Plasmid spread and chromosome mobilisation in *Escherichia coli* K12 populations

by

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ABSTRACT

The phenomenon of "epidemic spread" of a transmissible plasmid through a recipient population was re-examined. The phenomenon was studied quantitatively in the model system of F'lac in Escherichia coli K12. The rate of plasmid spread by re-transfer was rather low because of the low frequency of continuing transfer by recipients that had received F'lac.

The F'lac mating system was studied to answer the following questions:

(i) What is the effect of parental concentration on mating efficiency?
(ii) What proportion of parents are competent to mate?
(iii) How many cells can competent parents mate with?
(iv) How efficient is plasmid establishment in recipient cells after transfer?
(v) How long are the lags between rounds of donor transfer and between a recipient receiving F'lac and becoming a competent donor?

The variation in mating efficiency with parental concentration was incompatible with mating following bimolecular reaction kinetics and collision rate was not a limiting factor at concentrations above $5 \times 10^7$ cells/ml. The majority of both donors and recipients were competent to mate in 30', but whilst donors were fairly monogamous, recipients could mate with an average of 2-3 donors. Plasmid establishment after transfer was efficient and segregation had little effect on progeny growth rate. There was a lag of 30'-40' between rounds of donor competence and a lag of 90' before new progeny became competent donors.
The rates of segregation due to plasmid incompatibility were calculated under several simple models. Experiments with two colEl derivatives were in reasonable agreement with the predictions of a model based on random pool replication.

We studied the dependence on the recA function of the recombination needed for Hfr formation. We obtained Hfr clones from a recA F⁺ strain, but the rate of Hfr formation was only about 1% that in an isogenic rec⁺ strain, so Hfr formation in rec⁺ strains is probably mostly mediated by the host's normal recombination system. Our experiments suggested that "Type II" F⁺ strains were not defective in Hfr formation as had been previously reported, but had some secondary defect. The recA mutation reduced chromosome transfer in the same proportion as Hfr formation so the relationship between the two processes remains unclear.
I declare that I composed this thesis myself and that the work in this thesis is my own.

J.A. Cullum 9th August, 1978

Publication

Some work, which is not described in this thesis, was also done in collaboration with Dr. M. Vicente. This work was published in *Journal of Bacteriology* and the paper is bound into the back of the thesis.
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CHAPTER 1

INTRODUCTION

The development of simple techniques for detecting covalently closed circular DNA has revealed that plasmids are very common in most species of bacteria and are responsible for a wide range of functions. In addition all plasmids are probably capable of being transferred to other bacterial strains by one or more of the following processes (see Reanney, 1976):

(i) Conjugational transfer by own mating system.
(ii) Mobilisation by another conjugation system.
(iii) Transduction.
(iv) Transformation.

The most studied examples of plasmid spread in nature have been cases of spread of drug resistance factors after the use of antibiotics (see Falkow, 1975). In this sort of situation a bacterial population that was formerly drug-sensitive becomes infected with drug resistance factors. This process could be due to two rather different mechanisms:

(i) A large amount of plasmid transfer results in the spread of the plasmid through the population by the infection of plasmidless cells.
(ii) The plasmid confers a selective advantage on the new host so that after some initial transfer to introduce the plasmid into the new host strain selection results in cells carrying the plasmid becoming the predominant component of the population.

These two mechanisms are not mutually exclusive, but it is useful when considering the role of transfer systems to distinguish these
cases of "transfer-driven" and "selection-driven" plasmid spread. In the cases of transductional and transformational transfer the first mechanism is probably unimportant because these processes occur at a low frequency and transfer results in the destruction of the donor cells. However, the first mechanism could, in principle, be important in the case of conjugational transfer. Such an example seems to be furnished by the "epidemic spread" of ColI in _Salmonella typhimurium_ that can occur in the laboratory under certain conditions (Ozeki et al., 1962).

We studied "epidemic spread" quantitatively using F'lac in _E. coli_ K12 as a model system. We supported this study with investigations into the behaviour of each parent in F'lac matings. We measured the proportion of cells of each parent competent to mate and the number of cells with which each competent parent could mate. The influence of parental concentration on mating and the gaps between periods of competence were also studied.

Incompatibility may form a barrier to the establishment of a plasmid in a cell carrying a related plasmid. We calculated the rates of plasmid segregation due to incompatibility under several simple models and compared them with experimental data we obtained with two colEl derivatives.

We also studied another aspect of plasmid behaviour in populations: the interactions of F with the chromosome to form Hfr cells and to transfer chromosomal genes. We studied the effect of the recA mutation on these interactions.

**Epidemic spread**

Most plasmids isolated from nature transfer at a very low frequency. Sometimes this is due to a repression system and
derepressed mutants can be isolated that transfer efficiently (Meynell et al., 1968). When a repressed plasmid enters a new host cell there may be several generations during which transfer can occur at a high frequency before fertility drops to the normal low repressed level (Stocker et al., 1963; Ozeki, 1965). The repression system of the R-factor R100 has been studied in detail (Finnegan and Willetts, 1971, 1973). This study took advantage of the fact that the R100 repression system will also repress the closely related transfer system of F (a property called "fertility inhibition"). This revealed that the repression system acted indirectly by switching off expression of the \textit{traJ} gene which is a positive control gene needed for expression of the other transfer genes, which are in a large operon (Helmuth and Achtman, 1975). Willetts (1974) suggested that when R100 enters a new host cell there is sufficient initial synthesis of the \textit{traJ} gene product to allow transfer for a considerable time.

The slowness of the onset of the fertility repression after transfer to a new host cell suggested a mechanism for transfer-driven plasmid spread by plasmids that normally have a low fertility. After transfer to a new host cell the plasmid might be re-transferred to several recipients during the period of fertility. Such re-transfer from recipients would lead to a sort of "chain reaction" and the proportion of recipient cells containing the plasmid would increase approximately exponentially until the proportion becomes high. Ozeki et al. (1962) explained the "epidemic spread" of ColI to a large proportion of the recipient population when colicinogenic and non-colicinogenic cells were incubated together for 20 hours by such a mechanism. Helmuth and Achtman (1975) pointed out that the control
of repression in F-like R-factors should allow such a process to operate.

Stocker et al. (1963) found that if they incubated a 1:20 mixture of colicinogenic and non-colicinogenic strains together for 18 hours 30-70% of the mixture became colicinogenic. If only one colicinogenic cell in five thousand was a competent donor (Ozeki et al., 1962) then each initial competent donor ultimately yields about $10^5$ progeny, which, perhaps, justifies the description "epidemic spread". However, the proportion of colicinogenic bacteria in the mixture only increased from 5% to 30-70% i.e. about 10 times. In the case of the R-factor 222 (also known as R100) in _Escherichia coli_ (Watanabe, 1963) the spread was even less.

We decided to re-investigate "epidemic spread" by studying plasmid spread by re-transfer in a quantitative manner. This subject is important not only for consideration of plasmid spread but also for the construction of high frequency transfer systems for repressed plasmids. The fact that repression is slow-acting suggested that there should be little difference in re-transfer behaviour between a repressed plasmid and an equivalent non-repressed plasmid; this was confirmed by experiments of Broda in which he compared re-transfer by a repressed plasmid and their derepressed mutants for the F-like R-factor R100 (Cullum et al., 1978a) and the I-like R-factor R64 (Broda, unpublished observations). We were therefore able to use the non-repressed plasmid F'lac as a model system; this plasmid was especially convenient as its transfer system has been intensively studied and many transfer-deficient mutants exist (Achtman et al., 1971). The matings to study re-transfer behaviour were for periods of about 20 hours. We also had to investigate some quantitative aspects of the F'lac mating system in short matings where re-transfer was
insignificant; the information from these studies was needed to analyse the re-transfer experiments quantitatively.

**Short matings**

There were five aspects of mating that we investigated:

(i) The effect of parental concentration on mating.
(ii) The proportion of parents competent to mate.
(iii) The number of partners with which a cell can mate within a short time.
(iv) The time between rounds of mating by a cell.
(v) The efficiency of plasmid establishment in recipients after transfer.

The simplest model for the effect of parental concentration on mating is to assume that mating follows the kinetics of a bimolecular reaction i.e. the rate of progeny formation is proportional to the product of parental concentrations (Walmsley, 1973). However, Collins and Broda (1975) showed that for Hfr matings the number of progeny formed changed much more slowly with concentration than predicted by this model. They found that motility decreased at higher concentrations so there were fewer collisions; however, this difference was not large enough to account for all the deviation from the model that was observed. Eckerson and Reynard (1977) measured aggregate formation at different concentrations in matings with an F-like R-factor Rl-19. Their results also showed a slower change with concentration than predicted by bimolecular reaction kinetics; the progeny yield at higher concentrations was less than that predicted by a line extrapolated from lower concentrations on the basis of this model. Collins and Broda (1975) measured mating aggregate formation using the number of progeny produced; this had the disadvantage of being rather indirect but had the important advantage of allowing a
wide range of concentrations to be used. Eckerson and Reynard (1977) used sectored colonies containing both parents as a measure of pair formation; this can only be used for conditions where a substantial proportion of cells are in aggregates; this is also true of Coulter counter methods.

Walmsley (1973) investigated the proportion of parents competent to form mating pairs in Hfr matings using a Coulter counter. He employed very short (7') matings to avoid the formation of aggregates larger than pairs. He varied the donor:recipient ratio and deduced the proportion of parents competent to form pairs from the amount of pair formation when one parent was in a minority and hence the limiting factor. He corrected his data for the non-completion of pair formation due to the short mating time; however, his correction was based on bimolecular reaction kinetics which are not supported by Collins and Broda (1975) (see above). We therefore decided to re-investigate the question of parental mating competence using F'lac matings.

Achtman (1975) showed that many of the cells in F'lac mating mixtures were in large aggregates. This suggested that multiple matings might be very common. However, Broda and Collins (1978) studied Hfr matings with a particular pair of strains and found that multiple mating was relatively rare. Multiple mating has different consequences for donor and recipient cells. Multiple mating by a donor cell will produce several progeny. Broda (1975) performed fluctuation tests for mating ability on cultures of the repressed F-like R-factor R100. He deduced that each spontaneously derepressed cell in the donor population gave rise to about 10 progeny in 60' minutes. We performed similar experiments using F'lac donors to establish how much variation there was between individual donor cells
in the population; this was also important for estimating the proportion of donors competent to mate. Multiple mating by recipients is not normally detected as a multiple mating event still produces only one progeny. The occurrence of multiple mating has been shown in matings of two donor strains with one recipient strain (Fischer-Funtuzzi and Di Girolamo, 1961); however, it is difficult to obtain quantitative information from such matings because plasmid incompatibility will reduce the yield of cells carrying two F' plasmids. We were able to estimate the number of donors mating with each recipient from competition experiments between two donor strains.

We wanted to discover if there was a significant lag period between a donor cell mating and being able to mate again. We also wanted to measure the time between a recipient receiving F'lac and being able to re-transfer it. Achtman et al. (1971) showed that, at 42°C, there was little re-transfer by recipients until 60' after the start of mating; the data of Willetts (1974) suggest that the lag is longer at 37°C. It was possible to measure re-transfer from recipients by adding a second recipient strain to a mating mixture after the donor strain had been killed with phage T6. This lag was long enough for re-transfer by recipients to be no problem when the gap between rounds of donor transfer were being measured.

One problem in interpreting the results of mating experiments is knowing the efficiency of plasmid establishment in a recipient cell after transfer. If a single donor cell could transfer plasmids to several recipient cells and there was a relatively low efficiency of inheritance, there should be a considerable variation in the number of progeny produced by different donor cells. Therefore experiments that measure amount of variation between progeny yield by different donor cells can give information about the efficiency
of plasmid establishment after transfer. It is possible for new progeny in F' matings to segregate F' daughter cells on division, as can be seen by the production of sectored colonies containing recipient cells with and without the plasmid (deHaan and Stouthamer, 1963). This will affect the apparent growth rate of new progeny. We therefore performed experiments to measure this apparent growth rate and to measure the amount of segregation occurring in our system by a sectored colony technique.

**Barriers to plasmid spread due to interaction with other plasmids**

The presence of other plasmids in recipient cells may be a barrier to spread of a plasmid by conjugation to recipients. A reduction in progeny yield can be due to at least three mechanisms:

(i) Restriction:

The plasmid in the recipient codes for a restriction enzyme that degrades the incoming plasmid DNA. Resident DNA in the recipient is protected by a modification system. An example of this is the restriction system coded for by prophage P1 that can reduce progeny yield in F' matings by a hundred-fold (Glover et al., 1963). Chromosomally coded restriction systems will have similar consequences for plasmid spread to plasmid coded ones.

(ii) Surface exclusion:

The resident plasmid has genes which greatly reduce the recipient ability of the cell carrying it. In the case of F' lac, there are surface exclusions genes which greatly reduce the amount of aggregate formation with F' donors (Achtman and Helmuth, 1975).
(iii) Incompatibility:

The two plasmids are unable to coexist in the same cell and, on division, cells containing only one plasmid type are segregated. Often both plasmids may be maintained in the same cell by selection and segregation occurs when selection is relaxed (Uhlin and Nordström, 1975; Cabello et al., 1976).

These three effects may be difficult to distinguish experimentally, especially if they occur in combination. Restriction need not be a barrier to plasmid spread by re-transfer because the plasmids which overcome the restriction barrier will be modified so that restriction will not affect the efficiency of re-transfer to other recipient cells. However, in nature there will probably be a mixed recipient population carrying different restriction systems.

The biological significance of surface exclusion is unclear. Achtman and Helmuth (1975) found surface exclusion genes in the large transfer operon. Thus, in an F-like R-factor surface exclusion will not be expressed normally because of the repression system (Helmuth and Achtman, 1975). In the case of F, surface exclusion can be overcome by using suitable growth conditions; this state is called "F" pheno" (Lederberg et al. 1952).

Incompatibility occurs between closely related plasmids and indeed is used to classify plasmids (Meynell et al. 1968). The mechanism of incompatibility and its importance as a barrier to plasmid spread are unclear.

We did not examine the effects of restriction and surface exclusion further. However, we did study the process of incompatibility by calculating segregation rates predicted by several simple models and comparing these with experimental data using a pair of colEl derivatives.
Incompatibility

Attempts to obtain mutants that relieve incompatibility between members of an incompatibility group have been unsuccessful although there are conditions when a specific pair of plasmids appear to coexist in the same cell (San Blas et al., 1974; DeVries et al., 1975). We therefore considered models in which incompatibility is the consequence of normal replication and segregation mechanisms and is not a separate function.

Jacob et al. (1963) suggested a model for replication and segregation in which there exist membrane sites which are responsible for the replication of a plasmid and these membrane sites divide at cell division and segregate one copy of the replicated plasmid to each daughter cell. Incompatibility would be due to competition for a limited number of membrane sites. If plasmids occupied the membrane sites throughout the cell cycle then an incoming incompatible plasmid would be unable to replicate because the membrane sites would all be occupied by the resident plasmid. However, in the case of autonomous plasmids, segregation is often fairly symmetrical between two plasmids (Echols, 1965; Uhlin and Nordström, 1975; Cabello et al., 1976; Timmis et al., 1977). Thus, the attachment to any site is not permanent. If there is only one site per cell then, if a cell contains two incompatible plasmids (one copy of each), one will win the competition for the site and the other will not be replicated and enter only one of the daughter cells. Therefore, the proportion of cells carrying both plasmids will fall by half each generation. This is much faster segregation than that observed even for the low copy number plasmids: R1 (Uhlin and Nordström, 1975) and F' plasmids (Jamieson and Bergquist, 1977). If there were more than one site per cell then two plasmids with the same replication and segregation system should be able to
coexist in the same cell.

The essential feature of the model of Jacob et al. (1963) is the tight coupling between replication and segregation. Because of the failure of such models, we consider models in which replication and segregation are independent. Such independence has also been suggested for *Staphylococcus aureus* plasmids (Novick and Schwesinger, 1975).

The model of Jacob et al. (1963) also predicts a "democratic" model of replication i.e. every plasmid copy is replicated once per generation. This can be tested by using a density shift experiment in which cells are grown on a medium that produces DNA of a certain density and then shifted to a new medium that labels the DNA with a different density. Then the first round of DNA replication after the shift produces DNA of an intermediate density and the second and subsequent rounds produce DNA of the new density. The democratic model predicts that twice-replicated plasmid DNA should not appear until one generation after the shift. However, when experiments were done using the plasmids NRl (Rownd, 1969), colEl (Bazaral and Helinski, 1970), Rl (Gustafsson and Nordström, 1975) and F' plasmids (Kline, 1974; Gustafsson et al. 1978) twice replicated DNA appeared much more quickly and the results were in better agreement with the random pool model of replication in which plasmids are selected at random from the pool in the cell for replication and returned to the pool after replication. In the case of the experiments with F' plasmids, Kline (1974) interpreted his results as due to a democratic replication model with the twice-replicated DNA being produced as a result of a disturbance of replication due to his bromouracil density label and Finkelstein and Helmstetter (1977) suggested that the results of Gustafsson et al. (1978) were due to a disturbance in cell growth caused
by the density shift. However, the interpretation in terms of the random pool model seems simpler and we mainly consider this model.

Positive control of plasmid replication is predicted by the model of Jacob et al. (1963). Pritchard et al. (1969) put forward an alternative negative control model for plasmid replication. Cabello et al. (1976) tested the predictions of these models by linking together two compatible plasmids of different copy number. The model of Jacob et al. (1963) predicts that such a plasmid should have a copy number equal to the sum of the copy numbers of the constituent plasmids and replication should occur from both origins. The model of Pritchard et al. (1969) predicts that the plasmid should have a copy number equal to that of its higher copy number constituent and replicate only from the origin of the higher copy number constituent. The data agreed with the predictions of the latter model but not the former. We assumed that replication control acted to restore the number of plasmid copies per cell to a constant number before division.

Two main models for segregation have been proposed (see Novick et al., 1975)

(i) Equal number segregation:

an equal number of plasmid copies are distributed to each daughter cell.

(ii) Random segregation:
plasmids can enter either daughter cell at random, on cell division. This model predicts that if there are N plasmids in a cell at division then a proportion $1/2^N$ of daughter cells will lack the plasmid. Thus, a plasmid will only be stable on this model if the copy number is reasonably high.
Novick et al. (1975) tested between these models by studying the segregation of plasmid-free cells when plasmid replication was blocked. If segregation is the same for non-replicating plasmids as for replicating plasmids then the random segregation model predicts an earlier appearance of plasmid-free cells than the equal number model. They found that a low copy number plasmid gave results in agreement with the equal number model. Hashimoto-Gotoh and Sekiguchi (1977) made similar conclusions from experiments with pSC101 which had 10-14 copies per cell. Novick et al. (1975) also interpreted the results of May et al. (1964) with a high copy number plasmid (about 32 per cell) as supporting equal number segregation. However, about 20% of the cells were plasmid-free at the start of the experiment so it would not have been easy to detect early new segregants. Thus, for higher copy number plasmids it is not clear which is the appropriate segregation model.

We considered random pool replication models with equal number segregation and with random segregation models. We also considered a democratic replication model. In all of the models that we considered incompatibility occurs as a result of the segregation mechanism rather than as a result of interference with replication of one of the plasmids. The inability of an F' plasmid to be maintained in an Hfr cell is due to prevention of its replication (Dubnau and Maas, 1968). However, in the case of this sort of incompatibility, between an integrated F and an autonomous F, it was possible to find a mutation in the integrated F that abolished incompatibility (DeVries and Maas, 1973). This inc mutation did not affect incompatibility between autonomous F' plasmids. In the case of RP4 an integrated copy of the plasmid did not show incompatibility towards the autonomous plasmid (Watson and Scaife, 1978). Thus, the incompatibility between an integrated and an autonomous F may well be a special situation and
not be a good analogy for incompatibility between autonomous plasmids.

**Hfr formation and chromosome transfer**

Many plasmids can integrate into the chromosome of their host cell (Datta and Barth, 1976a; Novick et al., 1975). The best studied case is that of F in *E. coli* K12, where integration results in Hfr strains that transfer certain chromosomal genes at a high frequency. Heteroduplex studies (Ohtsubo et al., 1974; Hu et al., 1975; Deonier and Davidson, 1976) have shown that F integration seems to involve reciprocal recombination between homologous sequences on F and on the chromosome. Thus, it seemed likely that the normal host recombination system might play an important role in F integration, as it does in F excision (Deonier and Mirels, 1977). However, three of the F regions involved in integration were homologous to "insertion sequences" (one to IS2 and two to IS3) which can take part in various types of recA-independent recombination events (see Starlinger and Saedler, 1976). The fourth sequence, the Y8 sequence, has been implicated in recA-independent recombination events leading to the break up of F' plasmids (Ohtsubo, et al., 1974; Palchaudhuri et al., 1976). There are two pieces of evidence that suggest a role for recA-independent processes in F integration.

(i) Hfr clones have been obtained with F' plasmids in recA cells (Broda and Meacock, 1971; DeVries and Maas, 1971). However, the frequency of such events is unknown and the relevance to F integration is unclear as DeVries and Maas (1971) found that F' plasmids tended to integrate in the chromosomal region from which the F' was derived.

(ii) Curtiss and Renshaw (1969a) found that certain F⁺ strains ("Type II" strains) seemed to be defective in Hfr formation;
this was a property of the host cell rather than of the F plasmid carried. They interpreted this as a defect in F integration. As Type II strains were still recombination-proficient, this suggested that F integration followed a different pathway from normal recombination and might be recA-independent.

Thus, it was unclear whether the IS sequences and the Yf sequence on F participated in recA-independent recombination activities to give F integration or whether they merely played a passive role as regions of homology for the host's normal recombination system. We tested between these theories by comparing the rates of Hfr formation in isogenic rec+ and recA strains. The rate of Hfr formation was reduced about a hundred-fold in the recA strain which supported the second theory.

Type II F+ strains give comparable frequencies of chromosomal transfer to normal (Type I) strains (Curtiss and Renshaw, 1969b). Clowes and Moody (1966) reported that the recA mutation reduced chromosome transfer thirty-fold in an AB1157 strain; this strain was probably Type II as it was descended from the Type II strain W945 (Bachmann, 1972). Moody and Hayes (1972) reported that, in a Type I strain (W1655), the recA mutation reduced chromosomal transfer about two hundred-fold. We performed experiments to test whether there was a difference in the effect of recA mutations on chromosome transfer in Type I and Type II strains, but found no evidence for any difference. As Hfr cells yield predominantly F+ recombinants and it appeared that Hfr cells accounted for about 15% of chromosome transfer by F+ populations (Curtiss and Stallions, 1969), the percentage of recombinants that remained F- in matings with a Type I strain should be about 10-15% higher than for a Type II strain.
However, Curtiss and Renshaw (1969b) found that there was no difference between Type I and Type II strains. We confirmed this observation and showed that it was true for recA strains also. Fluctuation tests with Type II strains suggested that Hfr clones were formed, but were difficult to isolate because of some secondary effect.
CHAPTER 2

MATERIALS AND METHODS

Materials

L-broth (per litre): 10g tryptone (Difco), 5g yeast extract (Difco)
10g NaCl, pH 7.2.

Care was taken to keep L-broth detergent-free because of the

L-broth agar: L-broth solidified with 1.5% agar (Difco).

Lactose tetrazolium agar: 1% lactose and 0.002% tetrazolium dye
(2,3,5-Triphenyl-2H-tetrazolium Chloride; Eastman) were added to
L-broth agar.

Giemsa agar (per litre): 10g tryptone (Difco), log yeast extract
(Difco), 8g NaCl, 15g agar (Difco). After melting, 0.002M CaCl₂,
1% glucose and 12.4 ml. of Giemsa dye (Gurr R66) were added.

BBL bottom agar (per litre): 10g B.B.L. trypticase, 5g NaCl, 10g
agar (Difco).

BBL top agar (per litre): as BBL bottom agar, except contains 6.5g
agar.

Spizizen salts (X5) (per litre): (NH₄)₂SO₄ 2g, K₂HPO₄ 14 g, KH₂PO₄ 6g,
tri-sodium citrate 1g, MgSO₄.7H₂O 0.2 g.

Minimal agar: Spizizen salts (X5) were added 1:4 to 2% agar (Davis N.Z.)
together with appropriate supplements. 1 µg/ml thiamine and a further
1.25mM MgSO₄ were added to all minimal agar.

Minimal top agar: 0.7% agar (Difco).

Supplements were used at the following final concentrations:

Sugars (glucose, lactose, arabinose) 2 mg/ml

Amino acids (except lysine) 20 µg/ml; lysine 100 µg/ml.

Adenine 50 µg/ml

Streptomycin sulphate (Glaxo) 200 µg/ml; nalidixic acid (Sigma) 40 µg/ml;
ampicillin (Beechams) 50 \( \mu g/ml \); Kanamycin (Winthrop) 50 \( \mu g/ml \); tetracycline (Lederle) 10 \( \mu g/ml \) (20 \( \mu g/ml \) to L-broth agar).

Coulter counter diluent: 9% NaCl, 0.08% sodium azide. This was filtered through a 0.22 \( \mu \) Millipore filter and refiltered during dispensing.

Phage buffer (per litre): \( Na_2HPO_4 \) 7g, \( KH_2PO_4 \) 3g, NaCl 5g, 1mM MgSO_4, 0.1 mM CaCl_2, 0.001% gelatin.

Dilutions: Bacterial dilutions were made in L-broth.

Bacterial Strains

The bacterial strains used are shown in Table 2.1.

Construction of ED2510: ED2510 was made by P1 transduction of pML2 from ED2521 into ED3887. A plate lysate of P1 vira (from P. Broda) was grown on ED2521. A volume of this lysate was added to 0.5 ml of a fresh overnight culture of ED3887 to give a multiplicity of infection of about 0.1; 5 mM CaCl_2 was present in the mixture. After 20' incubation at 37°C, the bacteria were pelleted using a bench centrifuge and resuspended in 1 ml of 0.1M citrate buffer pH 7.0. Dilutions (in the citrate buffer) were plated on L-broth agar containing streptomycin and kanamycin. A clone was purified by streaking to single colonies twice.

Construction of ED2516 and ED2517: These strains were constructed by flask matings of the recipient strain ED3826 with ED2510 and GWl37 respectively.

Culture Conditions

Strains were stored on L-broth agar slopes at 4°C. Before use they were streaked to single colonies and a 5 ml. standing overnight culture in L-broth inoculated from a single colony. After checking the phenotype, the overnight culture could be stored at 4°C for up to
Table 2.1  Bacterial strains

1. Strains used in F'lac mating experiments

(a) Plasmid-carrying strains:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid(s) carried</th>
<th>Host strain</th>
<th>Sourcea</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED2510</td>
<td>F'lac traI65, pML2</td>
<td>JC6256</td>
<td>This thesis</td>
</tr>
<tr>
<td>ED2516</td>
<td>pML2</td>
<td>ED3826</td>
<td>This thesis</td>
</tr>
<tr>
<td>ED2517</td>
<td>pDS1107</td>
<td>ED3826</td>
<td>This thesis</td>
</tr>
<tr>
<td>ED2525</td>
<td>F'lac traI40</td>
<td>JC3272</td>
<td>W</td>
</tr>
<tr>
<td>ED2526</td>
<td>F'lac traA</td>
<td>JC3272</td>
<td>W</td>
</tr>
<tr>
<td>ED3887</td>
<td>F'lac traI65, colEl</td>
<td>JC6256</td>
<td>W</td>
</tr>
<tr>
<td>GW137</td>
<td>F'lac traI65, pDS1107</td>
<td>JC6256</td>
<td>JW</td>
</tr>
<tr>
<td>JC6582</td>
<td>F'lac tra^</td>
<td>JC6255</td>
<td>W</td>
</tr>
<tr>
<td>M172</td>
<td>F'lac traH88</td>
<td>JC6255</td>
<td>W</td>
</tr>
</tbody>
</table>

(b) Relevant phenotypic propertiesb of plasmid-less strains:

<table>
<thead>
<tr>
<th>Strain</th>
<th>His</th>
<th>Lys</th>
<th>Str</th>
<th>Nal</th>
<th>T6</th>
<th>Su</th>
<th>ColEl</th>
<th>Sourcea</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED2196</td>
<td>-</td>
<td>+</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>-</td>
<td>S</td>
<td>W</td>
</tr>
<tr>
<td>ED3826</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>-</td>
<td>R</td>
<td>W</td>
</tr>
<tr>
<td>JC3272</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>-</td>
<td>S</td>
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</tr>
<tr>
<td>JC6255</td>
<td>+</td>
<td>+</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>+</td>
<td>S</td>
<td>W</td>
</tr>
<tr>
<td>JC6256</td>
<td>+</td>
<td>+</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
<td>S</td>
<td>W</td>
</tr>
</tbody>
</table>

2. Strains used in Hfr formation experiments

(a) F^+ strains:

<table>
<thead>
<tr>
<th>Strain</th>
<th>recA allele</th>
<th>Type</th>
<th>Parent strainc</th>
<th>Sourcea</th>
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<tbody>
<tr>
<td>ED877</td>
<td>-</td>
<td>I</td>
<td>X637</td>
<td>B</td>
</tr>
<tr>
<td>ED879</td>
<td>+</td>
<td>I</td>
<td>X637</td>
<td>B</td>
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<tr>
<td>ED957</td>
<td>+</td>
<td>II</td>
<td>X852</td>
<td>B</td>
</tr>
<tr>
<td>ED969</td>
<td>+</td>
<td>II</td>
<td>X852</td>
<td>B</td>
</tr>
</tbody>
</table>
Table 2.1 (continued)

(b) F\textsuperscript{−} strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1157</td>
<td>thr leu thi lacY galK ara xyl mtl proA his argE str tsx sup37</td>
<td>B</td>
</tr>
<tr>
<td>Χ478</td>
<td>ara leu ton proC lacZ tsx purE trp xyl lys mtl metE thi str</td>
<td>B</td>
</tr>
</tbody>
</table>

3. Other strains:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB259</td>
<td>An HfrH strain (see Bachmann, 1972)</td>
</tr>
<tr>
<td>ED2521</td>
<td>A C600 (see Bachmann, 1972) derivative</td>
</tr>
<tr>
<td></td>
<td>carrying the plasmid pH2</td>
</tr>
<tr>
<td>ED2520</td>
<td>An E.coli B/r/l strain</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Strains were kindly provided by the following people:

B Dr. P. Brdka

F Dr. D. Finnegan

JW Dr. J. Watson

W Dr. N. S. Willetts

\textsuperscript{b} All strains require tryptophan and carry the lac deletion X74. The abbreviations of properties are as follows:

His, Lys: \(+/−\) require/do nor require histidine and lysine respectively.

Str, Nal, T6, ColE1: R/S are resistant/sensitive to streptomycin, nalidixic acid, phage T6, colicin E1 respectively.

These strains are described in Achtman et al. (1971) except for ED3826 which is a colicin E1-resistant derivative of JC3272.

\textsuperscript{c} Χ637 and Χ852 are cycloserine resistant lac\textsuperscript{−} strains. Χ852 is also thi\textsuperscript{−}. All the Χprefix strains are described in Curtiss and Renshaw (1969a). AB1157 is described in Bachmann (1972). The four F\textsuperscript{+} strains were made by P. Brdka. thyA mutants of the rec\textsuperscript{+} F\textsuperscript{−} parent strains were selected with trimethoprim and the strains mated with a recA Hfr strain with selection for Thy\textsuperscript{+} recombinants. rec\textsuperscript{+} and recA recombinants were then made F\textsuperscript{+} by mating with an F\textsuperscript{+} strain.
a month and used for several experiments. Exponentially growing cultures for experiments were produced by 1 in 100 dilutions from the overnight cultures into L-broth in side arm flasks. The flasks contained about one tenth flask volume of culture and were aerated in a shaking water bath (100 rpm) at 37°C. When necessary flasks were stored on ice when the cultures were at such an optical density that 30'-40' growth was required after restoration to 37°C before the experiment began.

**Preparation of Phage Lysates**

(i) **Assay of phage titres:** 0.1 ml of a suitable phage dilution in phage buffer and 0.1 ml. of an overnight culture of the bacterial indicator strain were added to 2.5 ml. of BBL top agar and the top layer poured on to BBL bottom agar. For P1 and MS2, 5mM MgSO$_4$ and 5mM CaCl$_2$ were present in the top layer. After overnight incubation at 37°C the number of phage plaques was counted. The following strains were used as indicator strains: for T6, ED2520; for P1, AB1157; for MS2, ED879.

(ii) **Plate lysates:** 0.1 ml. of a phage lysate, diluted to about 10$^7$ pfu/ml in phage buffer, and 0.5 ml. of a fresh overnight culture of bacteria were added to 3 ml. of BBL top agar containing 5mM CaCl$_2$ and 5mM MgSO$_4$. This top layer was poured on to thick (about 40 ml. agar per 90 mm diameter petri dish) fresh L-broth agar plates that had not been dried. After overnight incubation, the top layer was scraped off into a bottle containing 0.5 ml. chloroform and the petri dish rinsed with two 1 ml. portions of phage buffer. The top layer was vortex-mixed and then agar was pelleted for 10 minutes in a bench centrifuge. The supernatant (i.e. the phage lysate) was collected and stored at 4°C with chloroform added. ED879 was
used to prepare lysates of MS2. MS2 was kindly provided by Dr. N. S. Willetts.

(iii) Preparation of high titre phage T6: Cultures of ED2520 were grown with vigorous shaking at 37°C in 500 ml. portions of L-broth containing 0.1% glucose in 5 litre flasks. When the cultures reached about 2 x 10^8 cells/ml., phage T6 (provided by P. Broda) was added at a multiplicity of infection of 0.1. After vigorous shaking for a further 4-5 hours, 2 ml. of chloroform were added to each flask and the flasks stored at 4°C overnight. The cell debris was pelleted by 30' centrifugation at 5,000 rpm in an MSE 6 x 250 ml. rotor. The phage were then pelleted by 60' centrifugation at 19,000 rpm in a Beckman type 19 rotor. The pellet was resuspended by adding 2 ml. of phage buffer per 250 ml. centrifuge bottle and gentle shaking overnight at 4°C. The final phage titre was above 10^{12} pfu/ml.

**Tube Mating**

1 ml. of appropriate dilutions of exponentially growing donor and ED2196 recipient cultures were mixed in 18 mm x 150 mm tubes and incubated (37°C, linear shaker 4 cm throw, 180 shakes/min.) for the required mating times. 0.1 ml. samples of appropriate dilutions were plated, using top agar, on selective agar. Nalidixic acid was used as a counter selective agent that prevented F' lac transfer occurring on the plates (Barbour, 1967). The platings were performed as rapidly as possible and were completed within 8' of the end of the mating time.

**Matings to study variations between individual donor cells**

0.1 ml. of the required JC6582 dilutions were dispensed into a series of 12 mm x 75 mm tubes which were incubated without shaking. After 5' incubation, 0.1 ml. of an exponentially growing ED2196
culture was added to each tube. After 30' mating, 0.1 ml. of phage T6 (about $10^9$ pfu) was added to each tube. The rapid dispensing of the cultures and the phage was achieved by using a Colworth Droplette. After 5', the total contents of each tube was plated using top agar, on agar selective for Lac$^+$/NaI$^R$ progeny. Plating all the tube cultures took 7'.

**Flask Matings**

Appropriate volumes of donor and recipient cultures were mixed in conical flasks of about ten times the mating mixture volume. The mixture was aerated by shaking (100 rpm).

**Matings to study Lac$^+$/Lac$^-$ sectored colonies**

We mixed equal volumes of exponentially growing cultures of the donor and recipient strains, at a concentration of about $1.5 \times 10^8$ cells/ml. After 20' and 30' mating, samples were diluted in L-broth blended and spread on fresh lactose tetrazolium agar and fresh L-broth agar (both containing streptomycin). The experiment was performed in a 37°C room, with media and equipment prewarmed to minimise disturbance to the cells.

After overnight incubation, the lactose tetrazolium plates were examined for the presence of sectored Lac$^+$/Lac$^-$ colonies (i.e. white/red sectors). Dilutions were chosen to give 50-100 colonies per plate.

Colonies on the L-broth agar were tested for β-galactosidase production as follows. Whole well isolated colonies were put into tubes containing 2 ml. L-broth, which were vortex-mixed to emulsify the colonies; the tubes were stored at 4°C whilst the test for β-galactosidase activity was performed. 0.1 ml. samples were added to tubes containing 1 ml. of minimal medium supplemented with casamino acids, tryptophan and 1% lactose. These tubes were incubated at 37°C for 2 hours. The production of β-galactosidase was then tested using a procedure adapted from Pardee et al. (1959). One drop of toluene was added to each tube and the tubes were shaken vigorously at 37°C.
for 30' to evaporate the toluene. Then 0.3 ml. of M/75 o-nitrophenyl-β-D-galactoside (BDH) was added to each tube and incubation at 37°C continued. Most colonies containing Lac+ cells gave a yellow colour within 15'. Incubation was continued overnight to detect smaller proportions of Lac+ cells. Tests showed that this procedure would detect at least as few as 1% Lac+ cells.

Appropriate dilutions of the emulsified colonies which produced β-galactosidase were plated on lactose tetrazolium agar to estimate the proportions of Lac+ and Lac− cells.

**Coulter counter measurement of aggregate-forming ability**

We used a Coulter counter model ZB, with a 30μ orifice, that was connected to a Coulter channelizer with a hundred channels to measure the aggregate-forming ability of a donor culture. An account of the operation of such an apparatus is given by Achtman(1975).

Samples of the donor cultures, diluted to the same optical density as the recipient, were mated for 30' in tubes with equal volumes of an exponential culture of ED2196 (about 1.5 x 10^8 cells/ml.). A sample from the tube was gently diluted into L-broth containing 1.87μ diameter latex spheres (Coulter). Blended (with a mating interrupter - Low and Wood, 1965) and unblended samples of the bacteria-latex mixture were examined in the Coulter counter.

For each sample, two peaks were seen with the channelizer. The peak at the larger particle size was due to the latex, and the other peak was due to bacteria. The concentration of latex was high enough for the contribution of cell aggregates to the latex peak of the unblended mixture to be negligible. The bacteria in the blended samples are present almost entirely as single cells and there was a cut off size between the bacterial and latex peaks where the number of particles was approximately zero. The particles in the unblended
mixture of less than this cutoff size must have been mainly single cells, since there were very few particles of less than half the cutoff size in the sample i.e. they were too small for pairs to have made a large contribution. The number of particles in the cell peaks of the blended (C+) and unblended (C-) mixtures and the latex peaks of the blended (L+) and unblended (L-) mixtures were measured. The volumes of blended and unblended mixtures sampled by the Coulter counter were, in general, different because the mixtures were sampled until one of the channels of the Channelyser reached the maximum count of 10,000 particles. Control experiments showed that the latex is unaffected by blending; therefore the ratio of L+ to L- gives the ratio of volumes sampled in the blended and unblended samples. Then, the numbers of single cells (i.e. not in aggregates) in the two samples C+ and C- allows one to calculate the proportion of cells in aggregates as: 1 - (L+)(C-)/(L-)(C+).

The use of an internal latex control avoids sampling errors in dilution and in the volume sampled by the Coulter counter. The method is also insensitive to drift in machine settings and any bias by the Channelyser against larger particles.

The method used by Achtman (1975) was inappropriate for our experiment (Fig. 5.1) as the proportion of cells in the donor culture found in aggregates changed appreciably over the course of the experiment (from 7.5% to 17%) and the formula of Achtman (1975) gives only the number of parental "particles" involved in mating aggregates. Also, his assumption that every particle, regardless of size, was equally likely to participate in mating aggregates might not be appropriate for cultures entering stationary phase.

**Fluctuation test**

A standing overnight culture was diluted to about 200 cells/ml
and 0.5 ml. samples were dispensed into a series of small tubes (12 mm x 75 mm). A parallel bulk culture (volume at least 50 ml) was also prepared from the same dilution. These cultures were incubated until they contained about $2 \times 10^8$ cells/ml. Then 0.1 ml. samples were dispensed into small tubes (the samples were previously diluted 1 in 20 for rec$^+$ strains), and the parent cultures were then stored at $4^\circ$C. The samples were incubated for 5 minutes and then 0.1 ml. per tube of an exponentially growing culture of AB1157 was added. After 80' mating, phage T6 was added to a final titre of about $10^{10}$/ml. After 5' further incubation, the total contents of each tube was plated on agar selective for Thr$^+$Ara$^+$Leu$^+$Str$^R$ recombinants using top agar. It was essential to select for all three nutritional markers to reduce the effects of background growth and mutations.

**Streak Matings**

0.5 ml. cultures were grown as described for the fluctuation tests. Loopfuls (0.003 ml.) of the cultures were streaked on agar selective for Thr$^+$Ara$^+$Leu$^+$Str$^R$ recombinants, after each plate had been spread with 0.5 ml. of an exponentially growing culture of AB1157 concentrated 10-fold by centrifugation. Twenty five streaks per plate were used.

**Sib Selection and Isolation of Hfrs**

The method of sib selection, as used previously (Broda 1967), was used to help isolate Hfr clones. 0.5 ml. portions of a suitable dilution of a culture were dispensed into small tubes and after incubation were mated with AB1157 in small tubes, as described for the fluctuation tests. When the number of recombinants was sufficiently high, clones were tested for the Hfr property by replica plating on to agar selective for Thr$^+$Ara$^+$Leu$^+$Str$^R$ progeny which had been spread with 0.1 ml. of an exponentially growing culture of AB1157. Otherwise,
the most fertile culture was used for another cycle of sib selection.

**Testing for MS2 sensitivity**

Colonies were patched on to L-broth agar using sterile toothpicks. After a few hours growth they were replica plated on to Giemsa plates spread with 0.1 ml of phage MS2 (about 3 x 10^{12} pfu/ml). After overnight incubation, the MS2^{S} (that is, F^{+}) clones gave purple-coloured patches that could be distinguished from the white patches given by F^{-} clones.

**Origin and direction of transfer of Hfr strains**

Hfr strains were mated with X'478 in a ratio of 1:100. Samples were taken at 20', 40' and 60' and dilutions were plated on agar selective for Pur^{+}, Met^{+}, Leu^{+}, Pro^{+} and Trp^{+} recombinants that were streptomycin-resistant.

**Matings to determine the efficiency of transfer of chromosomal genes**

Cultures of the parent F^{+} strains and AB1157 were grown to 1-2 x 10^{8} cells/ml. A 2.5 ml sample of the donor culture was added to 50 ml of the AB1157 culture. After 60' mating phage T6 was added to a final concentration of about 10^{10} pfu/ml to kill the donors. After 5-10' the cultures were centrifuged and the cells resuspended in 0.02-0.1 volume of L-broth. Dilutions were plated on agar selective for the Thr^{+}Ara^{+}Leu^{+}Str^{R} progeny, and agar selective for the AB1157 recipients.

When the progeny were to be tested for MS2 sensitivity, the matings were done with a 1:30 donor to recipient ratio, and control matings using r^{+} cultures to which 1% Hfr had been added were done under the same conditions. The Hfr strains used for this purpose were the rec^{+} strains AB259 and one of the recA Hfr strains that we had isolated from strain ED877. Twenty-five of the progeny colonies from each mating were tested for MS2 sensitivity as described above.
Correction for multiple mating

We used the method of Reeves (1960). A small proportion of Hfr cells was added to an F\(^+\) culture to increase the fertility about 100-fold so that the majority of recombinants (about 99%) were produced by Hfr donors and would be F\(^-\) in the absence of mating with F\(^+\) cells. However, about one third of recombinants were F\(^+\) in the mating conditions used. In matings using F\(^+\) donors alone about 20% of recombinants remained F\(^-\). Thus, in the absence of multiple mating, 30% of recombinants would be expected to be F\(^-\). The Hfr strains that we used were AB259 (for rec\(^+\) F\(^+\) strains) and a recA Hfr strain that we isolated from ED877 (for recA F\(^+\) strains).
CHAPTER 3
DONOR ABILITY IN F'lac MATINGS

In order to investigate donor ability we used low donor:recipient ratios so that the donor behaviour would be the limiting factor. Preliminary experiments showed that if a high recipient concentration (about $10^8$ cells/ml) was used then the number of progeny per donor reached a plateau by 30' of mating. Also re-transfer by recipients that had received F'lac did not become significant until 90' after the start of mating (see later).

Thus, in 30' matings with a high recipient concentration, the number of progeny per donor should not vary with the donor concentration when the donor:recipient ratio is low enough to make the donor the limiting factor. Table 3.1 shows that this occurs if the donor:recipient ratio is less than 1:10.

Effect of recipient concentration

If mating followed the kinetics of a bimolecular reaction, then at low donor:recipient concentrations, the number of progeny per donor should be proportional to recipient concentration. However, Fig.3.1 shows that in 30' matings (X) the number of progeny per donor reaches a plateau of about 1 when the recipient concentration is above $5 \times 10^7$ cells/ml. In addition, at lower concentrations, the number of progeny per donor varies more slowly with recipient concentration than expected on the theory of bimolecular reaction kinetics. This result is in agreement with those of Collins and Broda (1975) for Hfr matings. The plateau indicates that donor fertility rather than collision becomes the limiting factor above $5 \times 10^7$ cells/ml; this makes it easier to interpret mating experiments with a high recipient concentration.
Table 3.1  Effect of donor:recipient ratio on progeny yield in matings at high recipient concentration.

<table>
<thead>
<tr>
<th>Donor:recipient ratio&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Donor viable count&lt;sup&gt;b&lt;/sup&gt; (/ml)</th>
<th>Progeny viable count&lt;sup&gt;b&lt;/sup&gt; (/ml)</th>
<th>Progeny/donor ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.0 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.1 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
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</tr>
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<td>1.8 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.83</td>
</tr>
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<td>1:10&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>1.2 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.95</td>
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<td>2.3 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
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<td>0.83</td>
</tr>
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<td>1:1</td>
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<td>8.5 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.44</td>
</tr>
</tbody>
</table>

<sup>a</sup> 1 ml of dilutions of the donor (JC6582) culture were mixed with 1 ml of undiluted recipient (ED2196) culture in tubes to give the ratios shown. Both cultures were growing exponentially at the same optical density. The viable count of the recipient culture was 1.8 x 10<sup>8</sup> cells/ml.

<sup>b</sup> Viable counts after mating.
Figure 3.1  Effect of recipient concentration on F′lac matings.

JC6582 was mated with ED2196 in tubes for 20' and 30'. The recipient concentration at the start of mating was varied between $2 \times 10^5$ cells/ml and $2 \times 10^8$ cells/ml. The donor concentration was about $2 \times 10^4$ cells/ml. Selection was for Nal$^R$ Lac$^+$ colonies.

Number of progeny per donor after 20' mating 0
Number of progeny per donor after 30' mating X
20' matings (Fig. 3.1:0) gave similar results to 30' matings except that the plateau was at about 0.4 progeny per donor. This shows that many donors are unable to mate in 20' but can mate in 30'. We do not know whether this is due to a delay in aggregation or a delay in progeny formation after mating aggregate formation as observed by de Haan and Stouthamer (1963).

Donor competence and multiplicity of mating

Fig. 3.1 showed that about 1 progeny per donor was produced in 30' matings at high recipient concentrations. This could be due to every donor mating with one recipient or to a sub-population of donors competent to mate which each mated with several recipients. We distinguished these two cases using a fluctuation test similar to that used by Broda (1975). A dilution of a donor culture was dispensed in a series of tubes, the dilution being chosen to give an average of about one donor per tube. Recipient culture was added to each tube and, after mating, the total contents of each tube were plated out on to agar selective for progeny cells. The less the variation in fertility between individual donor cells, the less the variance in progeny numbers between the tubes should be. Table 3.2 shows the results of such experiments. Parallel control matings with a hundred times the donor concentration showed that the donor numbers increased four to five times during the experiments and an average of about one progeny per final donor was produced i.e. each donor entering a tube produced an average of about 4-5 progeny. The average number of progeny per tube at the lower donor concentration was about one hundredth that at the higher concentration (Table 3.2) which showed that mating occurred with the same efficiency in both cases.

A reconstruction experiment showed that the number of donors entering each tube followed a Poisson distribution approximately.
Table 3.2 Variations in donor fertility in duplicate JC6582 x ED2196 matings.

<table>
<thead>
<tr>
<th>Control matings</th>
<th>Experiment A</th>
<th>Experiment B</th>
</tr>
</thead>
<tbody>
<tr>
<td>(donor concentration x 100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average number of donors entering tubes</td>
<td>92.3</td>
<td>87.8</td>
</tr>
<tr>
<td>Average number of donors after mating</td>
<td>470</td>
<td>396</td>
</tr>
<tr>
<td>Average number of progeny per tube</td>
<td>398-</td>
<td>456</td>
</tr>
<tr>
<td>Number of progeny per donor after mating</td>
<td>0.85</td>
<td>1.15</td>
</tr>
<tr>
<td>Recipient concentration (x 10^-8/ml)</td>
<td>1.5</td>
<td>2.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tubes with low donor numbers</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average number of progeny per tube (x)</td>
<td>3.88</td>
<td>4.47</td>
</tr>
<tr>
<td>Variance of number of progeny (s^2)</td>
<td>10.1</td>
<td>18.8</td>
</tr>
<tr>
<td>s^2/x</td>
<td>2.61</td>
<td>4.47</td>
</tr>
<tr>
<td>Number of tubes</td>
<td>50</td>
<td>49</td>
</tr>
</tbody>
</table>
If the distribution of the number of progeny produced by each donor entering a tube has a mean $\mu$ and a variance $\sigma^2$ and the number of donors entering each tube has a Poisson distribution, mean $\lambda$, then the mean and variance of progeny numbers produced by each tube will be $\lambda\mu$ and $\lambda^2\mu + \lambda\sigma^2$ (see Appendix). The ratio (variance/mean) will therefore be $\mu + \sigma^2/\mu$. Our control experiments (Table 3.2) show that $\mu$ is 4-5. If a proportion $p$ of donors are competent to mate and each produce exactly $\mu/p$ progeny then the (variance/mean) ratio will be $\mu/p$. As the observed variance/mean ratios (2.67 and 4.47) are comparable to $\mu$, this indicates that most donors are competent to mate; any variation between donors raises the ratio. Thus, it appears that each donor entering a tube seems to give rise to a number of progeny equal to its growth in the mating mixture. However, this method is not very sensitive to variation between donors because errors in estimating variance are large and because of the form of the dependence of the variance/mean ratio on the variance of donor fertility. Attempts were made to obtain both donors and progeny from mating mixtures by plating total mating mixtures on agar selective for Lac+ cells. Unfortunately the lactose selection was not strong enough to give unambiguous recovery of all the Lac+ cells in mating mixtures. This sort of experiment might be possible using an F' carrying a better selective marker and should give much better information about the variation in fertility between individual donor cells.

**Time between rounds of donor mating**

The fact that the number of progeny per donor reached a plateau after 20'-30' mating suggested that there might be a gap between donors mating and being able to mate again. The existence of such a gap was confirmed by triple matings of a donor strain with two recipient strains. In one mating mixture both recipient strains were present
at the start of mating (Fig. 3.2a). The number of progeny became comparable to donor number after 20'-30' mating. In the second mating mixture, the second recipient strain was added after 20' mating. The progeny from the second recipient did not approach the donor numbers until about 80' (Fig. 3.2b). This shows that there is a gap of at least 30'-40' between a donor mating and being able to mate again. The progeny from the second recipient were produced by mating with the donor because, as we show later, re-transfer from the recipient takes longer to occur.

The gap could have been due to a necessity for disaggregation of donor cells from mating complexes before they are able to mate again or to some "physiological" cause. Achtman (1977) showed that disaggregation occurred after about 60' of mating. If aggregation were responsible for the gap then blending of a mating mixture to disrupt aggregates should abolish the lag. Fig. 3.3 and 3.4 show that donors from blended mixtures do indeed seem to show a shorter lag. However, a control experiment showed that blending of a non-mated donor culture increased its fertility (Fig. 3.5). As the results in Fig. 3.4 and 3.5 could thus also be explained by the effects of blending on donor fertility, these experiments could not distinguish the effects of aggregation and any physiological effects on the gap between rounds of donor mating.
**Figure 3.2** Time between rounds of F'\text{lac} transfer by donors. JC6582 was mated in flasks with a mixture of approximately equal numbers of the two recipient strains, JC3272 and ED2196. In (a), both recipients were present at the start of mating. In (b), ED2196 was only added after 20' of mating.

JC6582 donor \hspace{1cm} X  
JC3272 recipient \hspace{1cm} \triangle  
ED2196 recipient \hspace{1cm} \triangledown  
JC3272 (F'\text{lac}) progeny \hspace{1cm} 0  
ED2196 (F'\text{lac}) progeny \hspace{1cm} \ast

**Figures 3.3 and 3.4** Effect of blending on time between rounds of donor transfer. Flask matings were performed between JC6582 and JC3272 for 25'. Blended (as in Low and Wood, 1965) and unblended samples were added to flasks containing ED2196 cultures.

<table>
<thead>
<tr>
<th>Unblended</th>
<th>Blended</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC6582 donor</td>
<td>X +</td>
</tr>
<tr>
<td>ED2196 recipient</td>
<td>\triangle \triangledown</td>
</tr>
<tr>
<td>ED2196 (F'\text{lac}) progeny</td>
<td>\square \diamond</td>
</tr>
</tbody>
</table>

**Figure 3.5** Effect of blending on donor fertility. Blended and unblended samples of a JC6582 culture were mated in flasks with JC3272.

<table>
<thead>
<tr>
<th>Unblended</th>
<th>Blended</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC6582 donor</td>
<td>X +</td>
</tr>
<tr>
<td>JC3272 recipient</td>
<td>\triangle \triangledown</td>
</tr>
<tr>
<td>JC3272 (F'\text{lac}) progeny</td>
<td>\square \diamond</td>
</tr>
</tbody>
</table>
Figure 3.2
Figure 3.3
Figure 3.4
Figure 3.5

LOG (NUMBER OF CELLS/ML) vs. TIME (MINUTES)
Effect of parental concentration on recipient mating ability

In order to study recipient mating ability we performed matings at high donor:recipient ratios so the recipients would be the limiting factor. We used a constant high concentration of F'mat donors and a range of donor:recipient ratios in 30' matings. Table 4.1 shows that at high donor:recipient ratios 95% of recipients received F'mat. The lower proportion of recipients that became Lac⁺ in 1:1 matings (45%) presumably reflects a shortage of donors. The recipient viable count showed no depression due to "lethal zygosis", in agreement with Skurray et al. (1976).

We then performed matings with a constant low (2 x 10⁴ cells/ml) recipient concentration and various donor concentrations. Table 4.2 shows that the proportion of recipients receiving F'mat is high (greater than 75%) for donor concentrations above 2 x 10⁷ cells/ml. The number of progeny per minority parent has a very similar dependence on the majority parent concentration for donors in the minority (Fig. 3.1) and recipients in the minority (Table 4.2); the yields at lower majority parent concentrations are probably governed by the collision rate. The progeny yield can not be greater than one progeny per recipient and the donor saturated value is also about one progeny per donor.

Multiple mating of recipients

We showed (Table 4.1) that the majority of recipients could receive F'mat in 30' matings. At high donor:recipient ratios there is the possibility of a recipient cell mating with several donors; this would not normally be detected as only one progeny cell would still
Table 4.1 Effect of donor:recipient ratio on the proportion of recipients that receive F'lac

<table>
<thead>
<tr>
<th>Donor viable count(^a)</th>
<th>Recipient viable count(^a)</th>
<th>Donor:recipient ratio(^b)</th>
<th>Proportion of recipients that become Lac(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 x 10(^8)</td>
<td>1.9 x 10(^8)</td>
<td>1:1</td>
<td>46%</td>
</tr>
<tr>
<td>2.2 x 10(^8)</td>
<td>2.2 x 10(^7)</td>
<td>10:1</td>
<td>90%</td>
</tr>
<tr>
<td>2.1 x 10(^8)</td>
<td>2.1 x 10(^6)</td>
<td>10(^2):1</td>
<td>92%</td>
</tr>
<tr>
<td>2.7 x 10(^8)</td>
<td>1.6 x 10(^4)</td>
<td>10(^4):1</td>
<td>95%</td>
</tr>
</tbody>
</table>

\(^a\) Viable count (/ml) after mating.

\(^b\) Ratio of culture volumes mixed at start of mating.
<table>
<thead>
<tr>
<th>Donor concentration</th>
<th>% of recipients that become Lac⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 x 10⁴</td>
<td>less than 0.5</td>
</tr>
<tr>
<td>2.9 x 10⁵</td>
<td>3.</td>
</tr>
<tr>
<td>2.8 x 10⁶</td>
<td>20</td>
</tr>
<tr>
<td>2.8 x 10⁷</td>
<td>75</td>
</tr>
<tr>
<td>2.1 x 10⁸</td>
<td>96</td>
</tr>
</tbody>
</table>

Dilutions of the donor JC6582 were mated with the recipient (ED2196) at a concentration of about 2 x 10⁴ cells/ml in large tubes. Dilutions of the mating mixture were plated on lactose tetrazolium agar containing nalidixic acid and the proportion of recipients that had become Lac⁺ was scored. Concentrations given are viable counts after mating.
be produced. It seemed likely that donor cells that could form aggregates with recipient cells but not transfer an F' plasmid would compete with F'\text{lac} donors. The more donors that a recipient was capable of mating with, the less the inhibition due to a competing donor strain should be. The measurement of the inhibition at different ratios of the F'\text{lac} donor strain and the competing donor strain would allow the estimation of the number of donors with which each recipient was capable of mating (see Appendix).

We used an F'\text{lac tral} mutant as a competing donor as tral mutants form mating aggregates but do not transfer F'\text{lac} (Achtman et al. 1971). Table 4.3 shows that an F'\text{lac tral} mutant competed with an F'\text{lac} donor, whereas an F'\text{lac traA} mutant that did not form mating aggregates had little effect on the F'\text{lac} donor strain JC6582. For further experiments we used ED2510 which contains an F'\text{lac tral} plasmid and also carries a colE1 derivative pML2 (Kan\textsuperscript{R}) which can be mobilised by the F'\text{lac tral} (Alfaro and Willetts, 1972). Transfer of pML2 provided an independent assay of the participation of ED2510 in mating. Coulter counter measurements showed that ED2510 and JC6582 had equal aggregate-forming ability with recipients (Table 4.3). We therefore made the simplifying assumption that ED2510 and JC6582 were equal competitors for aggregate formation. We also assumed that if one or more JC6582 cells were aggregated with a recipient cell then that recipient cell received F'\text{lac}. This was supported by the experiments of the previous chapter which showed that essentially all JC6582 donors could mate in 30'.

We studied the effect of different ratios of JC6582 and ED2510 on the proportion of recipients that became Lac\textsuperscript{+} in 30' matings. We mixed donor cultures at a concentration of about 2 \times 10^8 cells/ml and mated the donor mixture with recipients which were at a concentration
Table 4.3 Inhibition of JC6582 matings and aggregate-forming ability

<table>
<thead>
<tr>
<th>Strain</th>
<th>F'lac carried</th>
<th>Other plasmids carried</th>
<th>Inhibition % of JC6582 mating&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% of cells in aggregates with ED2196&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% of cells in aggregates in parent cultures&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC6582</td>
<td>tra&lt;sup&gt;+&lt;/sup&gt;</td>
<td>-</td>
<td>0</td>
<td>62</td>
<td>23</td>
</tr>
<tr>
<td>ED2525</td>
<td>traI</td>
<td>-</td>
<td>0.18</td>
<td>62</td>
<td>30</td>
</tr>
<tr>
<td>ED2526</td>
<td>traA</td>
<td>-</td>
<td>0.05</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>ED2510</td>
<td>traI</td>
<td>pML2</td>
<td>0.21</td>
<td>62</td>
<td>23</td>
</tr>
<tr>
<td>ED2196</td>
<td>F&lt;sup&gt;-&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14</td>
</tr>
</tbody>
</table>

<sup>a</sup> The quantity shown is one minus the relative proportion of recipients that become Lac<sup>+</sup> in 30' matings of a 1:1 mixture of donors with an equal volume of ED2196 culture diluted to about 2 x 10<sup>4</sup> cells/ml.

<sup>b</sup> A Coulter counter was used to measure the proportion of cells in aggregates. The culture being tested was blended to break up aggregates and the blended culture compared with unblended culture. The matings were 1:1 matings of donor and recipient cultures at the same optical density for 30'.
of about $2 \times 10^4$ cells/ml. Fig. 4.1 (X) shows the proportion of recipients that remain Lac for different ratios of the two donors. Whereas at low ratios of ED2510 only 5-10% of recipients remain Lac, at high ratios the majority of recipients remain Lac. We found that a Poisson distribution of the number of donors mating with each recipient gave a good fit to our data; Fig. 4.1 shows a theoretical curve corresponding to a Poisson distribution with a mean of 2.65 donors mating with each recipient. Thus, recipients mate with an average of 2-3 donors.

We also considered pML2 transfer by ED2510. Under conditions of excess recipients ED2510 only produced 0.1-0.2 Kan progeny per donor whereas JC6582 produced about 1 Lac progeny per donor. In 30' matings with $2 \times 10^8$ ED2510 cells/ml and $2 \times 10^4$ recipients/ml only 35% of recipients became Kan. This suggested that the lower fertility was due to inefficiency of progeny formation after aggregation rather than a failure of aggregation; a lower efficiency of aggregation by ED2510 donors would have still allowed a high proportion of recipients to receive pML2 when excess donors were present. This agrees with the Coulter counter measurements (Table 4.3) which showed that aggregate formation was as efficient in ED2510 as in JC6582. If it is assumed that the number of donors with which a recipient can mate follows a Poisson distribution, mean 2.65, and that only a certain proportion of ED2510 donors that form aggregates with a recipient can transfer pML2, then the proportion of recipients that should receive pML2 in matings in which different ratios of competing JC6582 donors are present can be calculated (see Appendix). Fig. 4.1 (O) shows the results of such an experiment and a theoretical curve corresponding to 0.13 pML2 transfers per aggregated ED2510 cell.
Figure 4.1  (i) Proportion of recipients that remained Lac\(^-\) when mated with a JC6582:ED2510 mixture plotted against the proportion of ED2510 in the donor mixture (X). The theoretical curve corresponds to a Poisson distribution (mean 2.65) of the number of donors mating with each recipient.

(ii) Proportion of Kan\(^R\) progeny that remained Lac\(^-\) (*) plotted against the proportion of ED2510 in the donor mixture.

(iii) Proportion of recipients that remained Kan\(^S\) after mating with an ED2510:JC6582 mixture plotted against the proportion of JC6582 in the donor mixture (O). The theoretical curve corresponds to a Poisson distribution (mean 2.65) of the number of donors mating with each recipient and an efficiency of Kan\(^R\) progeny formation per aggregated ED2510 donor of 0.13.

Figure 4.2  (i) Kinetics of progeny formation. JC6582 (about \(2 \times 10^8\) cells/ml) and ED2196 (about \(2 \times 10^4\) cells/ml) were mated in a tube. Samples were plated at different times on agar selective for Nal\(^R\) Lac\(^+\) progeny (O).

(ii) Time of aggregate formation. A series of parallel 30' matings of JC6582 and ED2196 were performed in tubes. 0.25ml of JC6582 culture (about \(2 \times 10^8\) cells/ml) and 1ml of a dilution of an ED2196 culture (about \(2 \times 10^4\) cells/ml) were used in each mating mixture. 0.75ml of an ED2510 culture (about \(2 \times 10^8\) cells per ml) was added to each tube at different times. The proportion of recipients that became Lac\(^+\) (X) is plotted against the time of addition of the ED2510 culture.
Figure 4.1
Figure 4.2
A second test of our assumptions was to select for Kan$^R$ progeny and find the proportion that also received F'$lac$ in matings with different mixtures of ED2510 and JC6582. If the number of donors with which each recipient could mate followed a Poisson distribution and the donors acted independently then the events of receiving F'$lac$ and receiving pML2 are independent (see Appendix). Therefore, the proportion of Lac$^+$ cells among Kan$^R$ (pML2) progeny should be equal to the proportion among unselected recipient cells. Fig. 4.1 (*) shows that the proportion of Lac$^-$ cells among the Kan$^R$ progeny was indeed comparable with that for the unselected recipients.

**Time of aggregate formation**

We took samples at different times from a mating of JC6582 with ED2196 in conditions of donor excess. Fig. 4.2 (O) shows that the appearance of progeny is spread out through the 30' with most progeny only appearing after 20'. Part of this spread could be due to a spread in times between aggregate formation and progeny formation as observed by de Haan and Stouthamer (1963). If all aggregates were formed rapidly then JC6582 matings should rapidly become insensitive to competition from ED2510. We tested this by performing a series of parallel matings using JC6582 and adding ED2510 at different times. Fig. 4.2 (X) shows that some sensitivity to ED2510 competition was retained throughout the 30' mating period. This suggests that aggregate formation occurred throughout this period and some recipients were unable to form aggregates until more than 20' had elapsed. It is difficult to interpret these experiments quantitatively as it is not known whether the number of donors with which a recipient can mate is different for recipients that form aggregates at different times.

**Apparent growth rate of new progeny and segregation of F$^-$ cells**

de Haan and Stouthamer (1963) found sectored Gal$^+/Gal^-$ colonies
produced by new progeny in F'gal matings. These appeared to be due to segregation of F^- cells on division of new progeny cells. Such segregation would decrease the apparent growth rate of new progeny. We attempted to measure the growth rate of new F'lac progeny after the donor in a mating mixture had been killed by addition of a high titre of phage T6. There was some reduction in the apparent growth rate of new progeny (Fig. 4.3), but the size of the effect was too small to be accurately measured by such experiments. We therefore measured the amount of segregation directly using a sectored colony method.

When blended mating mixtures were plated on to lactose tetrazolium agar, with streptomycin selection against the donor strain, some sectored Lac^+/Lac^- colonies were observed. These were not due to chance overlap as the proportion did not change when dilutions were varied to give between 20 and 150 colonies per plate. Use of lactose tetrazolium agar has two potential disadvantages for studying segregation:

(i) Lac^+ colonies were larger than Lac^- colonies which indicated that Lac^+ cells had a growth advantage. Therefore the proportion of Lac^+ cells in a colony cannot be used to estimate the time of any segregation. This problem can be overcome by using L-broth agar as F'lac does not affect the recipient growth rate in L-broth (Willetts and Finnegan, 1970; our results, next chapter).

(ii) Sectored Lac^+/Lac^- colonies will only be detected if they contain comparable proportions of Lac^+ and Lac^- cells. This can be overcome by testing colonies on L-broth agar for β-galactosidase production and finding the proportion of Lac^+ cells in enzyme-producing colonies by plating on
Figure 4.3  Growth of new progeny. JC6582 was mated in a flask with JC3272 for 17' and phage T6 was then added to kill the donors. The growth of the recipient and progeny was then followed. The data were smoothed by taking the geometrical mean of the value at each time point with those of the neighbouring two time points.

JC3272 (F'lac) progeny \((x \times 10^{-6})\)  
JC3272 recipient \((x \times 10^{-8})\)
lactose tetrazolium agar. The β-galactosidase assay could even detect colonies containing less than 1% Lac⁺ cells.

Mating within mixed colonies was not expected to be a problem (Uhlin and Nordström, 1975; Broda and Collins, 1978). This was supported by experiments using an amber transfer-deficient F'lac mutant that was transferred from the Su⁺ donor strain at the same frequency as a tra⁺ plasmid but was not re-transferred from the Su⁻ recipient (see next chapter). Any substantial intracolony mating by the tra⁺ plasmid should result in a reduction in sectored colonies on lactose tetrazolium agar compared to the mutant. However, no such difference was observed.

Table 4.4 shows the results of an experiment in which sectored Lac⁺/Lac⁻ colonies were detected on lactose tetrazolium agar and also on L-broth agar by using the β-galactosidase assay. The colonies with more than 98% Lac⁺ cells should appear Lac⁺ on tetrazolium agar; this small amount of segregation would have negligible effect on the apparent progeny growth rate. The colonies with between 25% and 75% Lac⁺ cells should appear sectored on lactose tetrazolium agar. Segregation of Lac⁻ cells by new progeny should reduce the apparent progeny growth rate by about one third in the 30' after mating.

The colonies with less than 2% Lac⁺ cells are interesting as they would correspond to unilinear inheritance of F'lac for at least 6 generations after plating. We do not know the basis for this behaviour; Lac⁺ and Lac⁻ clones from such colonies were stable but we did not investigate any of their other properties to see if they differed from normal progeny. This sort of behaviour probably also occurs in liquid culture as de Haan and Stouthamer (1963) still detected sectored colonies after 60' of growth in liquid medium.

It is likely that colonies with less than 2% Lac⁺ cells would appear
Table 4.4 Mixed Lac+/Lac- colonies, in matings between JC6582 and JC3272

<table>
<thead>
<tr>
<th></th>
<th>20' Mating</th>
<th>30' Mating</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) (\beta)-galactosidase test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of colonies tested</td>
<td>40</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>Number producing (\beta)-galactosidase</td>
<td>11</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>2) Proportion of Lac+ cells in colonies producing enzyme</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 98%</td>
<td>7</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>75% - 98%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25% - 75%</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2% - 25%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Less than 2%</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>3) Colonies on lactose tetrazolium agar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of colonies examined</td>
<td>204</td>
<td>215</td>
<td>419</td>
</tr>
<tr>
<td>Number of Lac+ colonies (including sectored colonies)</td>
<td>29</td>
<td>40</td>
<td>69</td>
</tr>
<tr>
<td>Number of sectored colonies</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

Note: If it is assumed that the colonies with less than 2% Lac+ cells would appear Lac- on the tetrazolium agar, the proportion of Lac+ colonies by \(\beta\)-galactosidase assay (15/60) is not significantly different from that (69/419) using lactose tetrazolium agar (\(\chi^2_1 = 2.6\); this is not significant at the 10% level).
Lac− on lactose tetrazolium agar, but it is not clear how such progeny would behave on minimal agar where there would be a "shift down" on plating. The proportion of Lac+ colonies is higher on the L-broth agar than on the lactose tetrazolium agar. However, Table 4.4 shows that, if the colonies with less than 2% Lac+ cells are classified as Lac−, the difference is not significant even at the 10% level, and therefore is probably due to sampling errors.

Re-transfer by recipients

There is a delay between a recipient receiving F'lac and being able to re-transfer the plasmid (Achtman et al., 1971; Willetts, 1974). We wished to measure the length of this lag. After an F'lac mating the donor strain was killed with phage T6. The mating mixture was added to a culture of a second recipient strain and the appearance of Lac+ progeny from this second recipient was monitored. Fig. 4.4 shows that it took more than 90' before the progeny numbers were comparable with the number of donors in the mixture i.e. the number of progeny from the first recipient. In matings with an established F'lac donor progeny numbers equal donor numbers after 20'−30' of mating. We estimated that there was a lag of about 90' for most recipient cells between receiving F'lac and being able to re-transfer it.
Figure 4.4  Time needed for retransfer of F'^{lac} by recipients.

JC6582 was mated with JC3272 for 20'. The primary donor, JC6582, was killed by the addition of sufficient phage T6 to give a final concentration of $10^{10}$ pfu/ml. At 30', a sample from the mating mixture was added to an ED2196 culture to measure retransfer of the plasmid by the JC3272 (F'^{lac}) progeny. Flask matings were used for this experiment.

<table>
<thead>
<tr>
<th></th>
<th>JC3272 recipient</th>
<th>ED2196 recipient</th>
<th>JC3272 (F'^{lac}) progeny</th>
<th>ED2196 (F'^{lac}) progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC3272 recipient</td>
<td>Δ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED2196 recipient</td>
<td></td>
<td>ν</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JC3272 (F'^{lac}) progeny</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>ED2196 (F'^{lac}) progeny</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 4.4
CHAPTER 5

PLASMID SPREAD BY RE-TRANSFER

Effect of growth phase on F'lac donor ability

We wanted to study plasmid spread by re-transfer under conditions of slow growth in late exponential phase. Stationary phase F'lac cells are poor donors and we wanted to measure how fast donor ability was lost on entry to stationary phase. We therefore followed a donor (JC6582) culture from exponential phase into stationary phase. We tested mating ability by mating samples for 30' with an excess of exponentially growing ED2196 recipient cells. Fig. 5.1 shows that the donor mating ability declined rapidly when the donor entered stationary phase at about 400'; it had declined about 40-fold by 600'. Thus, transfer of F'lac ceases when the donor numbers stop increasing.

We also measured the aggregate-forming ability of the donor cells using a Coulter counter. Fig. 5.1 (*) shows that the proportion of cells in aggregates after 30' 1:1 matings with ED2196 declined much more slowly than mating ability on entry to stationary phase; in fact there was no more than a three-fold drop in aggregate forming ability between 0' and 600' (Fig. 5.1). The formation of aggregates presumably indicates the existence of sex pili on the donor cells. Curtiss et al. (1969) found that the kinetics of mating ability loss by Hfr cells during starvation were much more rapid than the kinetics of pili loss.

Demonstration of plasmid spread by re-transfer

A higher rate of increase in progeny number than in recipient number in a mating mixture could be due to three causes:
Figure 5.1  Effect of growth phase on the progeny-forming ability and the aggregate-forming ability of an $F'^{lac}$ donor strain. The growth of a flask culture of JC6582 was followed from exponential phase into stationary phase. At intervals, samples were taken and mated in tubes for 30' with an excess of an exponentially growing culture of ED2196. The number of $\text{Nal}^R\text{Lac}^+$ progeny produced per donor was measured. Samples were also used to measure aggregate-forming ability with a Coulter counter. The values shown are not corrected for aggregates in the parent cultures. The proportion of cells in aggregates in the recipient culture remained at about 7.5% throughout the experiment, while the proportion in the donor culture rose from 7.5% to 17%.

<table>
<thead>
<tr>
<th>Donor culture viable count (cells/ml)</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of progeny per donor in matings</td>
<td>0</td>
</tr>
<tr>
<td>Proportion of cells in aggregates in matings</td>
<td>*</td>
</tr>
</tbody>
</table>
Figure 5.1
(i) A difference in progeny and recipient growth rates, i.e. the plasmid affects the host growth rate.

(ii) Re-transfer of the plasmid by recipients.

(iii) Continuing plasmid transfer by the original donors.

In order to separate these three effects we compared an F'lac tra+ with an amber transfer-deficient mutant F'lac traH88. The donor strains were Su+ so transfer by the original donors occurred in both cases. However, as the recipient strain was Su-, re-transfer by the recipients could only occur for the tra+ plasmid and not the traH88 plasmid (Table 5.1). The donor strains were T6S and the recipient strain was T6R so that addition of phage T6, after allowing time for some initial plasmid transfer, prevented continuing transfer by the original donor strain (Table 5.1).

We tested the effect of the plasmids on the recipient growth rate by following the growth of parallel cultures of JC3272 carrying either plasmid or plasmid-free from exponential phase to stationary phase. There was no detectable effect of the plasmids on growth rates. This is in agreement with the observation of Willetts and Finnegan (1970) that carrying F'lac did not affect the generation time of exponentially growing JC3272 cells in L-broth.

We compared the transfer abilities of the two donor strains JC6582 (carried F'lac tra+) and M172 (carried F'lac traH88) by mating a mixture of JC6582 and M172 with the Su− recipient strain JC3272. The two types of progeny can be distinguished as the tra+ progeny were sensitive to the male-specific phage MS2 whereas the traH88 progeny were resistant. We performed 30' and 60' matings in flasks with an excess of recipients. These experiments showed that M172 is at least 80% as efficient as JC6582 in producing F'lac progeny; i.e. suppression is efficient in agreement with Achtman et al. (1971).
Table 5.1. Types of transfer possible in mating mixtures

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Phage</th>
<th>Possibility of continuing transfer by original donors?</th>
<th>Possibility of re-transfer by recipients?</th>
</tr>
</thead>
<tbody>
<tr>
<td>F(^\prime)lac(^+) traH88</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>F(^\prime)lac(^+) traH88</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>F(^\prime)lac(^+) tra(^+)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>F(^\prime)lac(^+) tra(^+)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Fig. 5.2 shows the results of a 1200' mating of M172 with JC3272. Phage T6 was added to half the mating mixture at 60' to kill the donors. After T6 addition progeny numbers should increase by growth alone as no transfer is possible. The rate of increase of progeny and recipient numbers should be equal. This is not apparent from Fig. 5.2 because of sampling errors; however, a similar experiment (Fig. 5.3) with a more suitable choice of dilutions showed that the progeny:recipient ratio remained constant from 30' after addition of phage T6 until the culture entered stationary phase.

When continuing transfer by the original donor was allowed (Fig. 5.2 and 5.3, -T6), the progeny numbers remained comparable with the donor numbers and rose to about twice the donor numbers on entry to stationary phase. Thus, in the absence of re-transfer or any effect of the plasmid on the host's growth rate, the plasmid can only spread to a significant proportion of the recipient population if the donor:recipient ratio has been comparable to that proportion.

When re-transfer by recipients can occur (i.e. the tra\(^\text{+}\) plasmid is used) there should be some temporal separation of the two types of transfer; in the early times of a mating mixture re-transfer will be negligible (see previous chapter), but, later on, as re-transfer becomes important, the relative contribution of continuing transfer by the original donors should become negligible. This is because the ratio of progeny:original donor strain will become large. In fact, in our experiments using the tra\(^\text{+}\) plasmid (Fig. 5.4) transfer by the original donors became negligible after about 300' because the donor, but not the recipients and progeny, had entered stationary phase (Fig. 5.4, -T6). This is supported by the fact that the rate of increase in progeny numbers in the half of the culture to which T6 is added is very similar to that without T6. However, there was about
Figures 5.2 and 5.3  Matings of M172 (carries F'lac traH88) with JC3272. Exponentially growing cultures of the two strains were diluted in warm L-broth and mixed in flasks. After 60' (Fig. 5.2) or 30' (Fig. 5.3) the mating mixtures were split into halves and phage T6 was added to one half to give a final titre of about $10^{10}$ pfu/ml. This reduced the M172 viable count to less than 10$^2$ cells/ml for the duration of the experiments. The progeny were selected as Str$^R$ Lac$^+$ cells.

<table>
<thead>
<tr>
<th></th>
<th>-T6</th>
<th>+T6</th>
</tr>
</thead>
<tbody>
<tr>
<td>M172 donors</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>JC3272 recipients</td>
<td>Δ</td>
<td>▽</td>
</tr>
<tr>
<td>JC3272 (F'lac) progeny</td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

Figure 5.4  Mating of JC6582 (carries F'lac tra$^+$) with JC3272.

The same procedure was used as for the matings of Figure 5.2. The phage T6 reduced the JC6582 viable count to less than 10$^2$ cells/ml for the rest of the experiment.

<table>
<thead>
<tr>
<th></th>
<th>-T6</th>
<th>+T6</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC6582 donors</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>JC3272 recipients</td>
<td>Δ</td>
<td>▽</td>
</tr>
<tr>
<td>JC3272 (F'lac) progeny</td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>
Figure 5.2
Figure 5.3
Figure 5.4
a ten-fold lower final yield of progeny in the former culture because when the phage was added (at 60') transfer by the original donors was still important. The importance of the length of time during which re-transfer can occur is also illustrated by Fig. 5.4. If the exponential increase in progeny numbers observed in the interval from 300' to 900' had continued until 1200', the final yield of progeny would have been nearly ten times higher.

Quantitative estimate of re-transfer

We performed a 1200' mating of JC6582 with JC3272. At intervals we mated samples of the mating mixture with an exponentially growing culture of a second recipient strain to measure total donor ability in the mixture. Samples were also mated with the second recipient in the presence of phage T6 which prevented transfer by the original donor strain so that re-transfer by recipients could be measured. The viable counts of the donor, recipient and progeny are shown in Fig. 5.5. The donor strain entered stationary phase at about 300', but the progeny numbers increased at a rate greater than the recipient growth rate until the recipient entered stationary phase at about 700'. This indicated that most of the transfer in this period was re-transfer by recipients. The similarity of the amount of transfer to the second recipient in the presence and absence of phage T6 after 300' confirmed that transfer by the original donors was negligible (Fig. 5.6). Fig. 5.6 also shows that re-transfer by recipients declined rapidly as recipients entered stationary phase at about 700'.

We wished to distinguish between effects on plasmid spread due to the efficiency of re-transfer of progeny cells in which F'lac was established and the effect of the lag between receiving the plasmid and being able to re-transfer it (Fig. 4.4). We constructed a simple mathematical model to help separate these effects. We assumed that
Figure 5.5  Mating of JC6582 with JC3272. The same procedure was used as for Figure 5.2 except that no phage T6 was added.

JC6582 donors
JC3272 recipients
JC3272 (F' lac) progeny

Figure 5.6  Diluted samples from the mating mixture of Figure 5.5 were mated in tubes for 30' with the recipient strain ED2196. Matings were performed in the presence of about $10^{10}$ pfu/ml of phage T6 (X) and in the absence of phage T6 (O). The mating ability plotted is the ratio of the numbers of ED2196 (F' lac) progeny to JC3272 (F' lac) donors.
Figure 5.6
all recipients had a lag $\delta$ between receiving F'lac and becoming competent donors; in practice (Fig. 4.4) there appears to be some variation in the lag between individual recipients. We assumed that plasmid transfer occurred at a rate proportional to the number of competent donors; i.e. that the transfer frequency is independent of recipient concentration. Fig. 3.1 suggests that this assumption is reasonable when recipients are in excess and the recipient concentration is above $5 \times 10^7$ cells/ml. We assumed that any contribution to transfer by the original donor strain was negligible and that the progeny grew exponentially at a constant exponential growth rate ($k$). If the number of progeny at time $t$ is $x(t)$ then the number of competent donors (i.e. progeny that received F'lac before time $t - \delta$) is $x(t-\delta) \exp(k\delta)$. The rate of plasmid transfer is then $\alpha x(t-\delta) \exp(k\delta)$ where $\alpha$ is an effective mating rate; $\alpha$ takes into account the reduced apparent growth rate of new progeny (Fig. 4.3). Any variations due to "rounds of mating" (Fig. 3.2) would quickly be smoothed out by variations between individual cells.

The rate of increase of progeny numbers is the sum of a growth term and a re-transfer term:

$$\frac{dx(t)}{dt} = k x(t) + \alpha x(t-\delta) \exp(k\delta)$$

(5.1)

We show in the Appendix that, whatever the initial conditions, the solutions of equation (5.1) rapidly settle down to the form:

$$x(t) = C \exp(k+\lambda)t$$

(5.2)

where $\lambda$ is the solution of:

$$\lambda \exp(\lambda \delta) = \alpha$$

(5.3)

The larger $\lambda$ is the faster the plasmid spreads. As expected, (5.3) shows that increases in $\alpha$ (i.e. a higher mating frequency) raise the rate of spread, but increases in the lag $\delta$ between receiving the plasmid and being able to re-transfer it lower the rate of spread.
We wanted to interpret the mating rate $\alpha$ in terms of how often competent donors could transfer the plasmid. Consider a mating started with $x_0$ competent donors in which donors and progeny grew at the same exponential growth rate $k$. If re-transfer by recipients is ignored, the number of progeny ($y$) will satisfy:

$$ \frac{dy}{dt} = ky + \alpha x_0 e^{kt} \quad (5.4) $$

This equation has the solution:

$$ y = \alpha x_0 t e^{kt} \quad (5.5) $$

Thus, when $t = 1/\alpha$, the number of progeny will equal the number of donors. This "mating time" $1/\alpha$ can be thought of intuitively as the "time needed for each donor to mate once".

For F'lac, the progeny and recipient growth rates were equal so the progeny growth rate $k$ can be estimated from the recipient growth rate. The rate of plasmid spread ($\lambda$) can then be estimated by subtracting the recipient growth rate from the progeny increase rate. We measured the lag ($\delta$) as 90' (Fig. 4.4); this lag cannot be much larger than this in the growth conditions of Figs. 5.4 and 5.5 or re-transfer by recipients would not have been observed during the time span of the experiments. If $\delta$ and $\lambda$ are known, the mating rate $\alpha$ can be calculated using equation (5.3) (Table 5.2). This shows that the mating times ($1/\alpha$) are comparable to the generation times under these slow growth conditions (both about 300'). It is interesting to note that the mating times and generation times for faster growing cells (Chapter 3) are both about 30'.

Table 5.3 shows the theoretical effect of changes in the lag time ($\delta$) on the rate of progeny spread by re-transfer for mating time and generation time of 300'. It can be seen that the spread rate ($\lambda$) is not very sensitive to changes in the lag ($\delta$). In
Table 5.2 Calculation of Mating Rates

<table>
<thead>
<tr>
<th></th>
<th>Figure 5.4 Calculation based on progeny and recipient numbers in time from 400' to 700'</th>
<th>Figure 5.5 Calculation based on progeny and recipient numbers in time from 200' to 900'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient growth rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>constant ( k ) (x 10^3) (minute (^{-1}))</td>
<td>2.04</td>
<td>2.30</td>
</tr>
<tr>
<td>Rate constant of progeny numbers due to re-transfer ( \lambda ) (x 10^3) minute (^{-1}))</td>
<td>3.23</td>
<td>2.30</td>
</tr>
<tr>
<td>Mating rate ( \alpha ) assuming lag ( \delta ) of 90'. (x 10^3) (minute (^{-1}))</td>
<td>4.31</td>
<td>2.83</td>
</tr>
<tr>
<td>Generation time ( \log_2 (k) ) (minutes)</td>
<td>340</td>
<td>301</td>
</tr>
<tr>
<td>Mating time ( 1/\alpha ) (minutes)</td>
<td>232</td>
<td>353</td>
</tr>
<tr>
<td>Lag (δ) (min)</td>
<td>Rate of plasmid spread (λ) (min⁻¹) (x10³)</td>
<td>Doubling time for progeny numbers (min)</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>0</td>
<td>3.33</td>
<td>123</td>
</tr>
<tr>
<td>50</td>
<td>2.89</td>
<td>133</td>
</tr>
<tr>
<td>100</td>
<td>2.58</td>
<td>142</td>
</tr>
<tr>
<td>150</td>
<td>2.34</td>
<td>149</td>
</tr>
<tr>
<td>200</td>
<td>2.16</td>
<td>155</td>
</tr>
</tbody>
</table>

These calculations used values of 300' for both the mating time (1/α) and the generation time. λ was calculated from equation (5.3) using a Newton-Raphson iterative procedure.
particular, if there were no lag (i.e. $\delta = 0$) rather than one of about 100', the rate of spread ($\lambda$) would only be raised by about one third and the doubling time for progeny numbers would only be reduced by 13%. Thus, the main factor limiting plasmid spread is the inherent efficiency of plasmid transfer by competent donors and not the lag between a recipient receiving $F'$lac and being able to re-transfer it.

The mating ability of samples from a mating mixture (Fig. 5.6) should also depend on $\lambda$. If we assume that the transfer of cells to a faster growing recipient culture for 30' of mating does not affect the mating rate ($\alpha$), the lag ($\delta$) or the growth rate ($k$) then it is possible to construct a differential equation for the number of progeny with the second recipient. Substitution of values of $k$ and $\lambda$ from the results shown in Fig. 5.5 (Table 5.2) into the solution of the equation predicted a value of 0.14 second-recipient-progeny per first-recipient progeny (i.e. donors) in the mating mixture for the period 400'-700'. The observed values in this interval (Fig. 5.6, +T5) had an average of 0.23. Transfer to the better growth conditions for mating might explain why this value is 50% higher than the predicted value. However, despite this, the method of assessing mating ability by mating samples with the second recipient seems reasonably quantitative.
CHAPTER 6

INCOMPATIBILITY

Rate of segregation with random pool replication models

We considered a pair of incompatible plasmids and made the following assumptions:

(i) The population size is large enough for random fluctuations to be negligible.

(ii) Replication and segregation are independent.

(iii) The system for the control of plasmid copy number is such that all plasmid-carrying cells contain the same number of plasmid copies at division. We define this number as $2N$, so that $N$ is the average copy number at birth.

(iv) Two incompatible plasmids are indistinguishable to the replication and segregation mechanisms.

(v) Replication followed a random pool model.

(vi) Segregation followed an equal number model i.e. each daughter cell received $N$ plasmid copies on division.

All models that we considered contain assumptions (i)-(iii). Later in this section we examine the effect of changing assumptions (vi) and later in the chapter we study the effects of changing assumptions (iv) and (v).

The initial rate of segregation would depend on the distribution of the two plasmids among the cells of the population. However, after an initial period, the distribution of the plasmids between cells carrying both plasmids would settle down to a limiting distribution with a steady state rate of segregation of cells carrying only one plasmid type. We calculated (see Appendix) that the steady state rate of segregation was such that the proportion of cells carrying both
plasmids fell by a factor of \((N-1)(2N+1)/(N+1)(2N-1)\) each generation. Therefore, the half-time required for the proportion of cells carrying both plasmids to fall by a half is:

\[
t_\frac{1}{2} = \frac{-\log_e 2}{\log_e \left(\frac{(N-1)(2N+1)}{(2N-1)(N+1)}\right)}
\]

(6.1)

For large \(N\), \(t_\frac{1}{2} \approx N \log_e 2\) and \(t_\frac{1}{2} \approx 0.692N\) for \(N\) in the range 4-40.

This steady state rate of segregation should be observed experimentally in the situation where selection is relaxed after both plasmids have been maintained in a strain by selection. The other case where the rate of segregation can be measured is after one plasmid is introduced into cells containing the other plasmid by conjugation, transduction or transformation. Here the incoming plasmid would usually be at a numerical disadvantage compared to the resident plasmid.

We modelled this situation by considering a population of cells starting with 1 copy of the incoming plasmid and \(N-1\) copies of the resident plasmid. It was possible to calculate the rate of segregation for this case using a computer (see Appendix). Fig. 6.1 shows that for \(N=2\) there is a constant exponential rate of plasmid loss; this is identical to the steady state rate and the two plasmids behave symmetrically as the cells start with one copy of each plasmid. For higher copy numbers there is a rapid initial rate of loss and this rate drops to the steady state rate after 10-20 generations (Fig. 6.1). The initial rate of loss are very similar for copy numbers greater than \(N=10\), and the curves for \(N\) in the range 20-40 are too close over 20 generations to be distinguishable in Fig. 6.1.

We also considered a random segregation model (i.e. changed assumption (vi)) where each of the \(2N\) plasmid copies in a dividing cell have an equal opportunity of entering either daughter cell.
Figure 6.1  Theoretical curves for the rate of segregation due to incompatibility when one plasmid enters a cell carrying another incompatible plasmid under a random pool replication, equal number segregation model. We assumed that at generation 0 all cells at birth had 1 copy of the entering plasmid and N-1 copies of the resident plasmid. We used a computer to calculate the subsequent segregation using the transition probabilities of equation (A4.3). The curves for copy number at birth (N) in the range 21-40 are too close to that for N=20 to be shown in the figure.

Figure 6.2  Theoretical curves for the rate of segregation due to incompatibility when one plasmid enters a cell carrying another incompatible plasmid under a democratic replication, equal number segregation model. We calculated the curves as in Figure 6.1, except that we used the transition probabilities in equation (A4.9). The curve for N=15 is too close to that for N=20 to be shown in the figure.
Figure 6.1
Figure 6.2
This model produces a proportion \(1/2^{2N}\) plasmid free cells per generation; it would therefore be too unstable to apply to lower copy number plasmids. We calculated the steady state segregation rate using a computer (see Appendix) and found that the half-time was approximately \(0.692N-0.67\) in the range for \(N\) of 5-20. This would, in practice, be indistinguishable from the rate predicted by the equal number segregation model. The rate of segregation for the case of one plasmid entering a cell containing the other would also be indistinguishable from that predicted by the equal number model.

So far we have only considered the relationship of segregation rates to the copy number at birth \((N)\). In order to compare the predictions with experimental data it was necessary to relate this to the average copy number in an exponentially growing population \((\bar{N})\). If plasmid replication were spread uniformly through the cell cycle then the problem is analogous to that of relating cell length at birth \((L)\) to average length in an exponentially growing population \((\bar{L})\) when the cell length growth rate is constant; which is given as \(L=\bar{L}\log_2\) (Donachie et al., 1976). We therefore used the equation \(\bar{N}=1.44N\) for plasmid copy numbers; this will be reasonable provided replication is spread through the cell cycle, whatever the exact dependence. Even if plasmid replication occurs at a fixed time in the cell cycle this equation may still be reasonable provided this time is not too close to either cell birth or cell division.

**Experiments with colEl derivatives**

We measured the rate of segregation due to incompatibility using a pair of colEl derivatives, pML2 (\(\sim\text{Kan}^R\)) and pDS1107 (\(\sim\text{Amp}^R\)). These could be mobilised by an \(\text{F}^{\text{lac traI}}\) plasmid that was unable to transfer itself (Alfaro and Willetts, 1972). We were thus able to study incompatibility after conjugation in the absence of re-transfer
by the recipients as the colEl derivatives cannot transfer themselves. Continuing transfer by the original donors could be eliminated by killing them with phage T6.

Fig. 6.3 shows how the proportion of cells carrying both plasmids declines after one is introduced into cells carrying the other by conjugation. There is an initial rapid rate of loss followed by a lower rate of loss. The experimental data are in excellent agreement with a theoretical curve based on the assumption that one copy of the incoming plasmid enters cells containing the other (Fig. 6.3). The theoretical curve given is for N=20, but the curves for N in the range 20-40 are indistinguishable (see legend to Fig. 6.1).

We also measured the steady state segregation rate. We selected cells carrying both plasmids by growth on medium containing both kanamycin and ampicillin. We then followed the progress of segregation after selection was removed; Fig. 6.3 shows such an experiment. We found that even after growth in selective medium 20-70% of cells did not carry both plasmids as judged by the lower viable count on kanamycin ampicillin agar (KA) than on non-selective agar (N). The majority of these segregants carried only pML2 as the viable counts were indistinguishable between kanamycin agar (K) and N and between ampicillin agar (A) and KA. However, after growth in non-selective medium the viable counts on K became less than those on N and those on KA became less than those on A which indicated that segregation of cells carrying only pDS1107 had occurred. Thus, it seemed that the large initial asymmetry between the two types of segregants was due to the properties of the antibiotic resistances carried rather than properties of the segregation mechanism.

As segregation was very slow (Fig. 6.3) any differential effects of the two plasmids on the host growth rate could be important.
Figure 6.3  Rate of segregation due to incompatibility between the two colE1 derivatives pML2 and pDS1107.

(i) ED2510 (transfers pML2) was mated with ED2517 (carries pDS1107). After 40' mating, phage T6 was added to give a final concentration of about $10^{10}$ pfu/ml. This reduced the number of ED2510 donors to less than 1% of the number of ED2517 (pML2) progeny for the rest of the experiment. The culture was maintained between $2 \times 10^7$ and $4 \times 10^8$ cells/ml by serial dilution and, at intervals, samples were plated onto L-broth agar selective for StrR KanR AmpR progeny and StrR recipients. The ratios of the viable counts on these two media is shown (0). The theoretical curve corresponds to that for N=20 in Figure 6.1, i.e. it assumes a random pool replication, equal number segregation model.

(ii) GW137 (transfers pDS1107) was mated with ED2516 (carries pML2). The same procedure was used as in (i). The proportion of cells carrying both plasmids is shown ($x10^2$) ($\Delta$).

(iii) Steady state segregation rate. A StrR KanR AmpR progeny colony was selected after a mating of ED2510 with ED2517. It was streaked to single colonies on the same medium. One of these colonies was inoculated into L-broth containing kanamycin and ampicillin. When the culture was growing exponentially at about $2 \times 10^8$ cells/ml, it was spun down using a bench centrifuge and resuspended in antibiotic-free L-broth. Exponential growth was maintained by serial dilution as in (i). The proportion of cells carrying both plasmids ($\times$) was estimated as in (i) and samples were also plated on StrKan and StrAmp agar. The line drawn is a least squares fit to the points.

All the growth in these experiments was in flasks with shaking. The generation time was about 22'.

Both ED2516 (carried pML2) and ED2517 (carried pDS1107) had a generation time of about 22' in our growth conditions. We measured the differential growth rate of the two strains in a mixed culture; samples were plated on non-selective agar to find the proportion of each strain in the mixture at different times. The generation time of ED2516 was about 5% less than that of ED2517. A difference of this magnitude may have an appreciable effect on the estimate of the half-time for segregation. We calculated the half-time under three different assumptions about differential growth:

(i) We assumed all cells had the same generation time.

(ii) We assumed that cells carrying both plasmids had the same growth rate as ED2517.

(iii) We assumed that cells carrying both plasmids had the same growth rate as ED2516.

The half-time and the corresponding copy number estimates using our model are shown in Table 6.1. The higher estimates (assumption (ii)) are comparable to the value of 18 covalently closed circular DNA molecules per genome equivalent found by Cabello et al. (1976) if it is assumed that our cells contained about 4 genome equivalents of DNA (Cooper and Helmstetter, 1968).

The rates of segregation for cells carrying each type of plasmid can also be predicted under the assumptions above. Table 6.2 shows that the predictions are consistent with the experimental data. However, as the experimental estimates involve measuring the differences between viable counts on different selective agar, the data are not good enough to distinguish the different assumptions about the growth of cells carrying both plasmids.

Other replication models

We also calculated rates of segregation under a democratic
Table 6.1 Effect of differential growth rates on copy number estimates for CoIE1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Experiment</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

- **Proportion of cells carrying both plasmids at time 0':**
  - Experiment 1: 0.71
  - Experiment 2: 0.76
  - Experiment 3: 0.37

- **Proportion of cells carrying only pML2 at time 0':**
  - Experiment 1: 0.29
  - Experiment 2: 0.24
  - Experiment 3: 0.63

(i) Calculation on assumption of no differential growth rates:
- **Half-time ($t_{1/2}$) (generations):**
  - Experiment 1: 17.1
  - Experiment 2: 18.8
  - Experiment 3: 18.1

- **Copy number at birth ($N$):**
  - Experiment 1: 24.8
  - Experiment 2: 27.1
  - Experiment 3: 26.2

- **Average copy number ($\bar{N}$):**
  - Experiment 1: 35.7
  - Experiment 2: 39.2
  - Experiment 3: 37.7

(ii) Calculation on assumption that cells carrying both plasmids have same growth rate as ED2517:
- **Half-time ($t_{1/2}$) (generations):**
  - Experiment 1: 24.9
  - Experiment 2: 42.4
  - Experiment 3: 47.5

- **Copy number at birth ($N$):**
  - Experiment 1: 36.0
  - Experiment 2: 61.2
  - Experiment 3: 68.6

- **Average copy number ($\bar{N}$):**
  - Experiment 1: 51.9
  - Experiment 2: 88.3
  - Experiment 3: 98.9

(iii) Calculations on assumption that cells carrying both plasmids have same growth rate as ED2516:
- **Half-time ($t_{1/2}$) (generations):**
  - Experiment 1: 14.9
  - Experiment 2: 19.7
  - Experiment 3: 16.7

- **Copy number at birth ($N$):**
  - Experiment 1: 21.5
  - Experiment 2: 28.5
  - Experiment 3: 24.2

- **Average copy number ($\bar{N}$):**
  - Experiment 1: 31.0
  - Experiment 2: 41.1
  - Experiment 3: 34.9

A value of $t_{1/2}$ corrected for growth rate was used to estimate copy number at birth under the random pool replication, equal number segregation model. The average copy number in an exponentially growing population was estimated from the copy number at birth as described in the text. We assumed that cells carrying both plasmids had a generation time of 22.1' and that ED2516 grew 5% faster than ED2516.
Table 6.2 Asymmetric yields of segregants with various assumptions about growth rates.

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>400'</td>
<td>300'</td>
<td>400'</td>
</tr>
</tbody>
</table>

**pDS1107 segregants**

- **Observed** proportion of cells carrying pDS1107 alone:
  - Experiment 1: 0.12
  - Experiment 2: 0.18
  - Experiment 3: 0.09

- **Predicted** proportion of cells carrying pDS1107 alone if:
  - No correction for differential growth:
    - Experiment 1: 0.18
    - Experiment 2: 0.15
    - Experiment 3: 0.09

- Cells carrying both plasmids grow at same rate as:
  - (b) ED2517: 0.11, 0.06, 0.03
  - (c) ED2516: 0.16, 0.10, 0.07

**pML2 segregants**

- **Observed** proportion of cells carrying pML2 alone:
  - Experiment 1: 0.53
  - Experiment 2: 0.38
  - Experiment 3: 0.74

- **Predicted** proportion of cells carrying pML2 alone if:
  - No correction for differential growth:
    - Experiment 1: 0.47
    - Experiment 2: 0.39
    - Experiment 3: 0.72

- Cells carrying both plasmids grow at same rate as:
  - (b) ED2517: 0.54, 0.50, 0.79
  - (c) ED2516: 0.50, 0.46, 0.74

---

*a* Mean of estimates for three time points around the given time.

*b* We used the estimates of segregation rates ( mamma) from Table 6.1 and assumed a generation time of 22.1' for cells carrying both plasmids and, for (b) and (c), that ED2516 grew 5% faster than ED2517.
replication model. We used an equal number segregation model as a random segregation model leads to rapid plasmid loss (Table 6.3); this is because there is no compensating mechanism to restore copy number after unequal divisions. We used a computer (see Appendix) to calculate the steady state rate of segregation. We found that the half time was approximately:

$$t_{\frac{1}{2}} = 1.37 \, N - 0.96 \quad (N \text{ in the range 2-20}) \quad (6.2)$$

This segregation rate is about half that predicted by random pool models with the same copy number at birth. Fig. 6.2 shows that the rate of segregation when one plasmid enters cells containing the other plasmid is also less than in the random pool case (Fig. 6.1).

So far we have only considered models where the two plasmids are indistinguishable to the replication and segregation mechanisms. However, one plasmid might have an advantage over the other in selection for replication; e.g. there might be differences in the sites on the two plasmids that are recognised by replication-control proteins. We modelled such a case using a random pool replication model in which the two plasmids had an unequal chance of replication. This asymmetry caused an asymmetry in the number of segregants of each plasmid type. We calculated the proportion of final segregants of each type when the cells started with an equal number of copies of each type (see Appendix). Table 6.4 shows that there is appreciable asymmetry of segregation if the replication probabilities of the two plasmids are in the ratio 1.1:1; larger differences result in nearly all segregants carrying only the more successful plasmid.
Table 6.3  Plasmid loss with a democratic replication, random segregation model.

<table>
<thead>
<tr>
<th>Number of generations</th>
<th>% of plasmid-less cells if copy number at birth of starting populations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 5</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>7.82</td>
</tr>
<tr>
<td>10</td>
<td>22.4</td>
</tr>
<tr>
<td>15</td>
<td>34.3</td>
</tr>
<tr>
<td>20</td>
<td>43.4</td>
</tr>
<tr>
<td>30</td>
<td>55.8</td>
</tr>
<tr>
<td>40</td>
<td>63.8</td>
</tr>
</tbody>
</table>

These calculations use equation (1) of Dowman (1973) with $\phi_2 = 1$. The proportion of plasmid-free cells after $t$ generations ($F_t$) satisfies:

$$F_t^{1/N} = ((1 + F_t^{1/N})/2)^2.$$

N.B. Dowman (1973) equation (2) should be $\gamma_0 = 0$ not $\gamma_0 = 1$. 
Table 6.4  Effect of biased replication on asymmetric segregation

<table>
<thead>
<tr>
<th>Copy number at birth (N)</th>
<th>Ratio of probabilities of replication for the two plasmids</th>
<th>Percentage of segregants carrying only:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(i) More successful (ii) Less successfull</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plasmid plasmid</td>
</tr>
<tr>
<td>10</td>
<td>1.01:1</td>
<td>52</td>
</tr>
<tr>
<td>10</td>
<td>1.1:1</td>
<td>66</td>
</tr>
<tr>
<td>10</td>
<td>1.5:1</td>
<td>94</td>
</tr>
<tr>
<td>10</td>
<td>2:1</td>
<td>99</td>
</tr>
<tr>
<td>15</td>
<td>1.01:1</td>
<td>53</td>
</tr>
<tr>
<td>15</td>
<td>1.1:1</td>
<td>72</td>
</tr>
</tbody>
</table>
CHAPTER 7

HFR FORMATION AND CHROMOSOME TRANSFER

Rate of Hfr formation in a rec\(^+\) strain

A fluctuation test using tube matings of the rec\(^+\) strain ED879 gave a large variance between cultures (Table 7.1, lines 1-2) and yielded three highly fertile cultures, with 1430, 128 and 78 progeny compared to an average of 36.4 for the other 47 cultures. We isolated Hfr strains from each of these three cultures by sib selection. All three Hfr strains showed a gradient of transfer in matings with \( \times 478 \) with an early marker leu and a late marker proc. We estimated the rate of Hfr formation as \( 3.4 \times 10^{-6} / \text{cell/generation} \) (see Appendix). This is comparable to the value of \( 3 \times 10^{-6} / \text{cell/generation} \) found by Curtiss and Stallions (1969).

recA-dependence of Hfr formation

We wanted firstly to isolate Hfr strains from a recA F\(^+\) strain (ED877) and secondly to compare the rate of Hfr formation in this strain with that of an isogenic rec\(^+\) strain (ED879). Fluctuation tests for fertility of ED877 with 50 and 100 cultures using tube matings did not produce any highly fertile "jackpot" cultures (Table 7.1, lines 3-5). We therefore used a streak mating technique so that we could screen more cultures. We tested a further 449 cultures and found one highly fertile culture that yielded an Hfr strain. A second Hfr strain was isolated from a culture of slightly raised fertility using the sib selection method. Both Hfr strains were extremely sensitive to ultra-violet light. Matings with \( \times 478 \), a multiply auxotrophic F\(^-\) strain, showed a gradient of marker transfer metE, leu, trp; the high efficiency of metE transfer suggested that the origin of transfer was close to metE. Leu\(^+\) progeny were F\(^-\). Thus,
<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Number of cultures&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Donor viable count (x 10&lt;sup&gt;-8&lt;/sup&gt;) /ml</th>
<th>Mean number of progeny (x)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Variance in number of progeny (s&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>s&lt;sup&gt;2&lt;/sup&gt;/x</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ED879</td>
<td>50</td>
<td>4.1</td>
<td>67.0</td>
<td>38900</td>
<td>582</td>
</tr>
<tr>
<td>2. ED879 bulk culture</td>
<td>50</td>
<td>3.9</td>
<td>26.2</td>
<td>52.6</td>
<td>2.01</td>
</tr>
<tr>
<td>3. ED877</td>
<td>50</td>
<td>0.2</td>
<td>0.74</td>
<td>0.75</td>
<td>1.02</td>
</tr>
<tr>
<td>4. ED877 bulk culture</td>
<td>50</td>
<td>0.7</td>
<td>2.3</td>
<td>2.8</td>
<td>1.18</td>
</tr>
<tr>
<td>5. ED877</td>
<td>100</td>
<td>0.4</td>
<td>2.54</td>
<td>3.46</td>
<td>1.36</td>
</tr>
<tr>
<td>6. ED969</td>
<td>50</td>
<td>0.4</td>
<td>0.74</td>
<td>0.66</td>
<td>0.89</td>
</tr>
<tr>
<td>7. ED969</td>
<td>50</td>
<td>0.8</td>
<td>3.3</td>
<td>2.88</td>
<td>0.87</td>
</tr>
<tr>
<td>8. ED957</td>
<td>50</td>
<td>4.4</td>
<td>20.4</td>
<td>57.5</td>
<td>2.81</td>
</tr>
<tr>
<td>9. ED957 bulk culture</td>
<td>50</td>
<td>4.6</td>
<td>19.7</td>
<td>22.5</td>
<td>1.14</td>
</tr>
<tr>
<td>10. ED957</td>
<td>100</td>
<td>3.8</td>
<td>14.7</td>
<td>840</td>
<td>57.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of samples in case of bulk culture controls. The pairs of experiments for lines 1 and 2, 3 and 4, 6 and 7, 8 and 9 were performed in parallel.

<sup>b</sup> Samples from rec<sup>+</sup> cultures were diluted 1:20 before mating.
the two strains appear to be Hfr strains formed by the integration of F into the chromosome in a recA strain.

In streak matings with the ED877 cultures there was an average of less than one colony per streak. There were 8/449 cultures which gave four or more colonies per streak. Subcultures from four of these cultures were used in the more quantitative tube matings and showed a raised fertility. Our experience with the Type II strain ED969 also suggested that cultures with four or more colonies had a raised fertility and did not just represent random variations. We assumed that the higher fertility in the 8/449 ED879 cultures was due to Hfr cells. We estimated the rate of Hfr formation in ED877 as $4.6 \times 10^{-8}$/cell/generation (see Appendix). Thus, the rate of Hfr formation is about one hundred-fold lower in the recA strain ED877 than in the isogenic rec+ strain ED879.

**recA-dependence of chromosome transfer in Type I and Type II strains**

We wanted to compare the frequencies of chromosomal transfer by rec+ and recA derivatives of Type II strains, which seemed to be deficient in Hfr formation (Curtiss and Renshaw, 1969a), and normal Type I strains. Table 7.2 shows that Type I and Type II strains give comparable levels of chromosome transfer in agreement with Curtiss & Renshaw, 1969. We found (Table 7.2) that our recA strains gave chromosomal transfer at a frequency of about 1% that of isogenic rec+ strains, which is comparable to the value of 3% obtained by Clowes and Moody (1966) and of 0.5% obtained by Moody and Hayes (1972).

We also measured the proportion of chromosomal recombinants that remained F− in matings with low donor:recipient ratios. After correction for multiple mating (see Methods), this proportion was about 30% for all four strains (about 20% before correction); Curtiss and Renshaw (1969b) also found that Type I and Type II rec+ cells
Table 7.2 Efficiency of transfer by donor strains

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>ED879</th>
<th>ED877</th>
<th>ED957</th>
<th>ED969</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor type</td>
<td>I</td>
<td>I</td>
<td>II</td>
<td>II</td>
</tr>
<tr>
<td>recA allele</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Recipient viable count at start of mating (x 10^-8/ml)</td>
<td>1.3</td>
<td>2.0</td>
<td>1.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Initial ratio of donors to recipients (x)</td>
<td>0.15</td>
<td>0.063</td>
<td>0.12</td>
<td>0.059</td>
</tr>
<tr>
<td>Final ratio of Str^R Thr^+ Ara^+ Leu^+ progeny to recipients (y) (x 10^9)</td>
<td>690</td>
<td>2.6</td>
<td>480</td>
<td>4.0</td>
</tr>
<tr>
<td>Efficiency of mating (y/x) (x 10^8)</td>
<td>460</td>
<td>4.2</td>
<td>400</td>
<td>6.7</td>
</tr>
<tr>
<td>Proportion of recipient cells which become F^+</td>
<td>0.18</td>
<td>0.18</td>
<td>0.14</td>
<td>0.12</td>
</tr>
</tbody>
</table>
gave similar proportions of $F^-$ recombinants. As Hfr cells produce predominantly $F^-$ progeny, strains defective in Hfr formation should produce fewer $F^-$ recombinants, to the extent of in fact about 15% less (Curtiss and Stallions, 1969). Because of this puzzling lack of difference between Type I and Type II strains we decided to perform fluctuation tests to see if there was any evidence of heritable high fertility states in Type II strains.

ED969 (a Type II $\text{recA}$ strain) gave no fertile cultures in fluctuation tests with 50 cultures (Table 7.1, lines 6-7). The $\text{rec}^+$ strain ED957 gave slightly higher variance between 50 cultures than in a bulk culture control (Table 7.1, lines 8-9); two cultures gave 38 and 53 progeny respectively compared to the mean of about 20 progeny. However, the variance among separate cultures would always be expected to be higher than that between samples from the same bulk culture because of variations in the viable count between cultures. We performed a second fluctuation test with 100 cultures of ED957 and found quite a high variance (Table 7.1, line 10) due mainly to cultures with 288 and 82 progeny compared to a mean number of about 12. We attempted to isolate Hfr clones from these cultures by sib selection (Broda, 1967). A suitable dilution of the cultures was dispensed into 20 tubes and after growth samples from each tube were mated with a recipient. If there were a small number of Hfr cells in a population, correct choice of dilution should allow about one tube in the 20 to receive an Hfr cell. This tube would be much more fertile than the others and, provided Hfr and $F^+$ cells grow at the same rate, the culture should be enriched 10-100 times for Hfr cells. However, in the case of the fertile ED957 cultures, although 1-2 tubes out of 20 were of raised fertility after sib selection, their fertility was only comparable with that of the culture from which the tubes were inoculated. This suggested that cells with clonally
inherited high fertility did exist, but that their apparent growth rate was less than that of the \(^{+}\) population. This could have been due to instability or to real effects on growth rate.

We used a streak mating method to screen a further 900 cultures of the Type II recA strain ED969. There was an average of less than one colony per streak. We subcultured the 19 cultures which produce four or more (maximum six) colonies and 7 of the cultures which produced three colonies and tested the fertility of these subcultures using the more quantitative tube matings. These cultures gave an average of about 30 progeny per tube (minimum 12) compared to about 5 progeny per tube for a normal ED969 culture. Thus, the cultures which produced higher numbers of colonies in streak matings did have a raised fertility. We attempted to isolate Hfr clones by sib selection from two of the more fertile cultures but we encountered the same difficulties as with the rec\(^{+}\) strain ED957. Thus, both rec\(^{+}\) and recA Type II strains seem to produce higher fertility clones and the difficulty in isolating such clones seems to be a secondary effect.

If it was assumed that the higher fertility cultures of the Type II strains ED957 and ED969 were due to Hfr clones, then analogous calculations to the Type I cases gave rates of Hfr formation about half those for the corresponding Type I strains. As this did not take into account the reduced apparent growth rate of the high fertility cells in the Type II strains, there is no reason for considering the rate of Hfr formation to be any lower in the Type II strains than in the type I strains. The results of the sib selection could be accounted for if Type II Hfr cells had a 50% longer generation time than the \(^{+}\) parent strains.
CHAPTER 8

DISCUSSION

Parental behaviour in F'lac matings

The first event in mating is the collision of donor and recipient cells to form aggregates. We have used the formation of progeny to assay the number of aggregates. Although this method has the disadvantage of being rather indirect, it is much more sensitive than physical methods, and allows a much larger range of concentrations to be used. We measured the number of progeny produced per minority parent over a range of majority parent concentrations; we assumed that collision rate was the limiting factor at lower concentrations. Comparable results were obtained for donors in the minority (Fig. 3.1) and recipients in the minority (Table 4.2). These results were incompatible with the model of bimolecular reaction kinetics, as the progeny yield changed much more slowly with parental concentration than predicted by this model. Collins and Broda (1975) found that part, but not all, of the deviation from the predictions of this model in Hfr matings was due to a decrease in motility at higher concentrations. It should be possible to test the contribution of motility effects in F'lac matings by repeating our experiments with non-motile strains. We were unable to obtain non-motile derivatives of the (motile) donor strain JC6582 as the strain was resistant to \( \lambda \) phage that is usually used to select non-motile mutants (Meynell, 1961).

The work discussed above showed that the best mating conditions for studies on the behaviour of a particular parent were conditions where this parent is in the minority and hence the limiting factor and the majority parent should be at a high enough concentration for collision not to be the limiting factor, say \( 10^8 \) cells/ml (Fig. 3.1). We used these conditions to investigate the mating competence of donors
and recipients. The question of mating competence falls into two related parts:

(i) The proportion of cells in the population competent to mate.

(ii) The number of cells with which a competent cell can mate i.e. multiplicity of mating.

In the case of donors the contributions of these two aspects were assessed by measuring the fertility of bulk donor cultures and then studying the variation between individual donors (Table 3.2).

Table 3.2 shows that an average of about one progeny per final donor was produced in 30' matings. The small variance in progeny yields in matings with an average of one donor per tube (Table 3.2) showed that there was not much variation in fertility between individual donors. More information would have been obtained about the variation between individual donors if it had been possible to recover both donors and progeny from each mating tube. This was not possible with F'lac matings because the selection for Lac^+ cells was not strong enough; however, it should be possible to perform such experiments using a different F'. Thus, the majority of F'lac cells are able to transfer F'lac in 30' and there is a multiplicity of transfer of about one. Broda (1975) found that the ratio of variance to mean of progeny yield for matings with the repressed R-factor R100 was about 10, which corresponded to each spontaneously derepressed cell giving rise to about 10 progeny; this is not very different from our results which had ratios of 2.61 and 4.47 (Table 3.2); Broda (1975) used 60' matings so there would be more opportunity for growth.

95% of recipients were able to receive F'lac in 30' matings at high donor:recipient ratios (Table 4.1). The 5% of Lac^- colonies could correspond to cells that had not mated or to F^- segregants from cells that had received F'lac (de Haan and Stouthamer, 1963; Table 4.4).
Our conclusions that nearly all donors (Table 3.2) and recipients (Table 4.4) were competent to mate is in apparent contrast to the conclusions of Walmsley (1973) that in Hfr matings only 30-50% of donors and 30% of recipients were competent to form mating pairs. Part of this difference may be due to the use of a correction based on the model of bimolecular reaction kinetics for mating which is invalid (Collins and Broda, 1975; see earlier). Another reason is the short mating time (7') that he used; our results (Fig. 4.2) suggested that many recipients are unable to form aggregates even after 20' in a mating mixture.

We found (Table 4.3) that F'\text{lac} donors encountered competition from F'\text{lac tral} strains that could form aggregates but not transfer F'\text{lac}. We estimated that an average of 2-3 donors mated with each recipient from the yields of progeny in matings with different ratios of two competing donor strains (Fig. 4.1). Thus, unlike donors (Table 3.2), most recipients are capable of multiple mating with several donors. Large mating aggregates (Achtman, 1975) might be associated with multiple mating by recipients, but the limitations on donor multiple mating would place severe restrictions on the genetic complexity of mating events in such large aggregates. Broda and Collins (1978) studied the genetic complexity of mating aggregates in Hfr matings by performing matings with two donor strains and the two recipient strains and looking for mixed colonies containing cells of more than one donor strain or more than one recipient strain. They concluded that mating aggregates with more than one donor and recipient strain were rare. They suggested that large mating aggregates might be formed from clones of cells that had not separated on division. Also, large aggregates may be unstable and can break down on dilution (Achtman, 1977). The lower progeny yield in matings with 1:1 donor:recipient ratio...
(Table 4.1) is probably due to some recipients mating with more than one donor and hence "using up" donors; this would be accentuated by the inability of some recipients to form aggregates until a considerable time has elapsed (Fig. 4.2). Thus, in 1:1 matings as used by Achtman (1975) it is likely that considerable multiple mating by recipients occurs, but little multiple mating by donors despite fairly symmetrical distribution of donors and recipients in mating aggregates.

We observed much lower progeny yields in 20' matings than 30' matings at low donor:recipient ratios (Fig. 3.1). The fact that the yield per donor did not increase when recipient concentration was raised above 5 x 10^7 cells/ml for both 20' and 30' matings indicates that this is not solely due to a lack of time for sufficient collisions. We do not know whether this lower yield reflects an inability to pair with recipients or a delay after aggregation as observed by de Haan and Stouthamer (1963). This could be resolved by experiments in which a second recipient strain is added to the mating mixtures at various times; any non-aggregated donors would be able to mate with this second recipient strain. In the case of recipients, Fig. 4.2 shows that there is a considerable spread in times of aggregation as an F'lac tral strain competes with an F'lac donor even if it is added after 20' of mating. This spread in times was probably not collision-rate-determined as the data in Table 4.2 suggest that there is a concentration above which collision rate is not the limiting factor in mating.

In order for progeny colonies to be produced the plasmid must not only be transferred to the recipient cell, but must also become stably established and be efficiently inherited. If each donor cell transferred F'lac to a large number of recipients but the efficiency of subsequent inheritance was low, this would result in large variations
in fertility between individual donor cells. In fact, in experiments such as those of Table 3.2 the ratio of variance to mean of progeny yield should be proportional to the average number of transfers per progeny colony produced. As the variance in Table 3.2 is low, the efficiency of inheritance after transfer must be fairly high.

Segregation of F' cells by new progeny does occur (de Haan and Stouthamer, 1963; Table 4.4), but this does not have a large effect on the apparent growth rate (de Haan and Stouthamer, 1963; Fig. 4.3). Table 4.4 shows that there appeared to be unilinear inheritance of F'lac for over six generations by some new progeny. We do not know whether this is due to the state of the recipient cell or to the state of the plasmid transferred; it might be interesting to examine the structure of F'lac in such progeny colonies.

We showed (Fig. 3.2) that there was a gap of 30'-40' between a donor transferring F'lac and being able to mate again. Thus, continuing transfer by donors would occur about once per 60' in these exponential growth conditions. The lag could be due to a necessity for donors to disaggregate from mating aggregates before mating again or to some "physiological" limitation on mating; Achtman (1977) showed that disaggregation occurred after about 60' of mating. We attempted to distinguish these two theories by comparing the mating abilities with a second recipient of blended and unblended mating mixtures (Figs. 3.3 and 3.4). These experiments were inconclusive because blending also increased the fertility of unmated donor cultures (Fig. 3.5).

In the experiments discussed so far we have ignored re-transfer of F'lac from recipients. This was reasonable as there was a lag of about 90' after receiving F'lac before the majority of recipients were able to re-transfer the plasmid (Fig. 4.4). This may be the time
needed to synthesise sufficient transfer products, as there is probably a large pool of F-pilin in the cell (Beard and Connolly, 1975) and this pilus-subunit protein undergoes several modification steps after synthesis (see Tomoeda et al., 1975). Other factors also control fertility as is shown by the much faster decline of fertility than aggregate-forming ability (and hence presumably pilus formation) on entry to stationary phase (Fig. 5.1).

Re-transfer by recipients and "epidemic spread"

In our experiments, re-transfer by recipients could only occur efficiently in a limited range of concentrations and times. At lower recipient concentrations (below $5 \times 10^7$ cells/ml), the collision rate is the limiting factor in mating and the frequency of F'lac transfer is relatively low (Fig. 3.1). F'lac transfer is efficient at higher concentrations, but will stop rapidly when the culture enters stationary phase (Figs. 5.1 and 5.6). The minimum recipient concentration needed for efficient mating might well vary between different mating systems and between different bacterial strains; for instance, motility will probably be an important factor (Collins and Broda, 1975). The effect of growth conditions on mating ability also varies between plasmids (Burman, 1977). The rapid loss of mating ability when an F'lac donor entered stationary phase was probably not due to loss of pili as aggregates were still formed with recipients (Fig. 5.1). Hfr strains also experience a loss of mating ability which is much more rapid than the loss of pili on starvation (Curtiss et al., 1969).

In the early stages of mating mixtures transfer by the original donor strain will be the dominant effect on progeny numbers as, for F'lac, there is a lag of about 90' between a recipient receiving the plasmid and being able to re-transfer it (Fig. 4.4). Continuing transfer by the original donor strain may play an important role for several
generations; in Fig. 5.4, elimination of the donor strain with phage T6 at 60' results in about ten-fold lower progeny yield. In our experiments (Figs. 5.4, 5.5, 5.6) transfer by the original donor strain became unimportant after 300' because the donor strain entered stationary phase. In the general case, provided the progeny cells do not grow very much slower than the donors, re-transfer by recipients will make the ratio of donors to progeny so small that continuing transfer by donors would be negligible compared to re-transfer. When re-transfer by recipients cannot occur, continuing transfer by the original donor strain may play an important role in determining progeny yield throughout the experiment (Figs. 5.2, 5.3). The occurrence of a fertility-repression system has a large effect on transfer by the original donor strain because it reduces the number of competent donors to a very small proportion of the total donor numbers.

However, if repression is slow-acting after transfer of a plasmid to a recipient cell (Stocker et al., 1963), then the repression system should have little effect on the efficiency of plasmid spread by re-transfer. This was confirmed by experiments in which the repressed plasmids R100 and R64 were compared with derepressed derivatives (Cullum et al., 1978b; Broda, personal communication). We measured the rate of increase of progeny numbers in situations where continuing transfer by the original donor strain was negligible (Figs. 5.4 and 5.5, Table 5.2). We used a simple mathematical model to investigate the effect on the rate of plasmid spread of the lag between a recipient receiving F'lac and becoming a competent donor. Table 5.3 shows that the effect of this lag is fairly small and the rate of plasmid spread mainly reflected the inherent fertility of cells in which the plasmid was established. Table 5.2 shows that the mating time was similar to the generation time, both being about 300'. That is, cells in
which F'lac was established transferred the plasmid about one per
generation.

Perhaps the most striking feature about the results shown in
Fig. 5.4 and 5.5 is the small proportion of the recipients that
receive the plasmid. The initial donor:recipient ratio was about
1:1000 and about 10% of the recipients eventually carry F'lac.
In fact, as the donor strain grew faster than the recipient strain,
the donor:recipient ratio had risen to about 1:100 by the time the
donor strain entered stationary phase. When continuing transfer
by the original donor was prevented after 60' by addition of phage
T6 (Fig. 5.4) only about 1% of the recipients received F'lac.
It is possible that re-transfer occurs at a higher rate with other
plasmids; however, calculations from the data of Ozeki et al. (1962)
for ColI in Salmonella typhimurium showed that the rate in this system
was not much higher than that we observed with F'lac. It is likely
that in nature the efficiency of transfer would be even lower because
of barriers such as restriction systems. The R-factor RP4 does not
seem to have a repression system (Datta et al., 1971), but it never-
theless has a relatively low frequency of transfer. Mating systems
that transfer at a high frequency might be disadvantageous for three
reasons:

(i) Constant involvement in mating aggregates might reduce
access to nutrients.

(ii) Constant synthesis of large quantities of transfer products
might put the host cell at a disadvantage and provide
selection against the plasmid. The derepressed mutant
R100-1 of the F-like R-factor R100 does reduce the host's
growth rate, whereas the parent, R100, may even increase
the growth rate (Finnegan and Willetts, 1970). However,
the non-repressed plasmid F'\text{lac} did not affect the growth rate detectably.

(iii) The presence of sex pili would make the cell sensitive to male-specific phage.

Fertility repression is slow acting after transfer to a recipient cell in the best studied cases of ColI (Stocker \textit{et al.}, 1963) and R100 (Willetts, 1974). The indirect control of repression in F-like R-factors has been interpreted as a mechanism to allow "epidemic spread" of plasmids through a recipient population (Helmuth and Achtman, 1975; Achtman and Skurray, 1977). However, the low rate of spread by this means, even in our laboratory conditions, suggests that "epidemic spread" will be unimportant. Perhaps the indirect control of repression is primarily a mechanism to allow transfer to occur after spontaneous derepression of a cell carrying a repressed plasmid (Watanabe, 1963; Meynell \textit{et al.}, 1968), rather than a mechanism to allow "epidemic spread".

**Incompatibility**

The model with random pool replication and equal number segregation is arguably the simplest model for incompatibility which is not inconsistent with published data. We, therefore, calculated segregation rates for this model and compared them with experimental data. The model gave good agreement for the case of one colEl derivative being transferred into a cell in which another derivative was present (Fig. 6.3). As the segregation was relatively rapid the differential effect of the plasmids on the host growth rate was relatively unimportant. We also considered the steady state segregation rate and tested the model by comparing the copy number predicted by the model from the segregation rate with a published value. We assumed that cells containing both plasmids grew at the
same rate as cells containing only pDS1107 i.e. 5% slower than cells carrying only pML2 (Table 6.1). If cells carrying both plasmids grow any faster than this our estimate will be too high (Table 6.1). Our estimate is somewhat lower than an estimate based on the number of covalently closed circular DNA molecules (Cabello et al., 1976). However, it is unclear what growth conditions they used and under certain circumstances colEl copy number can increase considerably (Bazaral and Helinski, 1970).

It is interesting to note that considerable segregation due to incompatibility occurs even in selective medium and that in our case most segregants had lost only pDS1107. Growth in non-selective medium (Table 6.2) showed that this was not due to asymmetry in segregation; it must therefore be due to the particular antibiotics used. This means that observation of asymmetry between segregant types on selective medium does not necessarily reflect asymmetry of the incompatibility function. This leads to some doubt about the interpretation of the asymmetry between plasmids observed by Timmis et al. (1978) when investigating the incompatibility properties of DNA fragments cloned from R6-5.

It is possible to compare the predictions of the model with published data for copy number and segregation rates. There is the problem that segregation rate measurements and copy number measurements are often made under different growth conditions and growth conditions can affect the copy number of plasmids; this has been observed for colEl (Bazaral and Helinski, 1970), F'\text{lac} (Collins and Pritchard, 1973) and R1 (Engberg et al., 1975). In the case of two plasmids (F and pSC101) copy number has been determined by methods independent of extracting covalently closed circular DNA (Table 8.1). The agreement with our estimate based on published segregation rates is
Table 8.1 Calculation of copy numbers from published segregation data.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Half Time</th>
<th>Calculated $\bar{N}$</th>
<th>Calculated copy number per genome equivalent$^a$</th>
<th>Measured copy number per genome equivalent (by CCC DNA unless otherwise indicated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>1.8$^b$</td>
<td>3.7</td>
<td>1.8</td>
<td>1.2$^c$ (by hybridisation)</td>
</tr>
<tr>
<td>pSC101</td>
<td>6.7$^d$</td>
<td>13.9</td>
<td>3.5</td>
<td>5$^d$ 3-5.5$^e$ (segregation by temperature sensitive mutants)</td>
</tr>
<tr>
<td>colEl</td>
<td>14$^d$</td>
<td>29.2</td>
<td>7.5</td>
<td>18$^d$</td>
</tr>
<tr>
<td>Rl</td>
<td>4.0$^f$</td>
<td>8.3</td>
<td>2</td>
<td>0.4$^g$</td>
</tr>
<tr>
<td>R483</td>
<td>6.9$^h$</td>
<td>14.5</td>
<td>3.6</td>
<td>$^i$</td>
</tr>
</tbody>
</table>

$^a$ This used the relation between DNA content and growth rate of Cooper and Helmstetter (1968).

$^b$ Jamieson and Berquist (1977)

$^c$ Collins and Pritchard (1973)

$^d$ Cabello et al. (1976)

$^e$ Hashimoto-Gotoh and Sekiguchi (1977)

$^f$ Uhlin and Nordstrom (1975)

$^g$ Engberg et al. (1975)

$^h$ Datta and Barth (1976a)

$^i$ Barth et al. (1976).
fairly good (Table 8.1). Cabello et al. (1976) gave data for CoIE1 derivatives that gave a half time for segregation (Table 8.1) comparable with our measurements (18 generations). The points we discussed above with respect to our results also apply in this case. For the large plasmids Ri and R483 the segregation rate gave us copy number estimates several times those made by measuring the amount of covalently closed circular DNA (Table 8.1). At least part of this discrepancy can be explained by the efficiency of recovery of plasmid in the form of covalently closed circles. Thus, the predictions of our model are not inconsistent with the published data and yield the best results with F and pSC101 which are the cases where the published data probably gives the most accurate estimates of copy number.

The appropriate segregation model for high copy number plasmids is unclear (Novick et al., 1975). We therefore also considered the random segregation model and calculated the segregation rates for a random pool replication, random segregation model. This gave predictions very similar to those with equal number segregation. Thus, in practice, the rate of segregation of incompatible plasmids could not be used to distinguish the two models. However, the segregation rate could be used to distinguish random pool and democratic replication models; a democratic replication model gave a segregation rate about half that of a random pool model of corresponding copy number. The rate of loss when one plasmid entered cells carrying the other was also lower (Fig. 6.2). The random pool model gave a better fit to the data than the democratic model for experiments where one CoIE1 derivative was introduced into cells carrying the other by conjugation.
One use of our calculations is to predict the copy number of a plasmid from the segregation rate due to incompatibility. If it is assured that a random pool replication model is valid then the steady state rate of segregation will allow the calculation of copy number (Eq. (6.1)). Differential growth rates may affect this estimate; this will be more serious for high copy number plasmids where the rate of segregation is very low. This method of estimating copy number gives values under normal conditions, unlike methods based on the kinetics of segregation of plasmid-free cells by temperature-sensitive replication mutants at the restrictive temperature; the mutations may affect the copy number at the permissive temperature. The results of the latter experiments are even more difficult to interpret when the mutations are "leaky", i.e. some replication occurs at the restrictive temperature. Then the segregation rate at later times, when most plasmid-carrying cells have only one plasmid copy, allows the estimation of the amount of replication remaining and this must be extrapolated back to the start of the experiment to obtain the initial copy number. However, there are at least two different assumptions that could be made in extrapolating back:

(i) The amount of remaining replication per cell is constant and does not depend on the number of plasmid molecules present in the cell.

(ii) The amount of remaining replication per cell is proportional to the number of plasmids carried.

The methods used so far have made the second assumption. This gave results in agreement with covalently closed circular DNA measurements for pSC101 (Hashimoto-Gotoh and Sekiguchi, 1977). Durkacz and Sherratt (1973) made the same assumption when considering CoIE1
segregation from a temperature-sensitive-polA strain; if the first assumption were more appropriate for this case where a chromosomal mutation is used, then their estimate would be increased 2-4 times.

Experiments where one plasmid is introduced into cells carrying the other and the segregation rate measured are not suitable for finding the copy number of higher copy number plasmids. Fig. 6.1 shows that if the copy number at birth \( N \) is greater than 10 there is little variation with copy number over twenty generations. The ratio of the two types of segregants is also not useful because the appearance of segregants carrying only the minority plasmids is so slow.

In some cases segregation due to incompatibility is asymmetric with one plasmid being favoured (Macfarren and Clowes, 1967). The symmetric models we have discussed can be adapted in at least two ways to account for this:

(i) The probability of replication in the random pool model may not be the same for two plasmids; this could be due to differences in the plasmid sites recognised by the replication system. Table 6.4 shows that appreciable asymmetry occurs for copy numbers over 10 if there is a greater than 1.1:1 replication advantage.

(ii) One plasmid might carry genes that repress replication of the other plasmid.

In both of these cases mutations or perhaps even changes in the growth conditions should be capable of modifying the asymmetry. Changes in growth conditions can abolish asymmetry in the case of R483 (Datta and Barth, 1976a). In the second case it would, in principle, be possible for one plasmid to be incompatible with another that has an unrelated replication system; mutations should then allow both
plasmids to co-exist in a cell. In fact, inc mutations in Hfr strains which allow the maintenance of autonomous F' plasmids (DeVries and Maas, 1973) can be viewed as mutations abolishing incompatibility between the replicons of the chromosome and the autonomous F.

**Hfr formation and chromosome transfer**

We obtained a value for the rate of Hfr formation in a rec⁺ strain in good agreement with that of Curtiss and Stallions (1969). We showed that the rate of Hfr formation was reduced about one hundred-fold by the recA mutation. Thus it seems probable that the insertion sequences and the γS sequence (Fig. 8.1) function mainly as regions of homology that are acted upon by the host's recombination system. We did, however, isolate two Hfr clones from a recA strain. We do not know whether the structure of these Hfr strains is different from that of rec⁺ Hfr strains; it is probable that the small number of strains that have been studied by heteroduplex analysis (Ohtsubo et al., 1974; Hu et al., 1975; Deonier and Davidson, 1975) were formed by recA-dependent integration. A possible model for recA-independent integration is to consider the part of F carrying the known genes, which is between the two IS3 sequences (Fig. 8.1), as a transposon; many known transposons have repeated sequences at their ends (Kleckner, 1977). If this model were correct, F integration in a recA strain would involve insertion of the part of F between the IS3 sequences into the chromosome at a chromosomal location which need not have any homology with F; there would be a loss of a 15 kb section of F. This could be tested by heteroduplex analysis or, perhaps, by analysing the restriction fragments of Hfr cell DNA which hybridise with F. This transposon model would explain the insertion of an F''lac into the tsx gene observed by Broda and Meacock (1971).
**Figure 8.1** Map of the *Escherichia coli* K12 sex factor, F.

The transfer genes are in the region labelled "Tra". The replication and incompatibility functions are in the region labelled "rep". The genes determining inhibition of female-specific phage are in the region labelled "\( \phi \)". This map was simplified from Shapiro (1977).
The results of fluctuation tests and the attempts at sib selection of Hfr strains from Type II strains suggested that some sort of clonally inherited Hfr state did exist in Type II strains. Curtiss and Renshaw (1969a) also observed an increased variance in larger fluctuation tests with Type II rec\(^+\) strains, but they attributed it to variations in viable counts between tubes due to the use of a minimal medium. The similar frequencies of fertile cultures suggested that the Hfr formation rate need not be different in Type I and Type II strains. However, the apparent growth rate of Type II Hfr strains would have to be up to a third lower than that of the F\(^+\) parent strain. Broda (personal communication) isolated Hfr strains from an F\(^-\)-carrying Type II strain. These Hfr cells were stable, but the frequency of Hfr cells in the F\(^-\) population was about ten-fold lower in the Type II strain than in a Type I strain. This would be explicable if F integration in Type II strains affected the host's growth rate adversely; we do not know what the molecular basis for such an effect might be.

We found (Table 7.2) that a recA mutation reduced chromosome transfer about one hundred-fold; this was comparable to the reduction found by Clowes and Moody (1966) and Moody and Hayes (1972). Although this reduction could be interpreted as evidence for physical interaction between F and the chromosome, it could also be due to an indirect effect of the recA mutation. The comparable proportion of recombinants that remain F\(^-\) in matings with rec\(^+\) and recA F\(^+\) donors also suggest that the reductions in Hfr formation and chromosome transfer are of similar magnitude. Our evidence that there is not a genetic block in Hfr formation in Type II strains removes the strongest evidence for much of chromosomal transfer being due to a process fundamentally different from Hfr formation. The methods
for isolating Hfr strains select against any F integration that is unstable or affects the host's growth rate adversely; such integration events could well be in a majority. Thus the role of Hfr cells in chromosome transfer is still unresolved.
1. Mean and variance of progeny numbers per tube

If the number of donors entering each tube has mean $\lambda$ and variance $T^2$ and the donors act independently to produce a number of progeny with mean $\mu$ variance $S^2$ then a standard result in probability theory shows that the number of progeny per tube has mean $\lambda \mu$ and variance $\mu^2 T^2 + \lambda S^2$. This is seen by considering the probability generating functions $P$ of the number of donors entering each tube, $Q$ of the number of progeny per donor and $R$ of the number of progeny per tube. So:

$$P'(1) = \lambda \quad \text{and} \quad P''(1) = T^2 + \lambda^2 - \lambda$$

$$Q'(1) = \mu \quad \text{and} \quad Q''(1) = S^2 + \mu^2 - \mu$$

As $R$ is produced by a number of events with generating function $P$ and each event gives rise independently to a number of progeny with generating function $Q$, the three generating functions satisfy:

$$R(t) = P(Q(t))$$

So: $R'(t) = Q'(t)P'(Q(t))$

and $R''(t) = Q''(t)P(Q(t)) + (Q'(t))^2P''(Q(t))$

Therefore, the mean number of progeny per tube ($\bar{X}$) is:

$$\bar{X} = R'(1) = \lambda \mu \quad (\text{as } Q(1) = 1) \quad (A1.1)$$

and the variance of the number of progeny per tube ($V$) is:

$$V = R''(1) + R'(1) - (R'(1))^2$$

So: $V = \lambda S^2 + \mu^2 T^2 \quad (A1.2)$

If the distribution of the number of donors entering each tube is a Poisson distribution with mean $\lambda$, then $T^2 = \lambda$ and
2. Competition between JC6582 and ED2510 donors

We assumed that there was equal competition between JC6582 and ED2510 to form aggregates with recipients and that any recipients aggregating with one or more JC6582 cells became Lac\(^+\). We assumed that there was an excess of donors and that a proportion \(q\) of the donors were ED2510 cells and that a proportion \(p\) (= 1-\(q\)) were JC6582 cells. Then, if a recipient can aggregate with \(n\) donors it will only remain Lac\(^-\) if all donors are ED2510 and this has probability \(q^\text{n}\) provided individual donors act independently. If for \(n = 0,1,2, \ldots\) a proportion \(a_n\) of recipients can aggregate with \(n\) donors, the probability that a recipient remains Lac\(^-\) will be:

\[
P(\text{Lac}^-) = \sum_{n=0}^{\infty} a_n q^n
\]

We found that a Poisson distribution of the number of donors aggregating with each recipient gave a good fit to our data; i.e. \(a_n = \frac{\lambda^n e^{-\lambda}}{n!}\) with a mean of \(\lambda\) donors per recipient. In this case:

\[
P(\text{Lac}^-) = \sum_{n=0}^{\infty} \frac{\lambda^n e^{-\lambda} q^n}{n!}
\]

So: \(P(\text{Lac}^-) = e^{\lambda(q-1)} = e^{-\lambda p}\) (A2.1)

We assumed that a proportion \(\alpha\) of ED2510 cells that aggregated with recipients could transfer pML2 to give Kan\(^R\) progeny. If a recipient aggregates with \(n\) donors, of which \(k\) are ED2510, the probability that it remains Kan\(^S\) is \((1 - \alpha)^\text{k}\). Thus the probability that a recipient that has \(n\) mating sites remains Kan\(^S\) is:

\[
\sum_{k=0}^{n} \binom{n}{k} q^k p^{n-k} (1 - \alpha)^k = (1 - \alpha q)^n
\]

Therefore, the probability that a recipient remains Kan\(^S\) is:

\[
P(\text{Kan}^S) = \sum_{n=0}^{\infty} e^{-\lambda \alpha q} (1 - \alpha q)^n / n! = e^{-\lambda \alpha q}(A2.2)
\]

We finally show that the events of a recipient becoming Lac\(^+\) and
becoming Kan\textsuperscript{R} are independent by calculating the probability that a recipient receives neither plasmid, \( P(\text{Lac}^{-}\text{Kan}^{S}) \). If a recipient has \( n \) mating sites this occurs only if all donors aggregating are ED2510 cells and none of them transfer \( pML2 \). This has probability \( q^{n}(1 - \alpha)^{n} \). Therefore, \( P(\text{Lac}^{-}\text{Kan}^{S}) = \sum_{n=0}^{\infty} \lambda^{n}q^{n}(1 - \alpha)^{n}e^{-\lambda}/n! \)

\[ = \exp\lambda(q - 1)\exp(-\alpha\lambda q) \]

So: \( P(\text{Lac}^{-}\text{Kan}^{S}) = P(\text{Lac}^{-})P(\text{Kan}^{S}) \) \tag{A2.3} \]

Thus, the two events are independent.

3. Plasmid spread by recipient retransfer

The proof of the behaviour of the solutions of equation (5.1) falls into two parts. The first part uses several substitutions to identify the constants \( \lambda \) and \( C \) and the second part shows that a remainder term tends to zero. The equation (5.1) is:

\[
\frac{dx(t)}{dt} = kx(t) + \alpha \exp(k\delta).x(t - \delta) \tag{A3.1}
\]

The first substitution is \( x(t) = x(0).P(t).\exp(kt) \) so (A3.1) becomes:

\[
\frac{dP(t)}{dt} = \alpha .P(t - \delta) \tag{A3.2}
\]

We then put \( P(t) = Q(t).\exp(\lambda t) \) where \( \lambda \) is the solution of \( \lambda .\exp(\lambda \delta) = \alpha \). Substitution into (A3.2) gives:

\[
\frac{dQ(t)}{dt} + \lambda(Q(t) - Q(t - \delta)) = 0 \tag{A3.3}
\]

Integrating (A3.3) from 0 to \( t \) and changing the variable in the \( Q(t - \delta) \) term to \( t \) gives:

\[
Q(t) = Q(0) + \lambda\int_{t-\delta}^{t}dzQ(z) - \lambda\int_{t-\delta}^{t}dzQ(z) \tag{A3.4}
\]

Substitute \( R(z) = Q(z) - q \) where \( q = (Q(0) + \lambda\int_{t-\delta}^{t}dzQ(z))/(1 + \lambda \delta) \):

\[
R(t) = -\lambda\int_{t-\delta}^{t}dzR(z) \tag{A3.5}
\]

We can use the substitutions above to express \( x \) in terms of \( R \):

\[
x(t) = x(0).\exp(kt).\exp(\lambda t).(q + R(t)) \tag{A3.6}
\]

If we can show that \( R(t) \to 0 \) as \( t \to \infty \) then we obtain equation (5.2)
with \( C = q \cdot x(0) \). \( q \) is determined by the values of \( x(t) \) in the time interval \(-\delta \) to 0, which would be determined by the way the mating was set up.

We shall show that \( R(t) \) oscillates and the oscillations become smaller and die away as \( t \) becomes large. The first thing to note is that \( x(t) \) is a continuous function of \( t \). This is necessary biologically. Thus, \( R(t) \) is also continuous (see equation (A3.6)). As \( \lambda \) is positive, equation (A3.5) shows that if \( R(t) \) is positive then the integral must be negative and, hence, \( R(z) \) must take the value zero in the interval of length \( \delta \). This is also true if \( R(t) \) is negative. Thus \( R \) takes the value zero in any interval of length \( \delta \) so \( R(t) \) must oscillate about zero. \( R(t) \) satisfies equation (A3.3) (substitute \( Q(t) = q + R(t) \)). Multiply equation (A3.3) for \( R \) by the integrating factor \( \exp(\lambda t) \) and integrate from some value \( a \) (such that \( R(a) = 0 \)) to \( t \):

\[
R(t) = \lambda \exp(-\lambda t) \int_a^t \exp(\lambda s) R(s - \delta) ds
\]

(A3.7)

We shall now show that the size of the oscillations decreases exponentially as \( t \) increases. We consider \( t \) in the time interval \( T \leq t \leq T + \delta \) and show that the maximum absolute value of \( R \) in this interval \( (M') \) is less than the maximum value in the time interval \( T - 2\delta \leq t \leq T \) which we call \( M \), and in fact we show that \( M' \leq (1 - \exp(-2\lambda \delta))M \) so that the size of the oscillations decreases exponentially. We choose \( a \) in equation (A3.7) such that \( T - \delta \leq a \leq T \) (this is possible because of the oscillating behaviour of \( R \)). Then, if \( T \leq t \leq T + \delta \), the term \( s - \delta \) in equation (A3.7) satisfies \( T - 2\delta \leq s - \delta \leq T \) so that \( |R(s - \delta)| \leq M \). Therefore,

\[
|R(t)| \leq \lambda \exp(-\lambda t) \int_a^t \exp(\lambda s) |R(s - \delta)| ds
\]

As \( |R(s - \delta)| \leq M \) this shows that:

\[
|R(t)| \leq M \exp(-\lambda t) \int_a^t \exp(\lambda s) ds
\]
Integrating shows that:
\[ |R(t)| \leq M(1 - \exp(-\lambda(t - a))) \]
The choice of \( a \) is such that \( (t - a) \leq 2g \) so that:
\[ M' \leq M(1 - \exp(-2\lambda g)) \]
Therefore, \( R(t) \to 0 \) as \( t \to \infty \).
Solution of equation (5.1) for various initial conditions showed that the solutions rapidly attained the form of equation (5.2).

4. Incompatibility models

(a) Random pool replication equal number segregation model

If there are \( s \) copies of plasmid 1 and \( t \) copies of plasmid 2 then the probability that plasmid 1 is replicated next is \( s/(s + t) \) and the probability that plasmid 2 is replicated next is \( t/(s + t) \). If a cell has \( s \) copies of plasmid 1 and \( t \) of plasmid 2 then the probability that plasmid 1 is replicated \( k-s \) times to give \( k \) copies and plasmid 2 is then replicated to give \( 2N-k \) copies is:
\[
\left( \begin{array}{c}
k-1 \\ s+t \end{array} \right) \cdot \left( \begin{array}{c}2N-k-1 \\ k+t \end{array} \right) = \left( \begin{array}{c}k-1 \end{array} \right)! \left( \begin{array}{c}2N-k-1 \end{array} \right)! (s+t-1)! (s-1)! (t-1)! (2N-1)! \\
\left( \begin{array}{c}2N-1 \\ s+t \end{array} \right) 
\]
However, the probability of going from \( s \) to \( k \) copies of plasmid 1 and from \( t \) to \( 2N-k \) of plasmid 2 is independent of the order of replication of copies of the two plasmids. As there are \( \binom{2N-s-t}{k-s} \) possible orders the total probability of going from \( s \) to \( k \) and from \( t \) to \( 2N-k \) copies is:
\[ P(s \rightarrow k, t \rightarrow 2N-k) = \frac{(k-1)! (2N-k-1)! (s+t-1)! (s-1)! (t-1)! (2N-1)!}{(s+t-1)!} \\
\]
If there is equal number segregation, then the probability that there are \( s \) copies of plasmid 1 (hence \( N-s \) of plasmid 2) in a new-born cell given that the parent cell had \( k \) and \( 2N-k \) copies of plasmids 1 and 2 respectively is (from Whittle (1970), equation 4.5.1):
\[ P(s|k) = \frac{k}{s} \cdot \frac{2N-k}{N-s} \max(0, k-N) \leq s \leq \min(N, k) \\
\]
If replication and segregation are independent, from equations (A4.1) and (A4.2), the probability that there are \( j \) copies of plasmid 1 at birth given that there were \( i \) copies at birth the generation before is:

\[
p_{i,j} = \sum_{k=N-i}^{N-j} \binom{N-i}{k} \binom{N-j}{k} \frac{(2N-k)}{N-1} \quad 1 \leq i \leq N-1
\]

\[
\quad 0 \quad i=0 \text{ or } i=N \text{ and } i \neq j
\]

\[
1 \quad i=0 \text{ or } i=N \text{ and } i=j
\]

The matrix \( (p_{i,j}) \) was calculated by computer for \( N \) in the range 2-40. This allowed the calculation of the proportion of cells carrying both plasmids after starting from various initial distributions of plasmids. Calculation of the distribution of plasmids among cells carrying both showed that the distribution settled down to a uniform distribution with an equal proportion of cells carrying \( 1, 2, \ldots, N-1 \) copies of plasmid 1. If this were so then:

\[
\sum_{i=1}^{N-1} p_{i,j} = \lambda \quad j=1, 2, \ldots, N-1
\]

where \( \lambda \) is the ratio by which the proportion of cells carrying both plasmids decreases each generation during steady state segregation. We therefore used equation (A4.3) to confirm equation (A4.4) and calculate and hence the steady state segregation rate:

\[
\binom{2N-1}{N-1} \frac{(2N)}{N} \lambda = \sum_{k=0}^{N-1} \binom{k}{i-1} \binom{2N-k-1}{k} \frac{(2N-k)}{N-1} \frac{N-i}{N-j}
\]

\[
= \sum_{k=0}^{N-1} \binom{k}{j} \frac{(2N-k)}{N-j} \sum_{i=1}^{N-1} \binom{k-1}{i-1} \frac{(2N-k-1)}{N-i-1}
\]

\[
= \binom{2N-2}{N-2} \sum_{j=1}^{N} \binom{k}{j} \frac{(2N-k)}{N-j} = \binom{2N-2}{N-2} S_j
\]

as the sum over \( i \) is the coefficient of \( t^{N-2} = (t^{1-i}+1)^{N-1-i-1} \) in \( (1+t)^{N-2} = (1+t)^{k-1}(1+t)^{2N-k-1} \). \( S_j \) can be calculated as it is the coefficient of \( s^j t^{N-j} \) in \( J_j(s, t) \) where:

\[
J_j(s, t) = \sum_{k=0}^{\infty} (1+s)^{k} (1+t)^{2N-k}
\]

\[
= (1+s)^{j}(1+t)^{N-j} \sum_{k=0}^{\infty} (1+s)^{1}(1+t)^{N-1}
\]
\[ \beta_j(s, t) = (1+s)^j(1+t)^{N-j} \cdot \frac{(1+s)^{N+1} - (1+t)^{N+1}}{N+1} \]
\[ = (1+s)^j(1+t)^{N-j} \sum_{k=1}^{N+1} \frac{\binom{N+1}{k} s^k t^{N+1-k}}{s-t} \]
\[ = (1+s)^j(1+t)^{N-j} \sum_{k=1}^{N+1} \frac{\binom{N+1}{k} s^k (-1)^{N+1-k}}{s-t} \]

Thus, \( S_j = \sum_{a=0}^{2N} \sum_{b=0}^{N-j} \binom{j}{a} \binom{N+1}{b} (N+1)^{a+b} \)

This is the coefficient of \( s^{N+1} \) in \((1+s)^{2N+1}\), so \( S_j = \binom{2N+1}{N+1} \) and:

\[ \lambda = \frac{(N-1)(2N+1)}{(N+1)(2N-1)} \]  
(A4.5)

and: \( t_s = \log_2 \log_e \lambda \)  
(A4.6)

Expansion of \( \log_e \lambda \) in powers of \( 1/N \) showed that \( t_s \sim N \log_2 2 \) as \( N \to \infty \).

(b) Random segregation

We also considered a random segregation model i.e. the 2N plasmid copies are distributed at random with equal probability between the two daughter cells. Thus the probability of obtaining a daughter cell with \( s \) plasmid 1 copies and \( t \) plasmid 2 copies if the parent has \( k \) plasmid 1 copies and \( 2N-k \) plasmid 2 copies at division is:

\[ P(s, t, k, 2N-k) = \binom{2N}{s+t} \left( \frac{k}{s+t} \right) \binom{2N-k}{t} \left( \frac{s}{s+t} \right) \]

where the first term is the probability of producing a daughter cell with \( s+t \) plasmid copies and the second term is the probability of producing \( s \) plasmid 1 copies and \( t \) plasmid 2 copies given that the daughter cell receives a total of \( s+t \) copies. This simplifies to:

\[ P(s, t|k, 2N-k) = \binom{2N}{s+t} \left( \frac{k}{s+t} \right)^{2N-k} \]  
(A4.7)

0 \( \leq \) \( s \leq k \)  
0 \( \leq \) \( t \leq 2N-k \)

This segregation model produces plasmid-free cells at a frequency of \( \left( \frac{1}{2} \right)^{2N} \) per generation. Taking these cells into account in our calculations was inconvenient so we restricted attention to cells that carried plasmids. This introduces a normalising term \( 2^{2N-1} \) in the formulae.
below. Combining equations (A4.1) and (A4.7) shows that the probability that a plasmid-carrying cell at division contains \( j \) copies of plasmid 1 and \( 2N-j \) of plasmid 2 given that the generation before there were \( i \) and \( 2N-i \) copies is:

\[
q_{i,j} = \begin{cases} 
0 & i=0, j > 0 \text{ or } i=2N, j < 2N \\
1 & i=0, j=0 \text{ or } i=2N, j=2N \\
\frac{2^{2N-i-1}}{2^{2N-1}} & 1 \leq i \leq 2N-1, j=0 \\
\frac{2^{i-1}}{2^{2N-1}} & 1 \leq i \leq 2N-1, j=2N 
\end{cases} \tag{A4.8}
\]

We used a computer to calculate \( q_{i,j} \) for \( N \) in the range 2-20 and then calculated segregation rates.

(c) Democratic replication

We also considered a "democratic" replication model in which each plasmid copy is replicated once per generation. We used the equal number segregation model (equation (A4.2)). This gave the probability that there were \( j \) copies of plasmid 1 and \( N-j \) of plasmids 2 at birth given that there were \( i \) and \( N-i \) copies the generation before as:

\[
r_{i,j} = \begin{cases} 
\binom{2i}{i} \binom{2N-2i}{N-1} & 1 \leq i \leq N-1 \\
\binom{2N}{N} & \max(0,2i-N) \leq j \leq \min(2i,N) \\
1 & i=0, j=0 \text{ or } i=N, j=N \\
0 & \text{otherwise} 
\end{cases} \tag{A4.9}
\]

We wrote computer programs to calculate \( r_{i,j} \). We calculated the steady state segregation rate and the segregation rate for cells starting with the two plasmids in the ratio \( 1:N-1 \).
(d) Random pool replication with unequal chances of replication

To obtain equation (A4.1) we assumed that if there were i copies of plasmid 1 and j of plasmid 2 present in the cell then the probability that plasmid 1 is replicated next is \( i/(i+j) \) and that plasmid 2 is replicated is \( j/(i+j) \). We also considered a biased replication model where plasmids 1 and 2 had probabilities of replication per copy in the ratio of \( \alpha : 1 \) so the probabilities of replication become \( \alpha i/(\alpha i+j) \) and \( j/(\alpha i+j) \). We could not obtain an equation analogous to equation (A4.1) in this case as each order of replication has a different probability. We were, however, able to calculate the transition probabilities using a computer program that summed over all possible orders of replication. We were then able to study the segregation under this model for various values of \( \alpha \).

(e) Differential growth rates

We set up differential equations for the number of cells carrying plasmid 1 (x), plasmid 2 (y) and both plasmids (m). We assumed that cells with both plasmids segregated symmetrically into cells containing only one plasmid at a rate \( \alpha \) and that they grew exponentially with rate constant k. We assumed that cells carrying only plasmid 1 grew at rate \( k-\varepsilon \) and cells carrying only plasmid 2 grew at rate \( k+\delta \).

These assumptions gave the differential equations:

\[
\begin{align*}
\dot{x} &= (k-\varepsilon)x + \alpha m/2 \\
\dot{y} &= (k+\delta)y + \alpha m/2 \\
\dot{m} &= km - \alpha m \quad \text{(A4.10)}
\end{align*}
\]

The set of equations (A4.10) is linear and is easily solved analytically. We inserted the measured growth rate differences into the solution to obtain the desired corrections. The three cases that
we considered were:

(i) No correction: $\delta = \delta = 0$.

(ii) Cells carrying both plasmids grew like the plasmid 1 cells:

$$\delta = 0, \delta > 0.$$ 

(iii) Cells carrying both plasmids grew like the plasmid 2 cells:

$$\delta > 0, \delta = 0.$$ 

5. **Estimation of rates of Hfr formation from fluctuation tests**

We assumed that Hfr cells had the same growth rate as $F^+$ cells. The possibility that two processes contribute to chromosome transfer by $F^+$ strains meant that the methods of Luria and Delbrück (1943) could not be used to estimate the rate of Hfr formation. However, as the non-Hfr fertility should have only a small variance between cultures, the rate of Hfr formation could be estimated from the number of highly fertile "jackpot" cultures. The method is illustrated using values obtained for the Type I rec$^+$ strain ED879.

Cultures which gave rise to a large number of progeny should have a significant contribution from Hfr cells. We chose a cutoff value (in this case 59 progeny per mating tube) such that the "fertile" tubes with more than this number are likely to contain a large clone of Hfr cells. The probability of a single large clone will be much higher than that of two clones of half the size arising independently in the same culture. The difference between the number of progeny in a fertile tube and the mean number of progeny in the other tubes is considered to be due to the Hfr clone. Thus, in our case the mean number of progeny (ignoring the fertile cultures) is 36.4, so that the fertile tubes have more than 22.6 ($= 59 - 36.4$) progeny due to the Hfr clone. As the mating tube contained 0.1ml of a 1/20 dilution of the donor culture and the efficiency of mating is assumed to be 0.1, this
corresponds to more than $22.6 \times 10 \times 20 \times 10$ Hfr cells/ml in the culture, or (as the culture volume is 0.5 ml) $22.6 \times 10^3$ Hfr cells per culture. Therefore, the Hfr clone arose when there were no more than $2.05 \times 10^8 / 22.6 \times 10^3 = 9 \times 10^3$ cells in the culture, assuming Hfr and F\(^+\) growth rates are equal. This means that at most twice this number, i.e. $1.8 \times 10^4$, cell generations had passed in the fertile culture before the Hfr was formed. If $\alpha$ is the probability that an Hfr is formed in a single cell generation (i.e. $\alpha$ is the Hfr formation rate), then the probability that an Hfr is not formed in $1.8 \times 10^4$ cell generations, i.e. the culture is not a fertile culture, is given by $(1 - \alpha)^{1.8 \times 10^4}$. We estimated this probability as 0.94, because in 47 out of 50 cultures no such Hfr is formed, so we obtained a value of $\alpha$ of $3.4 \times 10^{-6}$ per cell per generation.

In the case of the recA strain ED877, we used the results from streak matings. 8/449 streaks had more than 3 progeny colonies. Reconstruction experiments gave an efficiency of mating of 0.7 progeny per donor. The cultures contained $2.5 \times 10^8$ cells/ml. Analogous calculations to those above gave a value for the rate of Hfr formation of $4.6 \times 10^{-8}$ per cell per generation.
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Cell Growth and Length Distribution in Escherichia coli

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The length growth rate of an exponentially growing population of Escherichia coli B/r was calculated from the population length and birth length distributions. Cell elongation took place at a constant rate that doubled at a certain length. This change in rate was responsible for a sudden drop in the frequency of classes of cells longer than that length. Asymmetry in cell partition was able to generate cells both shorter and longer than the expected twofold range, but did not greatly modify the length distribution in between.

Exponentially growing populations of Escherichia coli are generally considered to be formed by cells that divide in two once in every generation time. The simplest model for the age frequency distribution of such a population follows an exponential curve as described by Powell (10). If cell length were a linear function of cell age, the length distribution of such an exponential population should fit an exponential curve as well. In contrast, real distributions of cell length are of a more complicated shape; they show a peak with a sharp drop after it, and they are not bound between a twofold range of lengths. Between birth and division, cells of E. coli elongate at a rate dependent on the mass growth rate of the culture. Several growth laws have been postulated for bacterial populations following linear or exponential growth (2). As far as elongation is concerned, it has been proposed by Donachie et al. (4) that the rate of elongation for a given growth rate doubles at a certain length that is the same for all growth rates. It has been recently postulated (1) that this increase in the rate of elongation takes place by the addition of new elongation sites when a certain length is reached. To determine whether elongation takes place with a constant, exponential, or stepwise growth law, we have calculated the rate of elongation during the cell cycle by means of a different, and in some respects more sensitive, approach than that used by Donachie et al. (4).

We have also devised a computer program that allows us to simulate bacterial elongation and division in exponentially growing populations following different growth laws and takes into account the proportion of unequal partition observed in natural populations (7–9). This explains some of the differences between the observed length distribution and that produced by simple models.

MATERIALS AND METHODS

Strain and growth conditions. E. coli B/r A, ATCC 12407, was used for all the experiments. Cultures were grown at 37°C in Oxoid nutrient broth no. 2 and kept at a cell density lower than 3 × 10^7/ml by dilution with prewarmed medium. For time-lapse photography, the same medium, solidified by the addition of 1.5% agar, was aerated after melting by being passed through a sterile Pasteur pipette; a thin smear was placed on a glass slide that was then warmed at 37°C before use and kept at that temperature throughout the experiment. Generation time (r) was 20 min for liquid cultures (as measured by doubling in particles) and 22 min for cultures on agar slides (as estimated by doublings in total cell length).

Synchronous cultures were obtained by the membrane elution procedure of Helmstetter (6). The elution rate was fixed at 10 ml/min, and samples were taken for 1 min.

Particle counts. Particle counts were done in a Coulter Counter (model A).

Length measurements. Photographs of cells observed under phase-contrast optics were taken with a Zeiss Ultraphot microscope by the procedure of Donachie et al. (4). Samples from synchronous cultures were first concentrated 100-fold by centrifugation and suspension in a portion of supernatant fluid. Enlarged projections of the negatives were measured (3).

Computer program to model cell growth. The program to model cell growth was written in the IMP language and was run on an ICL4/75 computer. The culture being modeled was represented by a matrix. Each entry in the matrix represented the number of cells with a particular birth length that were of a particular age. Thus, the row of the matrix that an entry appeared in depended on its birth length, and the column depended on its age. At each time step, the entries corresponding to cells that divided were removed from the matrix, and the necessary numbers were added to the classes of newborn cells. (It was found that dividing the age into about 100 classes and the birth length into 150 classes gave satisfactory results.) The dividing-cell classes and the correct new-
born-cell classes were selected according to the growth and division laws being used.

When outputs of the length distribution and newborn cell length distribution were required, they could be calculated from the matrix by using the growth law to find the length from the birth length and age.

The length distribution settled down to a fairly constant distribution after 5 to 10 generations, starting from various initial distributions.

**Calculation of growth rate and length distributions.** To calculate the growth rate and length distributions, we wrote two computer programs that used equation 1 below. The first program used the observed birth length and population length distributions to calculate the growth rate; the second used the observed birth length distributions and assumed growth law to calculate the population length distribution. In both cases, cell division was ignored, so the calculations are only valid for lengths less than those at which significant amounts of cell division occur.

The length distributions were considered linear between the centers of the measured classes.

**RESULTS**

Calculation of length growth rate from length distribution and birth length distribution. Collins and Richmond (2) found an equation relating the growth rate \(g(x)\) of a cell of length \(x\) in an exponentially growing culture to the distribution of cell length \(\Lambda\), length of newborn cells \(\psi\) and the length of dividing cells \(\phi\). \((\Lambda, \psi, \phi\) and \(g\) are actually the probability density functions of those distributions.) This equation is:

\[
g(x) = \frac{K}{\Lambda(x)} \int_0^x (2\psi(y) - \phi(y) - \Lambda(y)) \, dy \tag{1}
\]

where \(K\) is the exponential growth rate constant. This assumes that the length growth rate is a function of the length alone and does not depend on the age or past history of a cell. The population must be growing exponentially with constant distributions of length and age.

The distribution of length of newborn cells was measured in samples from a membrane eluate (Fig. 1). Cell lengths were measured in samples from an exponentially growing culture to obtain its length distribution (Fig. 2a). It was apparent from the lengths of cells with septa that very few cells divided at a length of less than 5 \(\mu\)m. This was also shown by the lengths of newborn cells (dividing cells must be approximately twice as long as newborn cells). We were able to ignore dividing cells (i.e., \(\phi = 0\)), therefore, and use the measured length and cell length distributions to estimate \(g(x)\) (Fig. 2b). This gave a growth rate that was constant at lengths between 2 \(\mu\)m and 3.5 \(\mu\)m, doubled between about 3.5 \(\mu\)m and 4.5 \(\mu\)m, and was constant again at the new value until the neglect of dividing cells made the calculation invalid (about 5 \(\mu\)m).

This calculation of the growth rate supported models in which the growth rate doubles at a given length (here about 4 \(\mu\)m). The point at which the rate changed corresponded to the drop after the peak in the cell length distribution. A linear growth law would give a constant growth rate at all lengths, and an exponential growth law would give a straight line passing through the origin.

To find whether knowledge of the growth rate was sufficient to predict cell length distributions, a model was used in which all cells were born with length \(L_0\) to length \(2L_0\) and divided in half. For this, equation 1 became:

\[
\Lambda(x)g(x) = K [2 - \int_0^x \lambda(y) \, dy] \text{ for } L_0 < x < 2L_0 \tag{2}
\]

This can be solved analytically for simple forms of \(g(x)\). Figure 3 shows the solutions for the
FIG. 2. Rate of cell elongation during the cell cycle. The distribution of length in newborn cells shown in Fig. 1, the actual length distribution of an exponential culture shown in (a), and equation 1 (see text) were used to calculate the rate of elongation (b) for each cell length. Symbols: \( \bullet \), values obtained considering the whole distribution in Fig. 1 as newborn cells; \( \Delta \), values obtained assuming that cells longer than 6.5 \( \mu \)m were not newborn and therefore were excluded from the calculation; ---, rate of elongation for each cell length; - - - , course of rate of elongation as predicted by Donachie et al. (4) for \( \tau = 20 \) min. As cell division was ignored for this calculation, the results are only valid up to about 5 \( \mu \)m.

cases of linear growth, exponential growth, and growth that doubles its rate at about 4 \( \mu \)m.

None of the growth laws gave a very good fit to the observed distribution; this is not surprising, as the actual birth length distribution (Fig. 1b) showed a considerable spread. The result of a spread in birth lengths is to flatten the theoretical distributions. From equation 1, it can be seen that the value of \( \lambda \) will be smaller for shorter cells and larger for longer cells than that calculated on the assumption of constant birth length. This flattening means that the linear and exponential growth laws will not produce a clear peak, whereas the fit of the step growth law will be improved. We used equation 1 to calculate the population length distribution expected under the exponential and step growth laws when the observed spread in birth lengths is taken into account (Fig. 4). The birth length distribution in Fig. 1b was used, and cell division was ignored, so the calculation was not valid for lengths greater than 5 \( \mu \)m. As expected, the exponential growth law did not produce a high enough peak. The poor fit of the step growth law just after the peak probably reflected the fact that the actual change in growth rate (Fig. 2) is spread over 3.5 to 4.5 \( \mu \)m rather than being abrupt at 3.5 \( \mu \)m, as was considered for this calculation.

This simple model is useful for assessing qualitatively the effects of different changes in the growth law. We found that if the change in growth rate was spread over a range of lengths, rather than being instantaneous, then for a range similar to that of Fig. 2 (3.5 \( \mu \)m to 4.5 \( \mu \)m) a curve very similar to that in Fig. 3c was produced (except that the drop from the peak was not so abrupt), so the spread seemed to be of little importance. We also investigated in this way a variety of growth laws; one that gave a slightly better fit for the initial peak was a model in which the growth rate changed to 1.5 times its previous value rather than doubling (data not shown).

Asymmetric division. The deficiencies in the simple models that we have considered up to now suggested that any adequate model that aims at predicting the cell length distribution must have mechanisms to produce a spread in birth lengths. (As a consequence, it would also produce a tail of larger cells.) We thought that asymmetric division could be one such mechanism; when cells with a septum were examined, there were many in which the portions on either side of the septum were of different lengths.

The lengths of the two sister cells were measured in 205 dividing pairs of E. coli B/r in a sample of a population growing exponentially with a generation time of 20 min. Of these, 155 (76%) were symmetric by the criterion that the difference in length between the two sisters was smaller than 0.5 mm in the enlarged negative, that is, 0.33 \( \mu \)m in actual length. The remaining
FIG. 3. Comparison of theoretical and measured cell length distribution in E. coli populations. Symbols:
- , Observed cell length distribution (same as Fig. 2a); • , theoretical distributions \( \lambda (x) \) calculated using equation 2 (see text) for the growth laws. (a) Rate of elongation is constant (linear growth law):
\[
\lambda (x) = \frac{4 \ln^2}{L_b} \exp \left[ -\ln^2 \left( \frac{x}{L_b} \right) \right]
\]
(b) Rate of elongation is proportional to length (exponential growth law):
\[
\lambda (x) = \frac{2 L_b}{x^2}
\]
(c) Rate of elongation is constant up to the length \( 2A \), and then doubles (step growth law):
\[
\lambda (x) = \begin{cases} 
\frac{2 \ln^2}{A} \exp \left[ -\frac{\ln^2}{A} (x - L_b) \right] & \text{for } x < 2A \\
\frac{\ln^2}{2} \exp \left[ -\frac{\ln^2}{2} (2L_b - x) \right] & \text{for } x > 2A 
\end{cases}
\]
We used values of birth length \( L_b = 2.77 \mu m \) and \( A = 1.95 \mu m \).

50 pairs (24%) were asymmetric.

If we define the ratio of asymmetry as:
\[ z = \text{length of the long cell}/\text{length of the short cell} \]
we find an experimental value of \( z = 1.17 \) (standard deviation, 0.08) for the measured asymmetric pairs.

Asymmetry has also been observed in cells from cultures growing at slower rates, but such measurements are less accurate because the mean cell length decreases as generation time increases (4).

Length measurements of dividing cells failed to show any preferential grouping of either the long or the short sister within asymmetric pairs with the length of cells in symmetric pairs. This suggests that asymmetry is more likely caused by inaccurate positioning of the septum within a dividing cell rather than as a consequence of abnormal elongation in one of the sisters. A computer model was used to study the effects of asymmetric division. A certain proportion \( p \) of cells was allowed to divide in the ratio of 1:1, and the rest were allowed to divide symmetrically. These parameters for asymmetric division were considered to be the same for cells of all lengths.

Cell division was assumed to occur when cells had reached certain minimum length \( L_D \) and also reached a minimum age \( a_0 \). For the growth laws used, cells in the population that were born with length less than \( \frac{1}{2} L_D \) divided at length \( L_D \) at an age greater than \( a_D \) (i.e., had a longer generation time), and those with birth length greater than \( \frac{1}{2} L_D \) divided at age \( a_D \) with a length greater than \( L_D \).

A growth law according to which the cell length growth rate doubled at lengths \( 2A, 4A, 8A \), etc. was used. Results in Fig. 5 show the output of a computer run for two values of \( A \). They have a defect similar to that in the distribution calculated for Fig. 3c; the peak is too
This was not due to the particular values of the parameters used for simulating asymmetric division, as the peak shape proved to be insensitive to variations in these parameters (results not shown). The birth length distribution generated by the model contains over 90% of the cells in two length classes, being therefore much more compressed than the observed one (Fig. 1b). Asymmetric division alone cannot account, then, for the observed cell length distributions, as it does not produce a large enough spread in newborn cell lengths; it does, however, account for some of the cells in the “tails” of the distribution at short and long lengths.

Varying the value of \( \Lambda \) changes the shape of the distribution; however, values of \( \Lambda \) much different from those in Fig. 5 will produce the drop from the peak at a length class quite different from that observed in real populations. One special case in which the doubling in rate occurs at birth, as suggested by Donachie et al. (4), is shown in Fig. 6. Here the minimum division length is 4\( \Lambda \), so that the growth rate is constant over most of the cell cycle. In this case, the length distribution does not have a well-defined peak. A similar situation occurred when a simulation using an exponential growth law was run.

Higher limit for cell length. In our computer-modeled population, there was a lower limit for the length of a cell defined by the value

\[
L_{\text{min}} = L_D/(1 + z)
\]

that corresponded to the length of the short daughter of a cell that divides asymmetrically at

---

**FIG. 4.** Calculation of cell length distributions assuming the observed birth length distribution and the exponential and step growth laws. The calculation is not valid for lengths greater than 5 \( \mu m \) because it ignores cell division. Symbols: \( \triangledown \), exponential law; \( \bullet \), step law; \( \circ \), actual measured distribution.

**FIG. 5.** Distribution of length in a simulated population in which the rate of elongation was assumed to double at 3.8 \( \mu m \) when \( L = 1.37 \cdot L_D \) (a) or when \( L = 1.50 \cdot L_D \) (b). The simulation lasted 10 cycles. The computer was fed the values \( p = 24\% \) and \( z = 1.17 \). Symbols: \( \bullet \)–\( \bullet \), distribution of length in simulation; \( \circ \)–\( \circ \), actual distribution of length measured in a population of E. coli B/r, shown for comparison.
Fig. 6. Distribution of length in a simulated population (---) in which the rate of elongation was considered to double at length 2\(\lambda = 2.8 \mu\text{m}\) coincident with division and then at each successive doubling of total length. The simulation lasted 10 cycles. Parameters \(p\) and \(z\) were fixed as in Fig. 5. Distribution of length in actual population of \(E.\coli\) (---) shown for comparison.

length \(L_\nu\). However, no upper limit was imposed in the model, as it could not be defined in terms of cell elongation alone. In any case, the proportion of cells longer than 8\(\lambda\) after a simulation lasting 100 generations was less than 3% of the total, which made their contribution to the simulated distribution insignificant.

It seems possible, nevertheless, that although small increases in length will not shorten the generation time of a cell, increases in length large enough to contain additional units of initiation mass (5) can impose an upper limit to cell length. This follows from the reasoning that, in such long cells, additional rounds of DNA replication can be initiated and, once completed, will allow an additional division of the cell. Additional divisions could work as a compensating factor to limit the individual length of the progeny of very long cells.

One example of additional division is presented in Fig. 7, which shows a cell whose length at the time of cell separation was 13.50 \(\mu\text{m}\) instead of the minimum length at division of 5.30 \(\mu\text{m}\) for \(\tau = 22\) min (4). This cell divided for the second time 10 min after its first division; it divided for the third time approximately 20 min after the second division. At the time of the third division, some of the progeny lay within the length range of the control cell, which was dividing at approximately 22-min intervals, as expected.

**DISCUSSION**

The calculation of the rate of cell elongation shown in Fig. 2 agrees with the model of Donachie et al. (4) in that there is a doubling in rate at a certain cell length. They estimated, from a shift-up experiment with a population of homogeneous size, that the change occurs at 2.8 \(\mu\text{m}\). However, our results indicate that such a change occurs between 3.5 and 4.5 \(\mu\text{m}\). This spreading could be due either to a spread in each individual cell or to differences between the cells. It is not technically feasible to observe such spreading if the experimental approach of Donachie et al. (4) is used, which probably accounts for part of the discrepancy between the two results.

The results of Collins and Richmond for \(Bacillus\ cereus\) (2) are not incompatible with our model. They used a normal distribution for their distribution of newborn cells, which is not a good approximation when compared with our results in Fig. 1. They also introduced a mathematical smoothing step, thereby reducing the chances of finding abrupt changes in rate. Our results suggest that such changes in rate are responsible for the sudden drop observed in cell length distributions. In our simulations, some degree of smoothing was achieved by considering asymmetric divisions; this partially reproduced the tails at both ends of the distribution without affecting the peak and drop regions. Asymmetry in division was nevertheless insufficient to produce a spread in birth lengths as wide as the one found in a population from a membrane eluate (Fig. 1). It was found that, after a simulation lasting 100 cycles, 90% of the newborn cells fell within the two classes adjacent to the expected length at birth.

As the distribution of newborn cells is crucial in relating the growth laws to the distribution of exponential populations (Fig. 4), this failure to generate a large spread in the newborn population explains why our simulated populations do not show an excellent qualitative fit to the measured distribution. It is likely that some discontinuous events during the cell cycle, particularly at the the time of cell division, affect the patterns of partition or elongation in a way unaccounted for by our model, thus producing a wider spread in lengths at birth. In any case, asymmetry by itself can lead to fluctuations in the generation times of individual cells within a population. We explain these fluctuations by considering that cells born shorter than the normal birth length for the growth rate of the culture are delayed in division, whereas those whose birth length is the correct value or larger are not. Those cells longer than normal will not alter their generation times, at least for increases smaller than necessary.
Fig. 7. Division pattern of a cell whose length at division is 2.5 times the $L_d$ predicted by Donachie et al. (4) for $\tau = 22$ min (right-hand side of each frame) compared with a cell that falls within the predicted range of $L_d$ (left-hand side of each frame). Symbols (reflecting events observed in the enlarged negative rather than those visible in the print): ▽, Beginning of septum formation; ▽, completion of the septum; ●, cell separation. Frames were taken every 5 min.
accommodate additional units of initiation mass. However, bigger increases could shorten the generation time for an individual and serve at the same time as a correcting mechanism to impose a higher limit to cell length (Fig. 7).

That cells of the same length can have different ages provides a theoretical explanation for why methods to obtain synchronous populations based on size selection give far-from-perfect results. It follows, as well, that even cultures derived from a single cell will eventually lose synchrony after a few generations, as they will then contain cells whose birth length is smaller than expected for the generation time of the culture. These cells will suffer a delay before they can divide. This agrees with experimental evidence derived from the analysis of cultures obtained from a single cell in which synchrony was lost after no more than 16 generations (P. Meacock, personal communication). The details of the division process are not yet well enough known to allow us an interpretation of asymmetry in division at the molecular level. It is possible that, if septum formation is regulated by some effector present in only a small number of molecules per cell, local differences in its concentration could produce individual diversity at the time of partition.

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LITERATURE CITED