | + | **370** | + | . | *r* n.i. | **FC203** | – | P61 | + | → | $r^+$ |
| FC119 | + | **441** | – | → | $r^+$ | **FC204** | – | P61 | + | → | $r^+$ |
| FC119 | + | **D72** | – | → | $r^+$ | **FC205** | – | FC20 | + | → | $r^+$ |
| FC120 | + | **FC87** | – | → | $r^+$ | FC205 | – | **FC47** | + | ← | $r^+$ |
| FC121 | + | **FC87** | – | → | $r^+$ | **FC205** | – | FC211 | + | → | $r^+$ |
| FC122 | + | **FC87** | – | → | $r^+$ | **FC205** | — | FC212 | + | → | $r^+$ |
| FC123 | + | **FC1** | – | → | *r* | **FC205** | – | FC213 | + | → | $r^+$ |
| FC123 | + | **FC1** $_{m}a$ | – | → | $r^+$ | **FC205** | – | FC214 | + | → | $r^+$ |
| FC123 | + | **FC87** | – | → | $r^+$ | **FC205** | – | FC215 | + | → | $r^+$ |
| FC124 | + | **FC87** | – | → | $r^+$ | **FC205** | – | FC216 | + | → | $r^+$ |
| FC125 | + | **FC1** | – | → | m | **FC205** | – | FC217 | + | → | $r^+$ |
| FC125 | + | **FC29** | + | . | *r* n.i. | **FC205** | – | FC218 | + | → | $r^+$ |
| FC125 | + | **FC31** | + | . | m | **FC205** | – | FC219 | + | → | $r^+$ |
| FC125 | + | **FC36** | + | . | *r* | **FC205** | – | FC220 | + | → | $r^+$ |
| FC125 | + | **FC47** | + | . | m | FC205 | – | **196a** | + | ← | $r^+$ |
| FC125 | + | **FC58** | + | . | *r* n.i. | **FC205** | – | P61 | + | → | $r^+$ |
| FC125 | + | **FC87** | – | → | $r^+$ | **FC205** | – | X237 | 0 | . | *r* |
| FC125 | + | **FC88** | – | → | m | **FC206** | – | P61 | + | → | $r^+$ |
| FC125 | + | **FC90** | + | . | m | **FC207** | – | P61 | + | → | $r^+$ |
| FC125 | + | **FC238** | – | → | $r^+$ | **FC208** | – | P61 | + | → | $r^+$ |
| FC125 | + | **370** | + | . | *r* n.i. | **FC209** | – | P61 | + | → | $r^+$ |
| FC125 | + | **P61** | + | . | *r* n.i. | **FC210** | – | P61 | + | → | $r^+$ |
| FC126 | – | **FC42** | + | ← | $r^+$ | FC211 | + | **FC1** | – | → | m |
| FC127 | – | **FC42** | + | ← | $r^+$ | FC211 | + | **FC205** | – | → | $r^+$ |
| FC128 | + | **FC11** | – | → | $r^+$ | FC211 | + | **370** | + | . | *r* n.i. |
| **FC129** | – | FC47 | + | → | $r^+$ | FC212 | + | **FC205** | – | → | $r^+$ |
| **FC130** | – | FC47 | + | → | $r^+$ | FC213 | + | **FC1** | – | → | m |
| **FC131** | – | FC47 | + | → | $r^+$ | FC213 | + | **FC31** | + | . | *r* n.i. |
| **FC132** | – | FC47 | + | → | $r^+$ | FC213 | + | **FC36** | + | . | *r* n.i. |
| **FC133** | – | FC47 | + | → | $r^+$ | FC213 | + | **FC47** | + | . | m |
| **FC134** | – | FC47 | + | → | $r^+$ | FC213 | + | **FC90** | + | . | m |
| **FC135** | – | FC47 | + | → | $r^+$ | FC213 | + | **FC205** | – | → | $r^+$ |
| **FC136** | – | FC47 | + | → | $r^+$ | FC214 | + | **FC205** | – | → | $r^+$ |
| **FC137** | – | FC47 | + | → | $r^+$ | FC215 | + | **FC1** | – | → | *r* n.i. |
| FC138 | – | FC47 | + | . | $r^+$ | FC215 | + | **FC47** | + | . | m |


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 3 (*cont.*)

| mutant | sign | mutant | sign | direction | type | mutant | sign | mutant | sign | direction | type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FC215 | + | **FC87** | − | → | $r^+$ | **FC239** | − | FC222 | + | → | $r^+$ |
| FC215 | + | **FC88** | − | → | *r* | 176 | − | **FC0** | + | ← | *r* |
| FC215 | + | **FC90** | + | . | m | 176 | − | **FC23** $a_5$ | − | . | *r* |
| FC215 | + | **FC151** | − | → | $r^+$ | 176 | − | **FC29** | + | ← | $r^+$ |
| FC215 | + | **FC201** | − | → | $r^+$ | **176** | − | FC31 | + | → | $r^+$ |
| FC215 | + | **FC205** | − | → | $r^+$ | 176 | − | **FC36** | + | ← | $r^+$ |
| FC215 | + | **176** | − | → | m | **176** | − | FC38 | + | → | $r^+$ |
| FC216 | + | **FC205** | − | → | $r^+$ | 176 | − | **FC40** | + | ← | *r* |
| FC217 | + | **FC1** | − | → | m | 176 | − | **FC44** | + | ← | $r^+$ |
| FC217 | + | **FC31** | + | . | *r* n.i. | **176** | − | FC47 | + | → | $r^+$ |
| FC217 | + | **FC47** | + | . | m | 176 | − | **FC55** | + | ← | *r* |
| FC217 | + | **FC90** | + | . | m | 176 | − | **FC55** $a_5$ | + | ← | $r^+$ |
| FC217 | + | **FC205** | − | → | $r^+$ | 176 | − | **FC57** | + | ← | $r^+$ |
| FC217 | + | **370** | + | . | *r* n.i. | **176** | − | $a_6$ FC87 | − | . | *r* |
| FC218 | + | **FC205** | − | → | $r^+$ | 176 | − | **FC88** $a_5$ | − | . | *r* |
| FC219 | + | **FC205** | − | → | $r^+$ | 176 | − | **FC115** $a_5$ | + | ← | $r^+$ |
| FC220 | + | **FC205** | − | → | $r^+$ | **176** | − | FC215 | + | → | m |
| FC221 | + | **FC202** | − | → | $r^+$ | 196a | + | **FC10** | − | → | $r^+$ |
| FC222 | + | **FC1** | − | → | m | **196a** | + | FC20 | + | . | m |
| FC222 | + | **FC31** | + | . | *r* n.i. | 196a | + | **FC31** | + | . | m n.i. |
| FC222 | + | **FC36** | + | . | *r* n.i. | 196a | + | **FC36** | + | . | m n.i. |
| FC222 | + | **FC47** | + | . | m | **196a** | + | FC205 | − | ← | $r^+$ |
| FC222 | + | **FC90** | + | . | m | **244** | + | FC0 | + | . | *r* n.i. |
| FC222 | + | **FC202** | − | → | $r^+$ | **244** | + | $a_2$ FC9 | − | ← | $r^+$ |
| FC222 | + | **FC231** | − | → | $r^+$ | **244** | + | FC28 | + | . | *r* n.i. |
| FC222 | + | **FC232** | − | → | $r^+$ | **244** | + | FC32 | + | . | *r* n.i. |
| FC222 | + | **FC233** | − | → | $r^+$ | **244** | + | FC40 | + | . | *r* n.i. |
| FC222 | + | **FC234** | − | → | $r^+$ | 261 | + | **FC87** | − | → | $r^+$ |
| FC222 | + | **FC235** | − | → | $r^+$ | **360** | 0 | FC0 | + | . | *r* |
| FC222 | + | **FC236** | − | → | $r^+$ | **360** | 0 | $a_2$ FC9 | − | . | *r* |
| FC222 | + | **FC237** | − | → | $r^+$ | 360 | 0 | **FC10** | − | . | *r* |
| FC222 | + | **FC238** | − | → | $r^+$ | 360 | 0 | **FC41** | + | . | *r* |
| FC222 | + | **FC239** | − | → | $r^+$ | 370 | + | **FC1** | − | → | m |
| FC222 | + | **370** | + | . | *r* n.i. | 370 | + | **FC31** | + | . | *r* n.i. |
| FC223 | + | **FC31** | + | . | *r* n.i. | 370 | + | **FC47** | + | . | m |
| FC223 | + | **FC36** | + | . | *r* n.i. | 370 | + | **FC87** | − | → | $r^+$ |
| FC223 | + | **FC47** | + | . | m | 370 | + | **FC90** | + | . | m |
| FC223 | + | **FC90** | + | . | m | **370** | + | FC119 | + | . | *r* n.i. |
| FC223 | + | **FC202** | − | → | $r^+$ | **370** | + | FC125 | + | . | *r* n.i. |
| FC223 | + | **370** | + | . | *r* n.i. | **370** | + | FC211 | + | . | *r* n.i. |
| FC224 | + | **FC202** | − | → | $r^+$ | **370** | + | FC217 | + | . | *r* n.i. |
| FC225 | + | **FC202** | − | → | $r^+$ | **370** | + | FC222 | + | . | *r* n.i. |
| FC226 | + | **FC202** | − | → | $r^+$ | **370** | + | FC223 | + | . | *r* n.i. |
| FC227 | + | **FC202** | − | → | $r^+$ | **370** | + | FC236 | − | ← | *r* |
| FC228 | + | **FC202** | − | → | $r^+$ | **370** | + | $a_9a_{10}$ FC236 | − | ← | $r^+$ |
| FC229 | + | **FC202** | − | → | $r^+$ | **370** | + | $a_{10}$ FC236 | − | ← | *r* |
| FC230 | + | **FC202** | − | → | $r^+$ | **370** | + | FC238 | − | ← | *r* |
| **FC231** | − | FC222 | + | → | $r^+$ | **370** | + | $a_9$ FC238 | − | ← | $r^+$ |
| **FC232** | − | FC222 | + | → | $r^+$ | 370 | + | **514** | + | . | *r* n.i. |
| **FC233** | − | FC222 | + | → | $r^+$ | **370** | + | P61 | + | . | *r* n.i. |
| **FC234** | − | FC222 | + | → | $r^+$ | 375 | 0 | **FC6** | − | . | *r* |
| **FC235** | − | FC222 | + | → | $r^+$ | **375** | 0 | FC38 | + | . | *r* |
| FC236 | − | **FC47** | + | ← | *r* | **441** | − | FC119 | + | → | $r^+$ |
| **FC236** | − | FC222 | + | → | $r^+$ | 488 | + | **FC87** | − | → | $r^+$ |
| FC236 | − | **370** | + | ← | *r* | 514 | + | **FC1** | − | → | $r^+$ |
| FC236 | − | **370** $a_{10}$ | + | ← | *r* | 514 | + | **FC10** | − | → | $r^+$ |
| FC236 | − | **370** $a_9a_{10}$ | + | ← | $r^+$ | 514 | + | **FC31** | + | . | m |
| FC236 | − | **P61** | + | ← | *r* | 514 | + | **FC36** | + | . | m n.i. |
| FC236 | − | **P61** $a_{10}$ | + | ← | $r^+$ | **514** | + | FC47 | + | . | *r* n.i. |
| **FC237** | − | FC222 | + | → | $r^+$ | 514 | + | **FC54** | + | . | *r* |
| FC238 | − | **FC47** | + | ← | *r* | 514 | + | **FC54*** | + | . | m |
| **FC238** | − | FC125 | + | → | $r^+$ | 514 | + | **FC55** $a_5$ | + | . | *r* n.i. |
| **FC238** | − | FC222 | + | → | $r^+$ | **514** | + | FC87 | − | ← | $r^+$ |
| FC238 | − | **370** | + | ← | *r* | 514 | + | **FC90** | + | . | *r* n.i. |
| FC238 | − | **370** $a_9$ | + | ← | $r^+$ | **514** | + | 370 | + | . | *r* n.i. |
| FC238 | − | **EM84** | 0 | . | *r* | 556 | + | **FC1** | − | → | m |
| FC238 | − | **HB74** | 0 | . | *r* | 556 | + | FC47 | + | . | m |
| FC238 | − | **N24** | 0 | . | *r* | 556 | + | **FC151** | − | → | $r^+$ |
| FC238 | − | **NT332** | 0 | . | *r* | **739** | 0 | FC0 | + | . | *r* |


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 3 (*cont.*)

| mutant | sign | mutant | sign | direction | type | mutant | sign | mutant | sign | direction | type |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 739 | 0 | **FC10** | − | . | *r* | P61 | + | **FC203** | − | → | $r^+$ |
| 997 | + | **FC1** | − | → | $r^+$ | P61 | + | **FC204** | − | → | $r^+$ |
| 1018 | − | **FC90** | + | ← | $r^+$ | P61 | + | **FC205** | − | → | $r^+$ |
| **1074** | − | FC47 | + | → | $r^+$ | P61 | + | **FC206** | − | → | $r^+$ |
| 1651 | + | **FC10** | − | → | $r^+$ | P61 | + | **FC207** | − | → | $r^+$ |
| 2074 | 0 | **FC0** | + | . | *r* | P61 | + | **FC208** | − | → | $r^+$ |
| 2074 | 0 | **FC6** | − | . | *r* | P61 | + | **FC209** | − | → | $r^+$ |
| 2074 | 0 | **FC9** | − | . | *r* | P61 | + | **FC210** | − | → | $r^+$ |
| **2074** | 0 | FC23 | − | . | *r* | **P61** | + | FC236 | − | ← | *r* |
| **2074** | 0 | FC38 | + | . | *r* | **P61** | + $a_{10}$ | FC236 | − | ← | $r^+$ |
| **2074** | 0 | FC104 | + | . | *r* | P61 | + | **370** | + | . | *r* n.i. |
| A63 | + | **FC10** | − | → | $r^+$ | P61 | + | **NT332** | 0 | . | *r* |
| D10 | + | **FC87** | − | → | $r^+$ | **P61** | + | X237 | 0 | . | *r* |
| **D72** | − | FC119 | + | → | $r^+$ | UV104 | 0 | **FC1** | − | . | *r* |
| EM84 | 0 | **FC1** | − | . | *r* | **UV104** | 0 | FC47 | + | . | *r* |
| EM84 | 0 | **FC6** | − | . | *r* | UV357 | 0 | **FC1** | − | . | *r* |
| **EM84** | 0 | FC38 | + | . | *r* | **UV357** | 0 | FC47 | + | . | *r* |
| **EM84** | 0 | FC238 | − | . | *r* | **UV375** | 0 | FC0 | + | . | *r* |
| **F96** | − | FC38 | + | → | $r^+$ | UV375 | 0 | **FC10** | − | . | *r* |
| **F96** | − | FC47 | + | → | $r^+$ | X27 | 0 | **FC6** | − | . | *r* |
| HB74 | 0 | **FC6** | − | . | *r* | **X27** | 0 | FC38 | + | . | *r* |
| **HB74** | 0 | FC38 | + | . | *r* | X146 | − | **FC31** $a_6$ | + | ← | $r^+$ |
| **HB74** | 0 | FC238 | − | . | *r* | **X146** | − | FC34 | + | → | m |
| J158 | + | **FC87** | − | → | $r^+$ | X146 | − | **FC36** | + | ← | *r* |
| N24 | 0 | **FC1** | − | . | *r* | X146 | − | **FC36** $a_6$ | + | ← | $r^+$ |
| **N24** | 0 | FC38 | + | . | *r* | X146 | − | **FC90** | + | ← | $r^+$ |
| **N24** | 0 | FC238 | − | . | *r* | **X225** | − | FC34 | + | → | m |
| NB7017 | + | **FC10** | − | → | $r^+$ | X225 | − | **FC90** | + | ← | $r^+$ |
| NT332 | 0 | **FC6** | − | . | *r* | X237 | 0 | **FC205** | − | . | *r* |
| **NT332** | 0 | FC38 | + | . | *r* | X237 | 0 | **P61** | + | . | *r* |
| **NT332** | 0 | FC238 | − | . | *r* | X511 | 0 | **FC10** | − | . | *r* |
| **NT332** | 0 | P61 | + | . | *r* | **X511** | 0 | FC47 | + | . | *r* |
| **P53** | ? | FC6 | − | . | m | X511 | 0 | **FC105** | + | . | *r* |
| **P53** | ? | FC9 | − | . | *r* | X655 | 0 | **FC1** | − | . | *r* |
| **P53** | ? | FC41 | + | . | *r* | **X655** | 0 | FC38 | + | . | *r* |
| P61 | + | **FC1** | − | → | m | **X707** | − | FC40 | + | → | $r^+$ |
| P61 | + | **FC31** | + | . | *r* | **X707** | − | FC58 | + | → | m |
| P61 | + | **FC36** | + | . | *r* | X732 | + | **FC10** | − | → | $r^+$ |
| P61 | + | **FC47** | + | . | m | X732 | + | **FC11** | − | → | $r^+$ |
| P61 | + | **FC87** | − | → | $r^+$ | X763 | + | **FC10** | − | → | $r^+$ |
| P61 | + | **FC90** | + | . | m | X806 | + | **FC10** | − | → | $r^+$ |
| **P61** | + | FC125 | + | . | *r* n.i. | **X824** | + | FC1 | − | ← | $r^+$ |
| P61 | + | **FC201** | − | → | $r^+$ | **X833** | − | FC47 | + | → | $r^+$ |
| P61 | + | **FC202** | − | → | $r^+$ | **X833** | − | FC58 | + | → | m |

*Notes:*

UV104 is probably identical to 375 and is not therefore shown on the map in figure 2.
X237 is to the right of P61 just beyond the limit of our present map.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 4(*a*). DOUBLES $(+-) \leftarrow r^+$

| double | phenotype on B | method(s) of isolation | remarks† |
|---|---|---|---|
| (X824+FC1) | $r^+$ | X824 *v.* FC1 | |
| (FC41+FC1) | '$r^+$' | revertant of FC1 | |
| (FC41+$a_2$+FC9) | $r^+$ | FC41 *v.* $a_2$FC9 | |
| (FC106+$a_2$+FC9) | '$r^+$' | FC106 *v.* $a_2$FC9 | |
| (244+$a_2$+FC9) | $r^+$ | 244 *v.* $a_2$FC9 | |
| (FC73+$a_3$+FC23) | $r^+$ | revertant of (FC73+FC23) | |
| (FC0+FC18) | '$r^+$' | revertant of FC0 | |
| (FC0+FC21) | '$r$' | revertant of FC0 | |
| (FC0+FC7) | '$r^+$' | revertant of FC0 | |
| (FC0+FC8) | '$r^+$' | revertant of FC0 | |
| (FC0+FC14) | '$r^+$' | revertant of FC0 | |
| (FC0+FC15) | '$r^+$' | revertant of FC0 | |
| (FC0+FC23) | '$r^+$' | revertant of FC0 | |
| (FC0+$a_4$+FC88) | '$r^+$' | revertant of (FC0+FC88) | bromodeoxyuridine |
| (FC46+FC7) | $r^+$ | revertant of FC7 | |
| (FC48+FC7) | $r^+$ | revertant of FC7 | |
| (FC94+FC7) | $r^+$ | revertant of FC7 | acridine yellow |
| (FC95+FC7) | $r^+$ | revertant of FC7 | acridine yellow |
| (FC98+FC7) | $r^+$ | revertant of FC7 | acridine yellow |
| (FC103+FC7) | '$r^+$' | revertant of FC7 | |
| (FC91+FC7) | $r^+$ | revertant of FC7 | acridine yellow |
| (FC40+FC23) | $r^+$ | FC40 *v.* FC23 | |
| (FC42+FC126) | $r^+$ | revertant of FC42 | |
| (FC42+FC127) | $r^+$ | revertant of FC42 | |
| (FC55+$a_5$+176) | $r^+$ | revertant of (FC55+176) | |
| (FC55+$a_5$+$a_6$+FC87) | '$r$' | revertant of (FC55+FC87) | not characterized fully |
| (FC36+176) | '$r^+$' | 176 *v.* (FC36+FC54) | |
| (FC36+$a_6$+X146) | '$r$' | FC36$a_6$ *v.* X146 | |
| | | revertant of (FC36+X146) | 2-aminopurine |
| (FC36+$a_6$FC87) | '$r$' | revertant of (FC36+FC87) | |
| (FC57+176) | '$r^+$' | 176 *v.* (FC57+FC54) | |
| (FC57+$a_6$+FC87) | '$r^+$' | revertant of (FC57+FC87) | bromodeoxyuridine |
| (FC44+176) | $r^+$ | FC44 *v.* 176 | |
| (FC29+176) | $r^+$ | FC29 *v.* 176 | |
| (FC115+$a_5$+176) | $r^+$ | FC115$a_5$ *v.* 176 | |
| (FC54+$a_6$+FC87) | '$r$' | revertant of (FC54+FC87) | |
| | | revertant of (FC54+FC87) | 2-aminopurine |
| | | (FC54+FC87) *v.* $a_6$FC87 | |
| (FC31+$a_6$+X146) | '$r$' | FC31$a_6$ *v.* X146 | |
| (FC31+$a_6$+FC87) | '$r$' | revertant of (FC31+FC87) | |
| (FC90+X146) | '$r$' | (FC90+FC47) *v.* X146‡ | selected on B+K |
| | | FC90 *v.* X146 | |
| (FC90+X225) | '$r$' | (FC90+FC47) *v.* X225‡ | selected on B+K |
| | | FC90 *v.* X225 | |
| (FC90+1018) | '$r$' | (FC90+FC47) *v.* 1018‡ | |
| (FC90+FC87) | '$r$' | FC90 *v.* FC87 | |
| (514+FC87) | '$r^+$' | 514 *v.* FC87 | |
| (FC38+FC151) | $r^+$ | revertant of FC38 | |
| (FC38+FC87) | '$r^+$' | FC38+FC87 | |
| (FC47+FC205) | $r^+$ | FC47 *v.* FC205 | |
| (FC47+FC87) | $r^+$ | revertant of FC47 | |
| (FC47+$a_7$+$a_8$+FC202) | '$r^+$' | revertant of (FC47+FC202) | |
| (196a+FC205) | '$r^+$' | *r*196 *v.* FC205¶ | |
| (P61+$a_{10}$+FC236) | $r^+$ | revertant of (P61+FC236) | 2-aminopurine |
| (370+$a_9$+FC238) | '$r^+$' | revertant of (370+FC238) | 2-aminopurine |
| (370+$a_9$+$a_{10}$+FC236) | $r^+$ | revertant of (370+$a_9$+FC236) | 2-aminopurine |

† A revertant is spontaneous unless indicated otherwise in this column.

‡ A minus mutant crossed with a (++) double where the minus is very close to, or identical with, one of the plus mutants (see §6(*b*)(vii)).

¶ *r*196 is original deletion (see §11(*a*)). FC205 does not recombine with 196b. The wild-type double mutant was checked by backcrossing.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 4(*b*). DOUBLES (+ −) ← *r*

| double | method(s) of isolation | no. of barriers | remarks |
|---|---|---|---|
| (FC41+FC9) | FC41 *v.* FC9 | 1 | . |
| (FC41+FC82) | FC41 *v.* FC82 | 1 | . |
| (FC41+FC21) | FC41 *v.* FC21 | 2 | . |
| (FC41+FC88) | FC41 *v.* FC88 | 3 | . |
| (FC41+FC87) | FC41 *v.* FC87 | 5 | . |
| (FC73+FC21) | FC73 *v.* FC21 | 1 | . |
| (FC73+FC23) | FC73 *v.* FC23 | 1 | . |
| (FC73+FC88) | FC73 *v.* FC88 | 2 | . |
| (FC0+FC88) | FC0 *v.* FC88 | 1 | . |
| (FC0+176) | FC0 *v.* 176 | 2 | . |
| (FC0+FC87) | FC0 *v.* FC87 | 3 | . |
| (FC40+176) | (FC0+FC40+176 $+a_6$+FC87)† *v.* wild | 2 | . |
| (FC40+FC87) | FC40 *v.* FC87 | 3 | . |
| (FC42+FC87) | FC42 *v.* FC87 | 2 | . |
| (FC36+X146) | FC36 *v.* X146 | 1 | . |
| (FC36+FC87) | FC36 *v.* FC87 | 1 | . |
| (FC57+FC87) | FC57 *v.* FC87 | 1 | . |
| (FC55+176) | FC55 *v.* 176 | 1 | . |
| (FC55+FC87) | FC55 *v.* FC87 | 2 | . |
| (FC54+FC87) | FC54 *v.* FC87 | 1 | . |
| (FC31+FC87) | FC31 *v.* FC87 | 1 | . |
| (FC90+FC202) | FC90 *v.* FC202 | 2 | . |
| (FC47+FC202) | FC47 *v.* FC202 | 2 | . |
| (FC47+FC238) | FC47 *v.* FC238 | 3 | . |
| (FC47+FC236) | FC47 *v.* FC236 | 4 | . |
| (370+FC238) | 370 *v.* FC238 | 1 | selected on QA1 |
| (370+FC236) | 370 *v.* FC236 | 2 | . |
| (370+$a_{10}$+FC236) | 370 *v.* FC236 | 1 | selected on CR63 |
| | revertant of (370+FC236) with 2-aminopurine | | . |
| (P61+FC236) | P61 *v.* FC236 | 1 | . |

† This is a wild-type mutant of construction (++ −−) and behaves like a triple plus. When back-crossed, yields component doubles (see § 6(*b*)(v)).

TABLE A 4(*c*). DOUBLES (− +) → $r^+$

| double | phenotype on B | method of isolation | remarks† |
|---|---|---|---|
| (FC6+FC0) | $r^+$ | revertant of FC0 | . |
| (FC6+FC28) | '$r^+$' | revertant of FC6 | . |
| (FC6+FC35) | '$r$' | revertant of FC6 | . |
| (FC6+FC32) | '$r^+$' | revertant of FC6 | . |
| (FC6+FC33) | $r^+$ | revertant of FC6 | . |
| (FC6+FC36) | $r^+$ | revertant of FC6 | . |
| (FC6+FC29) | $r^+$ | revertant of FC6 | . |
| (FC6+FC31) | $r^+$ | revertant of FC6 | . |
| (FC6+FC30) | $r^+$ | revertant of FC6 | . |
| (FC6+FC38) | $r^+$ | revertant of FC6 | . |
| (FC6+FC39) | $r^+$ | revertant of FC6 | . |
| (FC86+FC0) | $r^+$ | FC86 *v.* FC0 | . |
| (FC86+FC40) | '$r^+$' | FC86 *v.* FC40 | . |
| (FC86+FC42) | $r^+$ | FC86 *v.* FC42 | . |
| (FC86+FC47) | $r^+$ | revertant of FC47 | . |
| (FC86+FC63) | '$r^+$' | FC86 *v.* FC63 | . |
| (FC10+FC106) | $r^+$ | revertant of FC10 | . |
| (FC10+FC105) | $r^+$ | revertant of FC10 | . |
| (FC10+X763) | $r^+$ | FC10 *v.* X763 | . |
| (FC10+FC0) | $r^+$ | revertant of FC0 | . |

† A revertant is spontaneous unless indicated otherwise in this column.




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 4(*c*) (*cont.*)

| double | phenotype on B | method of isolation | remarks† |
|---|---|---|---|
| (FC10+X732) | $r^+$ | FC10 *v.* X732 | . |
| (FC10+X806) | $r^+$ | FC10 *v.* X806 | . |
| (FC10+A63) | $r^+$ | FC10 *v.* A63 | . |
| (FC10+FC64) | '$r^+$' | revertant of FC10 | . |
| (FC10+NB7017) | $r^+$ | FC10 *v.* NB7017 | . |
| (FC10+FC62) | $r^+$ | revertant of FC10 | . |
| (FC10+1651) | $r^+$ | FC10 *v.* 1651 | . |
| (FC10+514) | $r^+$ | FC10 *v.* 514 | . |
| (FC10+FC63) | $r^+$ | revertant of FC10 | . |
| (FC10+196a) | $r^+$ | FC10 *v.* 196a | . |
| (FC11+FC73) | $r^+$ | revertant of FC11 | . |
| (FC11+FC0) | '$r^+$' | revertant of FC0 | . |
| (FC11+FC68) | '$r^+$' | revertant of FC11 | . |
| (FC11+FC72) | $r^+$ | revertant of FC11 | . |
| (FC11+FC128) | $r^+$ | revertant of FC11 | . |
| (FC11+X732) | $r^+$ | FC11 *v.* X732 | . |
| (FC11+FC67) | '$r^+$' | revertant of FC11 | . |
| (FC11+FC66) | $r^+$ | revertant of FC11 | . |
| (FC11+FC71) | $r^+$ | revertant of FC11 | . |
| (FC11+FC69) | $r^+$ | revertant of FC11 | . |
| (FC12+FC0) | '$r^+$' | revertant of FC0 | . |
| (FC19+FC0) | $r^+$ | revertant of FC0 | . |
| (FC22+FC0) | $r^+$ | revertant of FC0 | . |
| (FC27+FC0) | $r^+$ | revertant of FC0 | . |
| (FC74+FC42) | $r^+$ | revertant of FC42 | . |
| (FC75+FC42) | $r^+$ | revertant of FC42 | . |
| (FC76+FC42) | $r^+$ | revertant of FC42 | . |
| (FC77+FC42) | $r^+$ | revertant of FC42 | . |
| (FC78+FC42) | $r^+$ | revertant of FC42 | . |
| (FC80+FC42) | $r^+$ | revertant of FC42 | . |
| (FC81+FC47) | $r^+$ | revertant of FC47 | . |
| (FC85+FC47) | $r^+$ | revertant of FC47 | . |
| (FC1+997) | $r^+$ | (FC1+FC9) *v.* 997 | (FC9+997) is not made |
| (FC1+FC0) | '$r^+$' | revertant of FC0 | . |
| (FC1+FC40) | '$r$' | revertant of FC1 | . |
| (FC1+FC42) | not isolated | FC1 *v.* FC42 | orgy-crossed (see § 2(*d*)(iv)) |
| (FC1+514) | $r^+$ | FC1 *v.* 514 | . |
| (FC1+FC38) | '$r^+$' | FC1 *v.* FC38 | . |
| (FC1+FC123) | '$r^+$' | revertant of (FC1+FC123) | 2-aminopurine |
| (FC1+$_m$a+FC125) | $r^+$ | FC1$_m$a *v.* (1231 +FC125) | see § 8(*h*) |
| (FC9+FC0) | '$r^+$' | revertant of FC0 | . |
| (FC9+FC49) | '$r$' | revertant of FC9 | . |
| (FC9+FC40) | '$r$' | FC9 *v.* FC40 | . |
| (FC9+FC51) | '$r$' | revertant of FC9 | . |
| (FC9+FC104) | '$r^+$' | revertant of FC9 | . |
| (FC9+FC42) | $r^+$ | FC9 *v.* FC42 | . |
| (FC9+FC50) | $r^+$ | revertant of FC9 | . |
| (FC9+FC55) | $r^+$ | revertant of FC9 | . |
| (FC9+FC56) | $r^+$ | revertant of FC9 | . |
| (FC9+FC52) | $r^+$ | revertant of FC9 | . |
| (FC9+FC53) | $r^+$ | revertant of FC9 | . |
| (FC9+FC57) | $r^+$ | revertant of FC9 | . |
| (FC9+FC54) | $r^+$ | revertant of FC9 | . |
| (FC9+FC38) | $r^+$ | FC9 *v.* FC38 | . |
| (FC9+FC47) | $r^+$ | (FC1+FC9) *v.* (X511+FC47)‡ | . |
| (FC129+FC47) | $r^+$ | revertant of FC47 | proflavine |
| (FC132+FC47) | $r^+$ | revertant of FC47 | proflavine |

‡ A (− −) double crossed with a (0 +) double where X511, the zero sign mutant, maps extremely close to FC9, one of the minus mutants. The wild-type double mutant was checked by backcrossing.


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 4(*c*) (*cont.*)

| double | phenotype on B | method of isolation | remarks† |
|---|---|---|---|
| (FC136+FC47) | $r^+$ | revertant of FC47 | proflavine |
| (FC140+FC47) | '$r^+$' | revertant of FC47 | aminoacridine |
| (FC142+FC47) | $r^+$ | revertant of FC47 | aminoacridine |
| (FC82+FC40) | '$r^+$' | FC82 *v.* FC40 | . |
| (FC82+FC42) | $r^+$ | FC82 *v.* FC42 | . |
| (FC82+FC47) | $r^+$ | revertant of FC47 | . |
| (FC82+FC63) | '$r^+$' | FC82 *v.* FC63 | . |
| (FC131+FC47) | $r^+$ | revertant of FC47 | proflavine |
| (FC133+FC47) | $r^+$ | revertant of FC47 | proflavine |
| (FC135+FC47) | $r^+$ | revertant of FC47 | proflavine |
| (FC141+FC47) | '$r^+$' | revertant of FC47 | aminoacridine |
| (FC144+FC47) | '$r^+$' | revertant of FC47 | aminoacridine |
| (FC145+FC47) | '$r^+$' | revertant of FC47 | aminoacridine |
| (FC146+FC47) | '$r^+$' | revertant of FC47 | aminoacridine |
| (X833+FC47) | $r^+$ | X833 *v.* FC47 | . |
| (FC148+FC47) | $r^+$ | revertant of FC47 | aminoacridine |
| (FC147+FC47) | $r^+$ | revertant of FC47 | aminoacridine |
| (FC139+FC47) | '$r$' | revertant of FC47 | aminoacridine |
| (1074+FC47) | $r^+$ | 1074 *v.* FC47 | . |
| (X707+FC40) | $r^+$ | X707 *v.* FC40 | . |
| (FC152+FC38) | $r^+$ | revertant of FC38 | . |
| (FC21+FC38) | $r^+$ | FC21 *v.* FC38 | . |
| (FC7+FC42) | $r^+$ | revertant of FC7 | . |
| (FC7+FC92) | $r^+$ | revertant of FC7 | acridine yellow |
| (FC7+FC96) | $r^+$ | revertant of FC7 | acridine yellow |
| (FC7+FC45) | '$r^+$' | revertant of FC7 | . |
| (FC7+FC43) | '$r^+$' | revertant of FC7 | . |
| (FC7+FC44) | '$r^+$' | revertant of FC7 | . |
| (FC7+FC102) | '$r^+$' | revertant of FC7 | . |
| (FC7+FC90) | $r^+$ | revertant of FC7 | acridine yellow |
| | | FC7 *v.* FC90 | . |
| (FC7+FC47) | '$r^+$' | revertant of FC7 | . |
| (FC7+FC89) | $r^+$ | revertant of FC7 | acridine yellow |
| (FC137+FC47) | $r^+$ | revertant of FC47 | proflavine |
| (FC134+FC47) | $r^+$ | revertant of FC47 | proflavine |
| (FC83+FC47) | $r^+$ | revertant of FC47 | . |
| (FC84+FC47) | $r^+$ | revertant of FC47 | . |
| (FC88+FC36) | '$r^+$' | revertant of FC47 | . |
| (FC88+FC112) | '$r^+$' | revertant of FC88 | . |
| (FC88+FC113) | '$r$' | revertant of FC88 | . |
| (FC88+FC114) | '$r^+$' | revertant of FC88 | . |
| (FC88+FC116) | '$r$' | revertant of FC88 | . |
| (FC88+FC57) | '$r^+$' | FC88 *v.* FC57 | . |
| (FC88+FC29) | '$r^+$' | FC88 *v.* FC29 | . |
| (FC88+FC44) | '$r$' | FC88 *v.* FC44 | . |
| (FC88+FC115) | '$r^+$' | revertant of FC88 | . |
| (FC88+FC47) | $r^+$ | revertant of FC47 | . |
| (FC88+FC63) | $r^+$ | FC88 *v.* FC63 | . |
| (FC88+FC108) | $r^+$ | revertant of FC88 | . |
| (FC88+FC109) | $r^+$ | revertant of FC88 | . |
| (FC88+FC110) | $r^+$ | revertant of FC88 | . |
| (FC88+FC111) | $r^+$ | revertant of FC88 | . |
| (FC143+FC47) | '$r^+$' | revertant of FC47 | aminoacridine |
| (FC150+FC38) | $r^+$ | revertant of FC38 | . |
| (FC153+FC38) | $r^+$ | revertant of FC38 | . |
| (F96+FC38) | '$r^+$' | F96 *v.* FC38 | . |
| (F96+FC47) | '$r^+$' | F96 *v.* FC47 | . |
| (176+FC31) | '$r^+$' | 176 *v.* (FC54+FC31) | see footnote‡ table A 4(*a*) |
| (176+FC38) | $r^+$ | 176 *v.* FC38 | . |
| (176+FC47) | $r^+$ | 176 *v.* FC47 | . |
| (FC151+FC215) | '$r^+$' | FC151 *v.* FC215 | . |
| (FC205+FC212) | '$r^+$' | revertant of FC205 | . |





PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 4(*c*) (*cont.*)

| double | phenotype on B | method of isolation | remarks† |
|---|---|---|---|
| (FC205+FC214) | '$r^+$' | revertant of FC205 | . |
| (FC205+FC216) | '$r^+$' | revertant of FC205 | . |
| (FC205+FC218) | $r^+$ | revertant of FC205 | . |
| (FC205+FC219) | '$r$' | revertant of FC205 | . |
| (FC205+FC220) | '$r^+$' | revertant of FC205 | . |
| (FC205+P61) | $r^+$ | revertant of P61 | . |
| (FC205+FC217) | '$r^+$' | revertant of FC205 | . |
| (FC205+FC211) | '$r$' | revertant of FC205 | . |
| (FC205+FC20) | $r^+$ | FC205 *v.* FC20 | . |
| (FC205+FC213) | '$r^+$' | revertant of FC205 | . |
| (FC205+FC215) | '$r$' | revertant of FC 205 | . |
| (FC206+P61) | $r^+$ | revertant of P61 | . |
| (FC207+P61) | $r^+$ | revertant of P61 | . |
| (FC210+P61) | '$r^+$' | revertant of P61 | . |
| (FC232+FC222) | '$r$' | revertant of FC222 | . |
| (FC234+FC222) | '$r$' | revertant of FC222 | . |
| (FC239+FC222) | '$r$' | revertant of FC222 | . |
| (D72+FC119) | '$r^+$' | D72 *v.* FC119 | . |
| (FC87+370) | '$r^+$' | FC87 *v.* 370 | . |
| (FC87+FC58) | not isolated | FC87 *v.* FC58 | orgy-crossed (see § 2(*d*) (iv)) |
| (FC87+261) | '$r^+$' | FC87 *v.* 261 | . |
| (FC87+D10) | $r^+$ | FC87 *v.* D10 | . |
| (FC87+P61) | '$r^+$' | FC87 *v.* P61 | . |
| (FC87+FC117) | $r^+$ | revertant of FC87 | . |
| (FC87+FC118) | $r^+$ | revertant of FC87 | . |
| (FC87+FC120) | '$r^+$' | revertant of FC87 | . |
| (FC87+FC122) | $r^+$ | revertant of FC87 | . |
| (FC87+FC124) | $r^+$ | revertant of FC87 | . |
| (FC87+J158) | '$r^+$' | FC87 *v.* J158 | . |
| (FC87+FC125) | '$r$' | revertant of FC87 | . |
| (FC87+FC121) | '$r$' | revertant of FC87 | . |
| (FC87+FC123) | '$r$' | revertant of FC87 | . |
| (FC87+488) | '$r$' | FC87 *v.* 488 | . |
| (FC87+FC119) | '$r$' | revertant of FC87 | . |
| (FC87+FC215) | '$r^+$' | FC87 *v.* FC215 | . |
| (FC208+P61) | $r^+$ | revertant of P61 | . |
| (FC209+P61) | $r^+$ | revertant of P61 | . |
| (FC201+P61) | $r^+$ | revertant of P61 | . |
| (FC201+FC215) | '$r^+$' | FC201 *v.* FC215 | . |
| (FC203+P61) | $r^+$ | revertant of P61 | . |
| (FC204+P61) | $r^+$ | revertant of P61 | . |
| (FC231+FC222) | '$r^+$' | revertant of FC222 | . |
| (FC233+FC222) | $r^+$ | revertant of FC222 | . |
| (FC235+FC222) | $r^+$ | revertant of FC222 | . |
| (441+FC119) | $r^+$ | 441 *v.* FC119 | . |
| (FC202+FC223) | $r^+$ | revertant of FC202 | . |
| (FC202+FC225) | $r^+$ | revertant of FC202 | . |
| (FC202+FC226) | $r^+$ | revertant of FC202 | . |
| (FC202+FC227) | $r^+$ | revertant of FC202 | . |
| (FC202+FC228) | $r^+$ | revertant of FC202 | . |
| (FC202+FC230) | $r^+$ | revertant of FC202 | . |
| (FC202+P61) | $r^+$ | revertant of P61 | . |
| (FC202+FC221) | $r^+$ | revertant of FC202 | . |
| (FC202+FC20) | $r^+$ | FC202 *v.* FC20 | . |
| (FC202+FC229) | $r^+$ | revertant of FC202 | . |
| (FC202+FC224) | $r^+$ | revertant of FC202 | . |
| (FC202+FC222) | $r^+$ | revertant of FC202 | . |
| (FC237+FC222) | $r^+$ | revertant of FC222 | . |
| (FC238+FC125) | $r^+$ | FC238 *v.* FC125 | . |
| (FC238+FC222) | $r^+$ | revertant of FC222 | . |
| (FC236+FC222) | $r^+$ | revertant of FC222 | . |


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE A 4(*d*). DOUBLES (− +) → MINUTE

| double | method of isolation | double | method of isolation |
|---|---|---|---|
| (FC6+FC34) | revertant of FC6 | (FC1+FC222) | FC1 *v.* FC222 |
| (FC86+FC58) | FC86 *v.* FC58 | (FC82+FC58) | FC82 *v.* FC58 |
| (FC1+370) | FC1 *v.* 370 | (FC9+FC58) | revertant of FC9 |
| (FC1+FC58) | FC1 *v.* FC58 | (X833+FC58) | X833 *v.* FC58 |
| (FC1+P61) | FC1 *v.* P61 | (X707+FC58) | X707 *v.* FC58 |
| (FC1+FC217) | FC1 *v.* FC217 | (FC21+FC58) | FC21 *v.* FC58 |
| (FC1+FC211) | FC1 *v.* FC211 | (FC88+FC58) | FC88 *v.* FC58 |
| (FC1+FC125) | FC1 *v.* FC125 | (FC88+FC125) | FC88 *v.* FC125 |
| (FC1+FC20) | revertant of FC1 | (176+FC215) | 176 *v.* FC215 |
| (FC1+FC213) | FC1 *v.* FC213 | (X146+FC34) | X146 *v.* FC34 |
| (FC1+FC119) | FC1 *v.* FC119 | (X225+FC34) | X225 *v.* FC34 |

TABLE A 4(*e*). DOUBLES (− +) → *r*

| double | method of isolation |
|---|---|
| (FC1+FC215) | FC1 *v.* FC215† |
| (FC1+FC123) | FC1 *v.* FC123 |
| (FC88+FC215) | FC88 *v.* FC215 |

† This cross gave no minute plaques on K so the double was presumed *r* but not isolated.

TABLE A 4(*f*). DOUBLES (+ +) *r*

| double | method(s) of isolation | double | method(s) of isolation |
|---|---|---|---|
| (FC41+FC0) | FC41 *v.* FC0 | (FC91+FC47) | not isolated |
| (FC41+FC28) | not isolated† | (FC28+FC31) | not isolated |
| (FC41+FC40) | not isolated | (FC28+FC47) | not isolated |
| (FC41+FC32) | not isolated | (FC35+FC47) | not isolated |
| (FC41+FC55) | not isolated | (FC64+FC47) | not isolated |
| (FC41+FC31) | not isolated | (FC40+FC92) | not isolated |
| (FC41+FC47) | not isolated | (FC40+FC33) | not isolated |
| (244+FC0) | not isolated | (FC40+FC55) | (FC0+FC40+FC55) *v.* wild |
| (244+FC28) | not isolated | (FC40+FC36) | (FC0+FC40+FC36) *v.* wild |
| (244+FC40) | not isolated | (FC40+FC57) | (FC0+FC40+FC57) *v.* wild |
| (244+FC32) | not isolated | (FC40+FC54) | (FC0+FC40+FC54) *v.* wild |
| (FC106+FC0) | (FC106+$a_2$+FC9) *v.* (FC1+FC0) | (FC40+FC31) | (FC0+FC40+FC31) *v.* wild |
| (FC73+FC28) | not isolated | (FC40+FC90) | (FC0+FC40+FC90) *v.* wild |
| (FC73+FC40) | not isolated | (FC40+FC38) | (FC0+FC40+FC38) *v.* wild |
| (FC73+FC32) | not isolated | (FC40+FC47) | (FC0+FC40+FC47) *v.* wild |
| (FC73+FC47) | not isolated | (FC40+FC58) | (FC0+FC40+FC58) *v.* wild |
| (FC0+FC91) | (FC0+FC91+FC40) *v.* wild | (FC67+FC47) | not isolated |
| (FC0+FC40) | FC0 *v.* FC40 | (FC32+FC31) | not isolated |
| (FC0+FC32) | not isolated | (FC32+FC47) | not isolated |
| (FC0+FC33) | not isolated | (FC51+FC47) | not isolated |
| (FC0+FC55) | (FC0+FC40+FC55) *v.* wild | (FC104+FC55) | (FC104+FC55+FC54) *v.* wild |
| (FC0+FC36) | not isolated | (FC104+FC47) | not isolated |
| (FC0+$a_4$+FC36) | not isolated | (FC42+FC55) | (FC42+FC55+FC54) *v.* wild |
| (FC0+FC57) | (FC0+FC40+FC57) *v.* wild | (FC42+FC54) | not isolated |
| (FC0+FC54) | (FC0+FC40+FC54) *v.* wild | (FC42+FC31) | not isolated |
| (FC0+FC31) | (FC0+FC40+FC31) *v.* wild | (FC42+FC47) | not isolated |
| (FC0+FC38) | FC0 *v.* FC38 | (FC92+FC47) | not isolated |
| (FC0+FC47) | (FC0+FC40+FC47) *v.* wild | (FC96+FC55) | (FC96+FC55+FC54) *v.* wild |
| (FC0+FC58) | (FC0+FC40+FC58) *v.* wild | (FC96+FC54) | not isolated |
| (FC48+FC47) | not isolated | (FC96+FC31) | not isolated |
| (FC72+FC47) | not isolated | (FC33+FC31) | not isolated |
| (FC98+FC47) | not isolated | (FC33+FC47) | not isolated |

† *r* Mutant parents were crossed and failed to produce minute plaques on K, so the double was presumed *r* but not isolated.




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TABLE A 4(*f*) (*cont.*)

| double | method(s) of isolation |
|---|---|
| (FC45+FC47) | not isolated |
| (FC55+FC36) | (FC55+FC36+FC31) *v.* wild |
| (FC55+FC57) | FC55 *v.* FC57 |
| (FC55+FC54) | (FC42+FC55+FC54) *v.* wild |
| (FC55+$a_5$+FC54) | (FC55+$a_5$+FC54+FC31) *v.* wild |
| (FC55+FC31) | (FC55+FC36+FC31) *v.* wild<br>(FC55+FC57+FC31) *v.* wild |
| (FC55+$a_5$+FC31) | (FC55+$a_5$+FC54+FC31) *v* wild |
| (FC55+FC90) | (FC55+FC57+FC90) *v.* wild |
| (FC55+$a_5$+FC90) | not isolated |
| (FC55+$a_5$+514) | not isolated |
| (FC55+FC47) | (FC55+FC57+FC47) *v.* wild |
| (FC55+$a_5$+FC47) | not isolated |
| (FC56+FC47) | not isolated |
| (FC115+FC47) | not isolated |
| (FC36+FC54) | (FC55+FC36+FC54) *v.* wild |
| (FC36+FC31) | FC36 *v.* FC31<br>(FC36+FC31+$a_6$+FC47) *v.* wild |
| (FC36+FC223) | not isolated |
| (FC36+P61) | FC36 *v.* P61 |
| (FC36+FC125) | FC36 *v.* FC125 |
| (FC36+FC213) | not isolated |
| (FC36+FC222) | not isolated |
| (FC57+FC54) | (FC55+FC57+FC54) *v.* wild |
| (FC57+FC31) | FC57 *v.* FC31<br>(FC57+FC31+$a_6$+FC47) *v.* wild |
| (FC57+$a_6$+FC47) | not isolated |
| (FC44+FC47) | not isolated |
| (FC69+FC47) | not isolated |
| (FC29+FC58) | not isolated |
| (FC29+FC125) | not isolated |
| (FC54+FC31) | (FC57+FC54+FC31) *v.* wild<br>(FC54+FC31+$a_6$+FC47)† |
| (FC54+FC90) | (FC57+FC54+FC90) *v.* wild |
| (FC54+514) | (FC57+FC54+514) *v.* wild |
| (FC54+FC47) | (FC57+FC54+FC47) *v.* wild<br>(FC54+FC31+$a_6$+FC47)† |
| (FC54*+FC31) | not isolated |
| (FC102+FC90) | not isolated |
| (FC102+FC47) | not isolated |
| (FC31+$a_6$+FC47) | FC31 $a_6$ *v.* FC47 |
| (FC31+370) | not isolated |
| (FC31+FC223) | not isolated |
| (FC31+P61) | FC31 *v.* P61 |
| (FC31+FC217) | not isolated |
| (FC31+FC20) | not isolated |
| (FC31+FC213) | not isolated |
| (FC31+FC222) | not isolated |
| (FC90+514) | not isolated |
| (FC90+FC47) | FC90 *v.* FC47 |
| (514+FC47) | not isolated |
| (514+370) | not isolated |
| (FC58+FC125) | not isolated |
| (370+FC223) | not isolated |
| (370+P61) | not isolated |
| (370+FC217) | not isolated |
| (370+FC211) | not isolated |
| (370+FC125) | not isolated |
| (370+FC119) | not isolated |
| (370+FC222) | not isolated |
| (P61+FC125) | not isolated |

† These triples were not isolated. A mixture was made of ten wild-type progeny, half of which were expected to be the triple. In an orgy cross of these the *r* double was isolated (see § 2(*d*) (iv)).



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TABLE A 4(*g*). DOUBLES (++) MINUTE

| double | method of isolation | double | method of isolation |
|---|---|---|---|
| (FC91+FC92) | FC91 *v.* FC92 | (FC54*+514) | (FC54+514) *v.* FC54* |
| (FC91+FC33) | FC91 *v.* FC33 | (FC54*+FC47) | (FC54+FC47) *v.* FC54* |
| (FC91+FC55) | FC91 *v.* FC55 | (FC31+FC90) | FC31 *v.* FC90 |
| (FC36+FC90) | not isolated† | (FC31+514) | FC31 *v.* 514 |
| (FC36+514) | not isolated | (FC31+FC38) | FC31 *v.* FC38 |
| (FC36+FC30) | not isolated | (FC31+FC47) | FC31 *v.* FC47 |
| (FC36+FC38) | not isolated | (FC31+196a) | not isolated |
| (FC36+FC39) | not isolated | (FC31+FC58) | FC31 *v.* FC58 |
| (FC36+FC47) | FC36 *v.* FC47 | (FC31+FC125) | FC31 *v.* FC125 |
| (FC36+FC63) | not isolated | (FC90+FC58) | FC90 *v.* FC58 |
| (FC36+FC89) | not isolated | (FC90+370) | FC90 *v* 370 |
| (FC36+FC108) | not isolated | (FC90+FC223) | FC90 *v.* FC223 |
| (FC36+FC109) | not isolated | (FC90+P61) | FC90 *v.* P61 |
| (FC36+FC110) | not isolated | (FC90+FC217) | FC90 *v.* FC217 |
| (FC36+FC111) | not isolated | (FC90+FC125) | FC90 *v.* FC125 |
| (FC36+196a) | not isolated | (FC90+FC20) | FC90 *v.* FC20 |
| (FC36+FC58) | FC36 *v.* FC58 | (FC90+FC213) | FC90 *v.* FC213 |
| (FC43+FC47) | not isolated | (FC90+FC215) | FC90 *v.* FC215 |
| (FC52+FC47) | not isolated | (FC90+FC222) | FC90 *v.* FC222 |
| (FC53+FC47) | not isolated | (FC38+FC58) | FC38 *v.* FC58 |
| (FC62+FC47) | not isolated | (FC47+FC58) | FC47 *v.* FC58 |
| (FC66+FC47) | not isolated | (FC47+370) | FC47 *v.* 370 |
| (FC71+FC47) | not isolated | (FC47+FC223) | FC47 *v.* FC223 |
| (FC112+FC47) | not isolated | (FC47+P61) | FC47 *v.* P61 |
| (FC113+FC47) | not isolated | (FC47+FC217) | FC47 *v.* FC217 |
| (FC114+FC47) | not isolated | (FC47+FC125) | FC47 *v.* FC125 |
| (FC116+FC47) | not isolated | (FC47+FC20) | FC47 *v.* FC20 |
| (FC57+FC90) | (FC55+FC57+FC90) *v.* wild | (FC47+FC215) | FC47 *v.* FC215 |
| (FC57+FC47) | FC57 *v.* FC47 | (FC47+FC213) | FC47 *v.* FC213 |
| (FC29+FC90) | FC29 *v.* FC90 | (FC47+FC222) | FC47 *v.* FC222 |
| (FC29+FC47) | FC29 *v.* FC47 | (196a+FC20) | *r*196 *v.* FC20¶ |
| (FC54*+FC90) | revertant of (FC54+FC90)‡ | | |

† *r* Mutant parents were crossed and produced almost 50% minute plaques on K but the indicated double was not isolated.

‡ See § 9(*d*).

¶ *r*196 is original deletion (see § 11(*a*)). The wild-type double mutant was checked by backcrossing.

TABLE A 4(*h*). DOUBLES (−−) *r*

| double | method(s) of isolation |
|---|---|
| (FC1+FC9) | FC1 *v.* FC9 |
| (FC1+FC21) | FC1 *v.* FC21 |
| (FC1+FC23) | FC1 *v.* FC23 |
| | (FC1+FC23+$a_5$+176) *v.* wild |
| (FC9+FC21) | FC9 *v.* FC21 |
| (FC9+FC23) | FC9 *v.* FC23 |
| | (FC9+FC23+FC88) *v.* wild |
| (FC21+FC23) | (FC1+FC21+FC23) *v.* wild |
| (FC23+FC88) | (FC9+FC23+FC88) *v.* wild |
| (FC23+$a_5$+176) | (FC1+FC23+$a_5$+176) *v.* wild |
| (FC88+$a_5$+176) | (FC23+FC88+$a_5$+176) *v.* wild |
| (176+$a_6$+FC87) | (FC23+$a_5$+176+$a_6$+FC87) *v.* wild |



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TABLE A 5(*a*). TRIPLES (– – –)

| triple | type | phenotype on B | phenotype on K | method(s) of isolation | remarks |
|---|---|---|---|---|---|
| (FC10+FC21+FC23) | $r^+$ | not kept | | (FC21+FC23) *v.* FC10 | . |
| (FC6+FC21+FC23) | $r^+$ | not kept | | (FC21+FC23) *v.* FC6 | . |
| (FC86+FC21+FC23) | $r^+$ | not kept | | (FC21+FC23) *v.* FC86 | . |
| (FC11+FC21+FC23) | $r^+$ | not kept | | (FC21+FC23) *v.* FC11 | . |
| (FC100+FC21+FC23) | $r^+$ | not kept | | (FC21+FC23) *v.* FC100 | . |
| (FC1+FC9+FC21) | $r$ | not isolated | | (FC1+FC9) *v.* (FC1+FC21) | no $r^+$ |
| (FC1+FC9+FC23) | $r$ | not isolated | | (FC1+FC9) *v.* (FC1+FC23) | no $r^+$ |
| | | | | (FC1+FC9) *v.* (FC9+FC23) | no $r^+$ |
| (FC1+FC21+FC23) | $r^+$ | '$r^+$' | $r^+$ | (FC1+FC21) *v.* (FC1+FC23) | . |
| (FC1+FC21+176) | $r$ | not isolated | | (FC1+FC21) *v.* 176 | $r^+$ backcrossed gave no $r$ |
| (FC1+FC23+$a_5$+176) | $r^+$ | '$r^+$' | $r^+$ | (FC1+FC23) *v.* $a_5$176 | . |
| (FC9+FC21+FC23) | $r^+$ | not kept | | (FC21+FC23) *v.* FC9 | . |
| (FC9+FC23+FC88) | $r^+$ | '$r^+$' | $r^+$ | (FC9+FC23) *v.* FC88 | . |
| (FC23+FC88+$a_5$+176) | $r^+$ | '$r^+$' | $r^+$ | (FC23+FC88) *v.* $a_5$176 | . |
| (FC23+$a_5$+176+$a_6$+FC87) | $r^+$ | '$r^+$' | $r^+$ | (FC23+$a_5$+176) *v.* $a_6$FC87 | . |
| (FC88+$a_5$+176+$a_6$+FC87) | $r^+$ | '$r$' | $r^+$ | (FC88+$a_5$+176) *v.* FC87 | . |

TABLE A 5(*b*). TRIPLES (+ + +)

| triple | type | phenotype on B | phenotype on K | method of isolation | remarks |
|---|---|---|---|---|---|
| (FC106+FC0+FC40) | $r$ | not isolated | | (FC106+FC0) *v.* (FC0+FC40) | no $r^+$ |
| (FC106+FC0+FC55) | $r$ | not isolated | | (FC106+FC0) *v.* (FC0+FC55) | no $r^+$ |
| (FC106+FC0+FC57) | $r$ | not isolated | | (FC106+FC0) *v.* (FC0+FC57) | no $r^+$ |
| (FC106+FC0+FC54) | $r$ | not isolated | | (FC106+FC0) *v.* (FC0+FC54) | no $r^+$ |
| (FC106+FC0+FC38) | $r$ | not isolated | | (FC106+FC0) *v.* (FC0+FC38) | no $r^+$ |
| (FC0+FC91+FC40) | $r^+$ | '$r$' | $r^+$ | (FC0+FC40) *v.* FC91 | . |
| (FC0+FC40+FC55) | $r^+$ | '$r^+$' | $r^+$ | (FC0+FC40) *v.* FC55 | . |
| (FC0+FC40+FC36) | $r^+$ | '$r^+$' | $r^+$ | (FC0+FC40) *v.* FC36 | . |
| (FC0+FC40+FC57) | $r^+$ | $r^+$ | $r^+$ | (FC0+FC40) *v.* FC57 | . |
| (FC0+FC40+FC54) | $r^+$ | $r^+$ | $r^+$ | (FC0+FC40) *v.* FC54 | . |
| (FC0+FC40+FC31) | $r^+$ | $r^+$ | $r^+$ | (FC0+FC40) *v.* FC31 | . |
| (FC0+FC40+FC90) | $r^+$ | $r^+$ | $r^+$ | (FC0+FC40) *v.* FC90 | . |
| (FC0+FC40+FC38) | $r^+$ | $r^+$ | $r^+$ | (FC0+FC38) *v.* (FC0+FC40) | . |
| (FC0+FC40+FC47) | $r^+$ | $r^+$ | $r^+$ | FC(0+36+31+47) *v.* (FC0+FC40)† | . |
| (FC0+FC40+FC58) | m | '$r$' | m | (FC0+FC40) *v.* FC58 | backcross screened on (K+B) |
| (FC0+$a_4$+FC57+FC31) | $r^+$ | $r^+$ | $r^+$ | FC0$a_4$ *v.* (FC57+FC31) | . |
| (FC0+$a_4$+FC57+FC47) | $r^+$ | $r^+$ | $r^+$ | FC0$a_4$ *v.* (FC57+FC47) | . |
| (FC103+FC55+FC54) | $r$ | not isolated | | (FC40+FC55+FC54) *v.* FC103‡ | $r^+$ backcrossed gave no $r$ |
| (FC40+FC55+FC57) | $r$ | not isolated | | (FC40+FC55) *v.* (FC40+FC57) | no $r^+$ |
| (FC40+FC55+FC54)¶ | $r$ | $r$ | 0 | FC(0+40+55+54) *v.* wild | . |
| (FC40+FC55+FC38) | $r$ | not isolated | | (FC40+FC38) *v.* (FC40+FC55) | no $r^+$ |
| (FC40+FC57+FC54) | $r$ | not isolated | | (FC40+FC54) *v.* (FC40+FC57) | no $r^+$ |
| (FC40+FC57+FC38) | $r$ | not isolated | | (FC40+FC38) *v.* (FC40+FC57) | no $r^+$ |
| (FC40+FC54+FC38) | $r$ | not isolated | | (FC40+FC38) *v.* (FC40+FC54) | no $r^+$ |
| (FC104+FC55+FC54) | $r^+$ | $r^+$ | $r^+$ | (FC40+FC55+FC54) *v.* FC104‡ | . |
| (FC42+FC55+FC54) | $r^+$ | $r^+$ | $r^+$ | (FC40+FC55+FC54) *v.* FC42‡ | . |
| (FC96+FC55+FC54) | $r^+$ | '$r^+$' | $r^+$ | (FC40+FC55+FC54) *v.* FC96‡ | . |

† In this cross, barriers prevent the recovery of other triples. The structure of (FC0+FC40+FC47) was checked by backcrossing.

‡ (FC40+FC55+FC54) is an *r* triple because there is a barrier ($b_4$) between FC40 and FC55. FC103 is close to FC40 and failed to form a wild-type triple for the same reason. However, FC104, FC42, and FC96, also close to FC40, map to the right of this barrier and can make wild-type triples.

¶ Isolated as an *r* from the backcross of a (+ + + +) quadruple.


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TABLE A 5 (*b*) (*cont.*)

| | | | | | |
|---|---|---|---|---|---|
| (FC55+FC36+FC54) | $r^+$ | '$r^+$' | $r^+$ | (FC55+FC36) *v.* (FC55+FC54) | . |
| (FC55+FC36+FC31) | $r^+$ | '$r^+$' | $r^+$ | (FC55+FC54) *v.* (FC36+FC31) | . |
| (FC55+FC57+FC54) | $r^+$ | '$r^+$' | $r^+$ | (FC55+FC54) *v.* (FC55+FC57) | . |
| (FC55+FC57+FC31) | $r^+$ | '$r^+$' | $r^+$ | (FC55+FC57) *v.* (FC57+FC31) | . |
| (FC55+FC57+FC90) | $r^+$ | $r^+$ | $r^+$ | (FC55+FC57) *v.* FC90 | . |
| (FC55+FC57+FC47) | $r^+$ | $r^+$ | $r^+$ | (FC55+FC57) *v.* (FC57+FC47) | . |
| (FC55+FC54+FC31) | $r$ | not isolated | | (FC55+FC31) *v.* (FC55+FC54) | no $r^+$ |
| (FC55+$a_5$+FC54+FC31) | $r^+$ | $r^+$ | $r^+$ | FC55$a_5$ *v.* (FC54+FC31) | . |
| (FC36+FC31+FC47) | $r^+$ | $r^+$ | $r^+$ | (FC36+FC31) *v.* (FC90+FC47) | . |
| | | | | FC(0+36+31+47) *v.* FC47† | . |
| (FC36+FC31+$a_6$+FC47) | $r^+$ | '$r^+$' | $r^+$ | (FC31+$a_6$+FC47) *v.* FC36$a_5$ | . |
| (FC57+FC54+FC31) | $r^+$ | '$r^+$' | $r^+$ | (FC57+FC54) *v.* (FC57+FC31) | . |
| (FC57+FC54+FC90) | $r^+$ | '$r^+$' | $r^+$ | (FC57+FC54) *v.* FC90 | . |
| (FC57+FC54+514) | $r^+$ | '$r^+$' | $r^+$ | (FC57+FC54) *v.* 514 | . |
| (FC57+FC54+FC47) | $r^+$ | $r^+$ | $r^+$ | (FC57+FC54) *v.* (FC57+FC47) | . |
| (FC57+FC31+$a_6$+FC47) | $r^+$ | $r^+$ | $r^+$ | (FC57+FC31) *v.* (FC31+$a_6$+FC47) | . |
| (FC54+FC31+FC47) | $r^+$ | '$r^+$' | $r^+$ | (FC54+FC31) *v.* (FC31+FC47) | minutes screened |
| (FC54+FC31+$a_6$+FC47) | $r^+$ | not isolated | | (FC31+$a_6$+FC47) *v.* FC54 | orgy-crossed (see § 2 (*d*) (iv)) |
| (FC47+FC58+FC125) | $r$ | $r$ | 0 | (FC47+FC125) *v.* (FC38+FC58) | . |
| (FC47+FC58+FC222) | $r$ | $r$ | 0 | (FC47+FC222) *v.* (FC38+FC58) | . |
| (FC47+FC125+FC222) | $r^+$ | '$r$' | $r^+$ | (FC47+FC125) *v.* (FC47+FC222) | . |

† See § 10 (*c*) (ii) and table 18 (*a*).

TABLE A 5 (*c*). MULTIPLE MUTANTS

| quadruples (++++) | type | method(s) of isolation |
|---|---|---|
| (FC0+FC40+FC55+FC57) | *r* | (FC0+FC40+FC57) *v.* (FC0+FC40+FC55) |
| (FC0+FC40+FC55+FC54) | *r* | (FC0+FC40+FC55) *v.* (FC0+FC40+FC54) |
| (FC0+FC40+FC55+FC47) | *r* | (FC0+FC40+FC55) *v.* (FC36+FC31+FC47) |
| (FC0+FC40+FC36+FC31) | *r* | (FC0+FC40+FC36) *v.* (FC0+FC40+FC31) |
| (FC0+FC40+FC57+FC54) | m | (FC0+FC40+FC57) *v.* (FC0+FC40+FC54) |
| (FC0+FC40+FC57+FC31) | m | (FC0+FC40+FC57) *v.* (FC0+FC40+FC31) |
| | | (FC0+$a_4$+FC57+FC31) *v.* (FC0+FC40+FC57) |
| (FC0+FC40+FC57+FC90) | m | (FC0+FC40+FC57) *v.* (FC0+FC40+FC90) |
| (FC0+FC40+FC57+FC47) | m | (FC0+FC40+FC57) *v.* (FC0+FC40+FC47) |
| | | (FC0+$a_4$+FC57+FC47) *v.* (FC0+FC40+FC57) |
| (FC0+FC40+FC54+FC31) | *r* | (FC0+FC40+FC54) *v.* (FC0+FC40+FC31) |
| (FC0+FC40+FC54+FC47) | *r* | (FC0+FC40+FC54) *v.* (FC0+FC40+FC47) |
| (FC0+FC36+FC31+FC47) | *r* | (FC0+FC40+FC55) *v.* (FC36+FC31+FC47) |
| quintuple (+++++) | | |
| (FC40+FC55+FC36+FC31+FC47) | *r* | (FC0+FC40+FC55+FC36+FC31+FC47) *v.* wild |
| sextuple (++++++) | | |
| (FC0+FC40+FC55 +FC36+FC31+FC47) | $r^+$ | (FC0+FC40+FC55+FC47) *v.* (FC0+FC36+FC31+FC47) |



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TABLE A 5(*d*). MISCELLANEOUS

| mutant | type | method of isolation |
|---|---|---|
| (FC0+FC40+176) | *r* | (FC0+FC40+176+$a_6$+FC87) *v.* wild |
| (FC40+176+$a_6$+FC87) | *r* | (FC0+FC40+176+$a_6$+FC87) *v.* wild |
| (FC88+176+FC47) | *r* | (176+FC47) *v.* (FC88+FC47) |
| (FC88+FC115+FC47) | *r* | (FC88+FC115) *v.* (FC88+FC47) |
| (FC88+FC57+FC47) | m | (FC88+FC57) *v.* (FC88+FC47) |
| (FC88+FC44+FC47) | *r* | (FC88+FC44) *v.* (FC88+FC47) |
| (FC88+FC36+FC47) | *r* | (FC88+FC36) *v.* (FC88+FC47) |
| (FC88+FC29+FC47) | *r* | (FC88+FC29) *v.* (FC88+FC47) |
| (FC0+FC40+176 +$a_6$+FC87) | $r^+$ | (FC0+FC40) *v.* (176+$a_6$+FC87) |

TABLE A 6. PROOF OF BASE-SUBSTITUTION SIGN-ZERO MUTANTS

I. Ambers

| | double (*r*) | double (*r*) | triple ($r^+$) |
|---|---|---|---|
| | | | frameshift → |
| sign ... | (− 0) | (0 +) | (− 0 +) |
| | (FC6+2074)† | (2074+FC38) | (FC6+2074+FC38) |
| | (FC9+2074) | (2074+FC104) | (FC9+2074+FC104) |
| | (FC6+EM84) | (EM84+FC38) | (FC6+EM84+FC38) |
| | (FC6+HB74) | (HB74+FC38) | (FC6+HB74+FC38) |
| | (FC6+NT332) | (NT332+FC38) | (FC6+NT332+FC38) |
| | | | frameshift ← |
| sign ... | (+ 0) | (0 −) | (+ 0 −) |
| | (FC0+2074) | (2074+FC23) | (FC0+2074+FC23) |

II. Ochres

| | | | |
|---|---|---|---|
| | | | frameshift → |
| sign ... | (− 0) | (0 +) | (− 0 +) |
| | (FC10+360) | (360+FC0) | (FC10+360+FC0) |
| | (FC10+739) | (739+FC0) | (FC10+739+FC0) |
| | (FC10+UV375) | (UV375+FC0) | (FC10+UV375+FC0) |
| | (FC10+X511) | (X511+FC47) | (FC10+X511+FC47) |
| | (FC1+UV357) | (UV357+FC47) | (FC1+UV357+FC47) |
| | (FC6+X27) | (X27+FC38) | (FC6+X27+FC38) |
| | (FC6+375) | (375+FC38) | (FC6+375+FC38) |
| | (FC1+N24) | (N24+FC38) | (FC1+N24+FC38) |
| | | | frameshift ← |
| sign ... | (+ 0) | (0 −) | (+ 0 −) |
| | (FC41+360) | (360+$a_2$+FC9) | (FC41+360+$a_2$+FC9) |

III. No extragenic suppressor

| | | | |
|---|---|---|---|
| | | | frameshift → |
| sign ... | (− 0) | (0 +) | (− 0 +) |
| | (FC1+X655) | (X655+FC38) | (FC1+X655+FC38) |

† The sign zero mutants are listed in map order within the amber and ochre groups.



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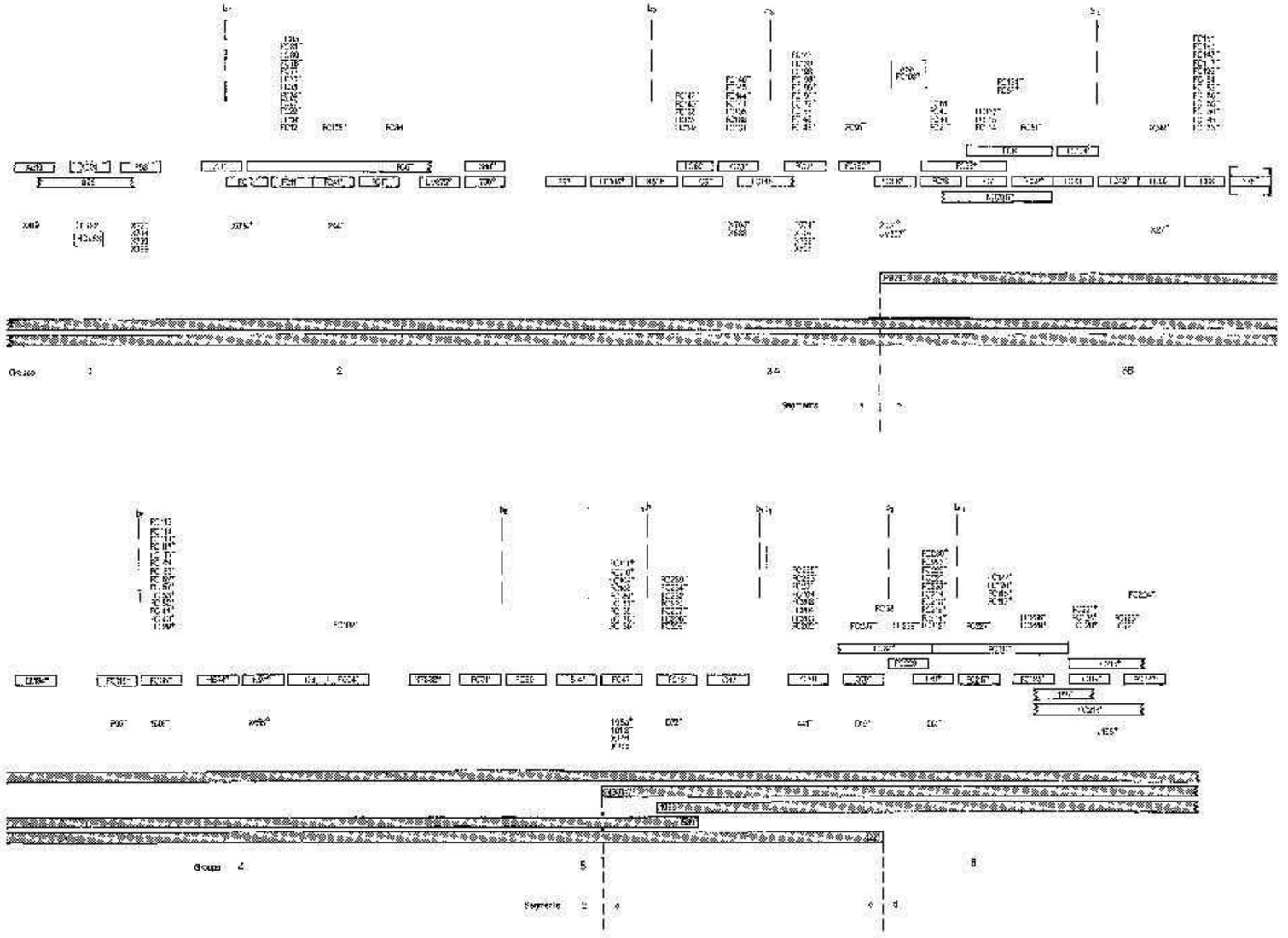

IGURE 2. The genetic map of part of the B cistron of the rII locus in phage T4 Benzer. The canonical set (see § 4 (*a*)) are shown boxed and are given the same extent unless known to cover multiple sites. As far as possible distances between mutants are taken from the result of u.v. crosses. With unreliably small distances (recombination $\sim 1 \times 10^{-6}$) the two mutants are butted up against one another. With unreliably large distances (recombination $> 60 \times 10^{-6}$), or a spot-cross plus result, the gap shown is of arbitrary length. All the other mutants used in this study are listed at the site of a canonical mutant with which they show recombination of $< 10^{-6}$ in a u.v. cross; FC mutants above the line, others below. The group numbers 1 to 8, the regions a, b, c and d (see § 4 (*b*)) and some of the mapping deletions are indicated.