Genet. Res., Camb. (1979), **33**, *pp.* 61–79
With 3 *text-figures*
Printed in Great Britain

Rate of segregation due to plasmid incompatibility

BY JOHN CULLUM* AND PAUL BRODA
Department of Molecular Biology, Kings Buildings, Mayfield Road, Edinburgh EH9 3JR, Scotland

(*Received* 18 *August*, 1978)

SUMMARY

We calculated the rates of segregation due to plasmid incompatibility under several simple models. A common feature of all the models that we considered is that incompatibility is caused by the inability of the segregation mechanism to distinguish between two incompatible plasmids.

We measured the rate of segregation due to incompatibility of a pair of ColE1 derivatives under two conditions: (1) One plasmid was introduced into cells carrying the other by conjugation. (2) Cells carrying both plasmids were maintained by selection and then selection was released.

Interpretation of the results was made more difficult by effects of the plasmids on the host cell's growth rate. These experiments gave results in agreement with the predictions of a random pool replication model. Published results were also in reasonable agreement with this model.

1. INTRODUCTION

Two closely related plasmids are usually unable to coexist in the same cell, a property called 'incompatibility'. San Blas, Thompson & Broda (1974) and De Vries *et al.* (1975) reported the maintenance of two F′ plasmids in the same cell. However, this was very specific for the particular pairs of F′ plasmids and no general incompatibility mutations for autonomous plasmids have been isolated. The lack of success in isolating incompatibility mutations makes it attractive to consider models in which incompatibility is an inevitable by-product of normal replication and segregation functions.

Jacob, Brenner & Cuzin (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

* Present address: Institut für Biologie III, Schänzlestrasse 1, 78 Freiburg i. Br., West Germany.

the resident plasmid. However, two incompatible plasmids can frequently be established in a cell by selection and, on release of selection, segregation is often fairly symmetrical between the two plasmids (Echols, 1963; Uhlin & Nordström, 1975; Cabello, Timmis & Cohen, 1976; Timmis, Andres & Slocombe, 1978). This suggests that the attachment to any site is not permanent although recombination between plasmids might account for some of the apparent displacement of the resident plasmid. 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 & Nordström, 1975) and F′ plasmids (Jamieson & 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, Brenner & Cuzin (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 been suggested for *Staphylococcus aureus* plasmids (Novick & Schwesinger, 1976).

The model of Jacob *et al.* (1963) also predicts a 'democratic' mode of replication, i.e. every plasmid copy is replicated once per generation. This can be tested by using a density shift experiment. 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 NR1 (Rownd, 1969), ColE1 (Bazaral & Helinski, 1970), R1 (Gustafsson & Nordström, 1975), and F′ plasmids (Kline, 1974; Gustafsson, Nordström & Perram, 1978) twice-replicated DNA appeared much more quickly and the results were in better agreement with the random pool model of replication. 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; Finkelstein & 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 simplest interpretation of these results is that the democratic replication model is invalid. The data are consistent with the random pool model but do not prove that this model is valid. In this paper we mainly consider the random pool model. We assumed that replication control acted to restore the number of plasmid copies per cell to a constant number before division.

Positive control of plasmid replication is predicted by the model of Jacob, Brenner & Cuzin (1963). Pritchard, Barth & Collins (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 Jacob model predicts that such a plasmid should have a copy number equal to the sum of the copy numbers of the con-

stituent plasmids and replication should occur from both origins. In contrast, the Pritchard model 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.

Two main models for segregation have been proposed (see Novick, Wyman, Bouanchaud & Murphy, 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 k plasmids in a cell at division then a proportion $1/2^k$ 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. The random segregation model predicts an earlier appearance of plasmid-free cells than the equal number model does under the assumption that equal number segregation continues after plasmid replication is blocked. A low copy number plasmid gave results in agreement with the equal number model. Hashimoto-Gotoh & Sekiguchi (1977) draw similar conclusions from experiments with pSC101, which had 10–14 copies per cell. Novick *et al.* (1975) also interpreted the results of May, Houghton & Perret (1964) with a high copy number plasmid (about 32 per cell) as supporting equal number segregation. However, in this case about 20% of the cells were plasmid-free at the start of the experiment so that it would have been difficult to detect early new segregants.

The equal number and random segregation models in a sense are extreme cases. For the low copy number plasmids some mechanism must exist to ensure that each daughter cell receives at least one plasmid copy. Another possible model to explain this is that a mechanism ensures the distribution of one copy to each daughter cell and the other copies are distributed at random. Such a mechanism is easier to imagine for higher copy number plasmids than an equal number mechanism that must pair up many plasmid copies and distribute them to daughter cells. This would be very difficult to distinguish experimentally from the equal number model. However, we found that the choice of segregation model seemed to be relatively unimportant in predicting the rates of segregation due to incompatibility. We did most of our work with an equal number segregation model as the calculations are easier than for a random segregation model. We compared the predictions with our own data and with published data. The effects of using other simple models instead were also investigated.

2. MATERIALS AND METHODS

(i) *Strains and Media*

Bacterial strains are shown in Table 1. Media, growth conditions and phage T6 preparations were as described in Cullum, Collins & Broda (1978). Kanamycin

Table 1. *Bacterial strains*

Strain	Description	Source*
ED2510	ED6256 (F'*lac traΔI337*)(pML2)	P1 transduction of pML2 from ED2521 to ED3887
GW137	ED6256 (F'*lac traI65*)(pDS1107)	Dr J. Watson
ED3826	JC3272 derivative resistant to colicin E1	Dr N. Willetts
ED2516	ED3826 (pML2)	ED2510 × ED3826
ED2517	ED3826 (pDS1107)	GW137 × ED3826
ED3887	JC6256 (F'*lac traΔI337*)(ColE1)	Dr N. Willetts
ED2521	C600 (pML2)	Dr D. J. Finnegan

* JC6256 and JC3272 are described in Achtman, Willetts & Clark (1971).

(Winthrop) and ampicillin (Beechams) were added to media at 50 μg/ml when required.

(ii) *Segregation rate measurements*

(*a*) Equal volumes of exponentially growing cultures at a concentration of about 5×10^7 cells/ml of ED2510 and ED2517 in one case and GW137 and ED2516 in the other case were mixed. After 40 min mating phage T6 was added to give a final concentration of about 10^{10} pfu/ml. The first samples were taken 10 min later (time zero). The cultures were maintained between 2×10^7 and 4×10^8 cells/ml by serial dilution. At intervals, dilutions of samples from the cultures were plated on L-broth agar containing either streptomycin or streptomycin, ampicillin and kanamycin. Samples were also plated on lactose tetrazolium agar containing kanamycin (for the ED2510 mating) or ampicillin (for the GW137 mating) to check that the number of Lac^+ donor cells surviving phage T6 was less than 1% of the progeny number.

(*b*) Cells containing both pML2 and pDS1107 were obtained by mating ED2510 with ED2517 and plating samples on L-broth agar containing streptomycin, kanamycin and ampicillin. After overnight incubation, colonies were streaked out onto the same medium. Samples from a purified colony were inoculated into 10 ml of L-broth in a side arm flask which contained ampicillin and kanamycin. When this culture reached about 2×10^8 cells/ml it was spun down in a bench centrifuge and resuspended in warm L-broth. The zero time was this time of resuspension. The culture was maintained between 2×10^7 cells/ml and 4×10^8 cells/ml by serial dilution. At intervals dilutions of samples from the culture were plated on streptomycin-containing L-broth agar which also contained ampicillin or kanamycin or both or neither.

3. RESULTS

(*a*) *Random pool replication models*

The main model that we considered contained the following assumptions:

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

(ii) Control of replication is such that all plasmid-carrying cells at division have

the same number of plasmid copies ($2N$); thus N is the average copy number at birth.

(iii) Replication and segregation are independent.

(iv) The replication and segregation mechanisms cannot distinguish between a pair of incompatible plasmids.

(v) Replication follows a random pool model.

(vi) Segregation follows an equal number model.

(vii) The plasmids do not affect the host cell's growth rate.

The first three assumptions are common to all the models that we consider. However, later we consider the effects of changing each of the last four assumptions. We only considered values of N greater than 1 because, if $N = 1$, all cells carrying two incompatible plasmids would segregate the two plasmids completely at the next division.

The initial rate of segregation in a population carrying two incompatible plasmids would depend on the distribution of the two plasmids between cells carrying both. However, after a few generations the distribution of the two plasmids would settle down to a limiting distribution with a corresponding constant steady state rate of segregation, which depends only on the copy number. This was confirmed by computer modelling. This rate is most easily expressed in terms of the half-time for segregation, i.e. the number of generations needed for the proportion of cells carrying both plasmids to fall by half. We calculated the half-time to be (see Appendix):

$$t_{\frac{1}{2}} = \log_e 2/\log_e ((N+1)(2N-1)/((N-1)(2N+1))), \quad (1)$$

where N is the copy number at birth. This is approximately equal to $N.\log_e 2$ for large values of N (say N greater than 10).

This steady state rate should be appropriate for experiments in which the rate of segregation is observed after both plasmids have been established in the same cells by selection. However, in nature the initial conditions are likely to be of one plasmid at a numerical disadvantage after entry of one copy into a cell in which the other plasmid is resident. The higher the copy number the greater this disadvantage will be. We modelled this situation by taking initial conditions in which there was 1 copy of one plasmid and $N-1$ of the other in cells at birth. For $N = 2$ (Fig. 1) the proportion of cells carrying both plasmids decreases exponentially; in fact, this is just the steady state case discussed earlier with one copy of each plasmid present. For higher copy numbers there is an initial high rate of segregation before the rate settles down to the steady state rate (Fig. 1). Such behaviour was seen by Novick & Brodsky (1972) after transfer of a plasmid into cells carrying an incompatible plasmid by transduction. It is interesting to note that the initial rate of segregation is almost independent of copy number for copy numbers above about 10. The way in which we can use such calculations to estimate copy number from segregation data will be discussed later.

In order for the calculations to be useful experimentally we have to relate the copy number at birth (N) to the average copy number in exponentially growing

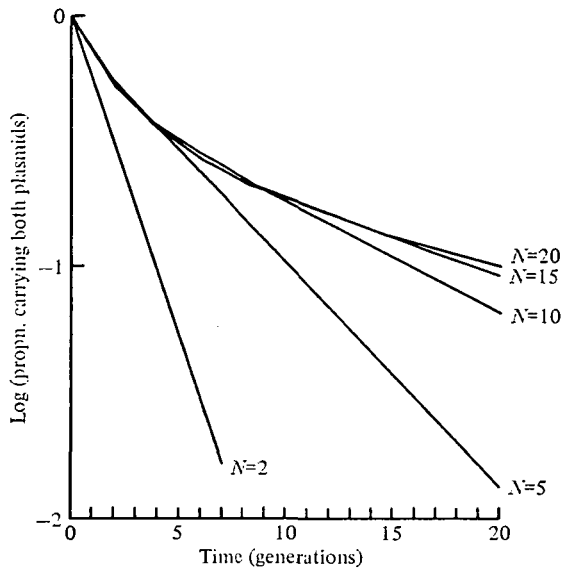

Fig. 1. Proportion of cells that would carry both plasmids, on the model of random pool replication and equal number segregation, starting from newborn cells carrying 1 copy of plasmid 1 and $N-1$ copies of plasmid 2. The model's predictions are shown for $N = 2, 5, 10, 15, 20$. The curves for $N = 30$ and $N = 40$ are too close to that for $N = 20$ to be shown here.

populations ($\bar{N}$). The relationship between N and $\bar{N}$ depends on the timing of plasmid replication in the cell cycle. The system in which this question has been most studied is that of F'*lac* in *Escherichia coli* B/r. Several groups obtained results which suggested that F'*lac* replicated at a particular time, though there was disagreement about the location of this time in the cell cycle (see Finkelstein & Helmstetter, 1977). However, using more direct methods two groups have recently obtained results that suggested that F'*lac* replication is spread throughout the cell cycle (Gustafsson *et al.* 1978; Andresdottir & Masters, 1978). In the case of the plasmids prophage P1 (Abe, 1974) and R1 (Gustafsson *et al.* 1978) replication may also be spread throughout the cell cycle.

If replication is spread uniformly through the cell cycle, the problem of relating $\bar{N}$ to N is analogous to that of relating average cell length ($\bar{L}$) to cell length at birth (L) that was considered by Donachie, Begg & Vicente (1976). They gave the equation $\bar{L} = L/\log_e 2$ and we used the analogous equation for copy number, $\bar{N} = 1{\cdot}44N$. This will be a reasonable approximation if replication is spread throughout the cell cycle, whatever the exact dependence. This equation might still be reasonable even if replication occurs at a particular point in the cell cycle provided it is not too close to cell birth or cell division.

We also considered a random segregation model (i.e. changed assumption (vi)) where each of the $2N$ plasmid copies in a dividing cell has an equal opportunity of entering either daughter cell. This model produces a proportion $(\frac{1}{2})^{2N}$ 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 $0{\cdot}692N - 0{\cdot}67$ 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.

(*b*) *Experiments with ColE1 derivatives*

We measured the rate of segregation due to incompatibility using a pair of ColE1 derivatives, pML2 (= Kan^R) and pDS1107 (= Amp^R). These could be mobilized by an F'*lac traI* plasmid that was unable to transfer itself (Alfaro & Willetts, 1972). We were thus able to study incompatibility after conjugation in the absence of retransfer by the recipients as the ColE1 derivatives cannot transfer themselves. Continuing transfer by the original donors could be eliminated by killing them with phage T6.

Figure 2 (○ and △) 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. 2). The theoretical curve given is for $N = 20$, but the curves for N in the range 20–40 are indistinguishable (see legend to Fig. 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. 2 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. This was probably due to the destruction of the ampicillin in the medium by β-lactamase, as the majority of these segregants carried only pML2 as the viable counts were indistinguishable between kanamycin agar (K) and non-selective agar, and between ampicillin agar (A) and KA. However, after growth in non-selective medium the viable counts on K became less than those on non-selective agar 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. 2, ×) any differential effects of the two plasmids on the host growth rate could be important. Both ED2516 (which carries pML2) and ED2517 (which carries pDS1107) had a generation time of about 22 min 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

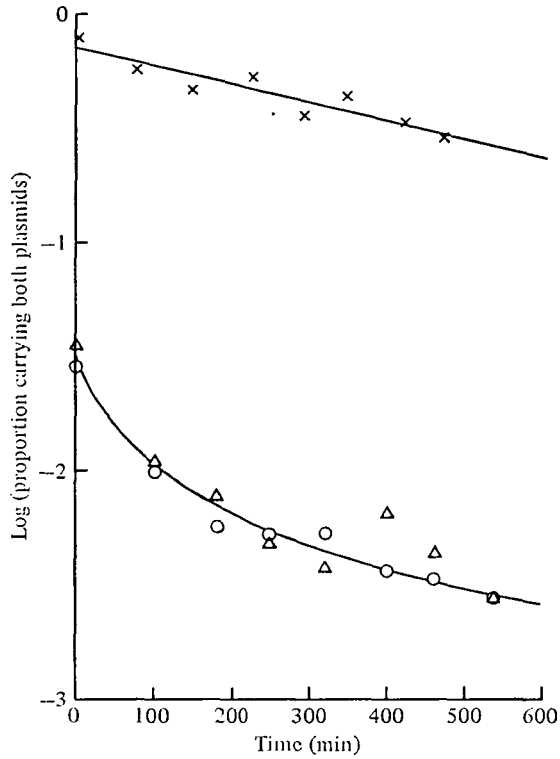

Fig. 2. Ratio of $Kan^R Amp^R$ cells (i.e. cells carrying both plasmids pML2 and pDS1107) to total cell numbers. The generation time was about 22 min. (i) From an ED2510 × ED2517 mating, ○; (ii) from a GW137 × ED2516 mating (× 10^2), △; (iii) starting from a culture grown up in the presence of kanamycin and ampicillin, ×. All $Kan^R Amp^R$ colonies still segregated $Kan^S Amp^R$ and $Kan^R Amp^S$ cells, showing that any stable recombinants could be neglected. The GW137 donor was less fertile than the ED2510 donor in matings with the non-coliginogenic recipient strain ED3826. The theoretical curve for (i) and (ii) above is based on the model used for Figure 1 with $N = 20$. It was fitted to the data using the number of cells containing both plasmids at time 0.

may have an appreciable effect on the estimate of the half-time for segregation. We used the results of three segregation experiments to calculate the copy number under three different assumptions about differential growth (see Appendix):

(i) If all cells had the same generation time, the average copy number ($\bar{N}$) would be 37·5 copies/cell.

(ii) If cells carrying both plasmids had the same growth rate as ED2517, the average copy number ($\bar{N}$) would be 79·7 copies/cell.

(iii) If cells carrying both plasmids had the same growth rate as ED2516, the average copy number ($\bar{N}$) would be 35·7 copies/cell.

Thus any reduction in growth rate of cells carrying both plasmids will have a large effect on the copy number estimate. The higher estimate (assumption (ii)) is 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 & Helmstetter, 1968).

The yields of segregants carrying each type of plasmid can also be predicted under each of the assumptions made above. The predictions were consistent with the experimental data. However, as the experimental estimates involve measuring the differences between viable counts on different selective agar, the data were not good enough to distinguish the different assumptions about the growth of cells carrying both plasmids. In practice, the growth rate may depend on the number of copies of each plasmid present.

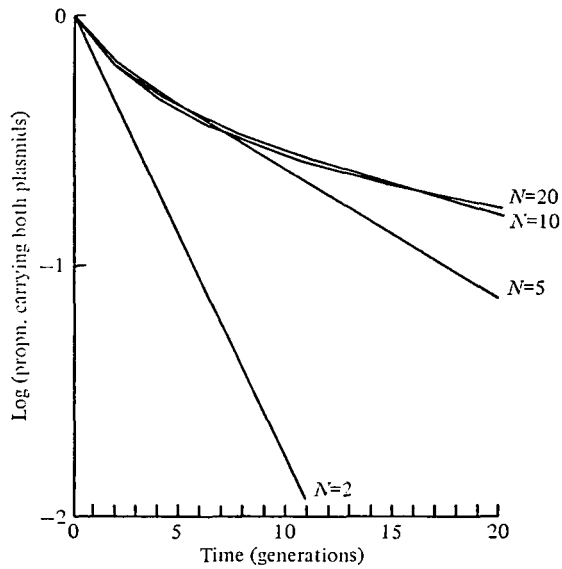

Fig. 3. Proportion of cells that would carry both plasmids, on the model of democratic replication and equal number segregation, starting from newborn cells carrying 1 copy of plasmid 1 and $N-1$ copies of plasmid 2. The model's predictions are shown for $N = 2, 5, 10, 20$. The curve for $N = 15$ is too close to that for $N = 20$ to be shown here.

(c) Other replication models

We also calculated rates of segregation under a democratic replication model. We used an equal number segregation model as a random segregation model leads to rapid plasmid loss; this is because there is no compensating mechanism to restore copy number after unequal divisions. This corresponds to equation (1) of Dowman (1973) with $p_2 = 1$. We used a computer (see Appendix) to calculate the steady state rate of segregation. We found that the half time was approximately

$$t_{\frac{1}{2}} = 1{\cdot}37N - 0{\cdot}96 \quad (N \text{ in the range } 2\text{–}20). \tag{2}$$

This segregation rate is about half that predicted by random pool models with the same copy number at birth. Figure 3 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. 1).

https://doi.org/10.1017/S0016672300018176 Published online by Cambridge University Press

In all the models we have discussed so far the two plasmids are indistinguishable to the replication mechanism. 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 recognized 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 2 shows that there is appreciable asymmetry of segregation if the replication probabilities of the two plasmids are in the ratio of 1·1:1; larger differences result in nearly all segregants carrying only the more successful plasmid.

Table 2. *Effect of biased replication on asymmetric segregation*

Copy number at birth (N)	Ratio of probabilities of replication for the two plasmids	Percentage of segregants carrying only	
		(i) More successful plasmid	(ii) Less successful plasmid
10	1·01:1	52	48
10	1·1:1	66	34
10	1·5:1	94	6
10	2:1	99	1
15	1·01:1	53	47
15	1·1:1	72	28

4. DISCUSSION

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 ColE1 derivative being transferred into a cell in which another derivative was present (Fig. 2). As the segregation is relatively rapid any differential effect of the plasmids on the host growth rate is relatively unimportant. We also considered the steady state segregation rate. If 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, then our estimate (80 copies/cell) is comparable to an estimate (18 copies/genome equivalent) 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 ColE1 copy number can increase considerably (Bazaral & Helinski, 1970). If the effect on growth rate on cells carrying both plasmids is less, the copy number estimate is lower.

It is interesting to note that considerable segregation due to incompatibility occurs even on kanamycin ampicillin medium and that in our case most segregants had lost pDS1107. Study of segregation in non-selective medium showed that this

was not due to asymmetry in segregation; it was probably due to the destruction of ampicillin removing selection for pDS1107. This means that observation of asymmetry between segregant types on selective medium does not necessarily reflect asymmetry of the incompatibility function. This in turn 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.

Table 3. *Calculation of copy numbers from published segregation data*

Plasmid	$t_{\frac{1}{2}}$	Calculated $\bar{N}$	Calculated copy number per genome equivalent[a]	Measured copy number per genome equivalent (by CCC DNA unless otherwise indicated)
F	1·8[b]	3·7	1·8	1·2[c] (by hybridization)
pSC101	6·7[d]	13·9	3·5	5[d] 3–5·5[e] (segregation by temperature-sensitive mutants)
ColE1	14[d]	29·2	7·5	18[d]
R1	4·0[f]	8·3	2	0 4[g]
R483	6·9[h]	14·5	3·6	1[i]

[a] This used the relation between DNA content and growth rate of Cooper & Helmstetter (1968).
[b] Jamieson & Bergquist (1977).
[c] Collins & Pritchard (1973).
[d] Cabello, Timmis & Cohen (1976).
[e] Hashimoto-Gotoh & Sekiguchi (1977).
[f] Uhlin & Nordström (1975).
[g] Engberg, Hjalmarsson & Nordström (1975).
[h] Datta & Barth (1976).
[i] Barth, Datta, Hedges & Grinter (1976).

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 plasmid copy number. This has been shown for ColE1 (Bazaral & Helinski, 1970), F′*lac* (Collins & Pritchard, 1973) and R1 (Engberg, Hjalmarsson & Nordström, 1975). In the case of two plasmids (F and pSC101), where copy number has been determined by methods independent of extracting covalently closed circular DNA, the agreement with our estimate based on published segregation rates is fairly good (Table 3). Cabello *et al.* (1976) gave data for ColE1 derivatives that gave a half time for segregation (Table 3) 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 R1 and R483 the segregation rates gave us copy number estimates several times those made by measuring the amount of covalently closed circular

DNA (Table 3). 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 give 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. It seems unlikely that such experiments could be used to choose between any segregation models which do not distinguish the two plasmids. However, the segregation rate could be used to distinguish random pool and democratic replication models. A democratic replication model gave segregation rates considerably less than those given by a random pool model of corresponding copy number (cf. equations (1) and (2) and Figs. 1 and 3). The latter model gave a better fit to our experimental data (Fig. 2, ○ and △). Measurements of segregation rate might prove useful in testing between random pool models and any other replication models that might be put forward.

One use of our calculations is to predict the copy number of a plasmid from the segregation rate due to incompatibility. If it is assumed that a random pool replication model is valid then the steady state rate of segregation will allow the calculation of copy number. However, 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 estimates of copy number under normal conditions. This is in contrast with methods based on the kinetics of segregation of plasmid-free cells by temperature-sensitive replication mutants at the restrictive temperature, in which 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, in principle, 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 plasmid copies 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 & Sekiguchi, 1977). Durkacz & Sherratt (1973) made the same assumption when considering ColE1 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. Figs. 1 and 3 show that if the copy number at birth (N) is greater than 10 there is little variation with copy number over twenty generations.

In some cases segregation due to incompatibility is asymmetric with one plasmid being favoured (Macfarren & 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 recognized by the replication system. Table 2 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 & Barth, 1976). 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 coexist in a cell. In fact, *inc* mutations in Hfr strains which allow the maintenance of autonomous F′ plasmids (De Vries & Maas, 1973) can be viewed as mutations abolishing incompatibility between the replicons of the chromosome and the autonomous F.

Since this paper was submitted for publication, two further papers in which models for replication and assortment have been considered have appeared (Novick & Hoppensteadt, 1978; Ishii, Hashimoto-Gotoh & Matsubara, 1978).

APPENDIX

(i) *Random pool replication, equal number segregation model*

If there are s copies of plasmid 1 and t copies of plasmid 2 in a cell 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

$$\left(\frac{s}{s+t}\cdots\frac{k-1}{k+t-1}\right)\left(\frac{t}{k+t}\cdots\frac{2N-k-1}{2N-1}\right) = \frac{(k-1)!(2N-k-1)!(s+t-1)!}{(s-1)!(t-1)!(2N-1)!}.$$

However, the probability of going from s to k copies of plasmid 1 and from t to $2N-k$ copies of plasmid 2 is independent of the order of replication of the 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

$$P(s \to k, t \to 2N-k) = \frac{\binom{k-1}{s-1}\binom{2N-k-1}{t-1}}{\binom{2N-1}{s+t-1}} \quad \text{for} \begin{cases} 1 \leqslant s \leqslant k \\ 1 \leqslant t \leqslant 2N-k. \end{cases} \tag{A 1}$$

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 at division, is (using Whittle (1970), equation 4.5.1)

$$P(s|k) = \frac{\binom{k}{s}\binom{2N-k}{N-s}}{\binom{2N}{N}} \tag{A 2}$$

for $$\max(0, k-N) \leqslant s \leqslant \min(N, k).$$

If replication and segregation are independent then, from equations (A 1) and (A 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

$$p_{i,j} = \begin{cases} \sum_{k=\max(i,j)}^{\min(N+i, N+j)} \dfrac{\binom{k-1}{i-1}\binom{2N-k-1}{N-i-1}\binom{k}{j}\binom{2N-k}{N-j}}{\binom{2N-1}{N-1}\binom{2N}{N}} & \text{for} \quad 1 \leqslant i \leqslant N-1 \\ 0 \quad \text{for} \quad i = 0 \quad \text{or} \quad i = N \quad \text{and} \quad i \neq j \\ 1 \quad \text{for} \quad i = 0 \quad \text{or} \quad i = N \quad \text{and} \quad i = j. \end{cases} \tag{A 3}$$

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. We were also able to calculate the steady state rate of segregation exactly. The limiting distribution of the number of plasmid 1 copies per cell at birth for the cells carrying both plasmids is a uniform distribution. We show this by proving that

$$\sum_{i=1}^{N-1} p_{i,j} = \lambda \quad \text{for} \quad j = 1, 2, \ldots, N-1. \tag{A 4}$$

Thus λ is the ratio by which the proportion of cells carrying both plasmids decreases in each generation during steady state segregation. We calculate λ and, hence, the steady state segregation rate. Substitution from equation (A 3) into the left hand side of equation (A 4) gives

$$\begin{aligned} \binom{2N-1}{N-1}\binom{2N}{N}\sum_{i=1}^{N-1} p_{i,j} &= \sum_{i=1}^{N-1}\sum_{k=\max(i,j)}^{\min(N+i,N+j)} \binom{k-1}{i-1}\binom{2N-k-1}{N-i-1}\binom{k}{j}\binom{2N-k}{N-j} \\ &= \sum_{k=j}^{N+j}\binom{k}{j}\binom{2N-k}{N-j}\sum_{i=1}^{k}\binom{k-1}{i-1}\binom{2N-k-1}{N-i-1} \end{aligned}$$

https://doi.org/10.1017/S0016672300018176 Published online by Cambridge University Press

$$= \binom{2N-2}{N-2} \sum_{k=j}^{N+j} \binom{k}{j} \binom{2N-k}{N-j}$$

$$= \binom{2N-2}{N-2} S_j$$

as the sum over i is the coefficient of

$$t^{N-2} \; (= t^{i-1+N-i-1}) \quad \text{in} \quad (1+t)^{2N-2} \; (= (1+t)^{k-1} \, (1+t)^{2N-k-1}).$$

Thus we need to show that S_j is independent of j and we do this by calculating S_j. S_j is the coefficient of $s^j t^{N-j}$ in $\mathscr{S}_j(s, t)$, where

$$\mathscr{S}_j(s,t) = \sum_{k=j}^{N+j} (1+s)^k \, (1+t)^{2N-k}$$

$$= (1+s)^j \, (1+t)^{N-j} \sum_{m=0}^{N} (1+s)^m \, (1+t)^{N-m}$$

$$= (1+s)^j \, (1+t)^{N-j} \, \frac{(1+s)^{N+1} - (1+t)^{N+1}}{s-t}$$

$$= (1+s)^j \, (1+t)^{N-j} \sum_{k=1}^{N+1} \binom{N+1}{k} \frac{s^k - t^k}{s-t}$$

$$= (1+s)^j \, (1+t)^{N-j} \sum_{k=1}^{N+1} \binom{N+1}{k} \sum_{m=0}^{k-1} s^m t^{k-m-1}.$$

Thus,
$$S_j = \sum_{a=0}^{j} \sum_{b=0}^{N-j} \binom{j}{a} \binom{N-j}{b} \binom{N+1}{N+1-a-b}.$$

This is the coefficient of s^{N+1} in $(1+s)^{2N+1}$. Therefore

$$S_j = \binom{2N+1}{N+1}.$$

This is independent of j, so this proves that equation (A 4) is correct and the value of λ is

$$\lambda = \frac{(N-1)\,(2N+1)}{(N+1)\,(2N-1)}. \tag{A 5}$$

The half time for segregation ($t_{\frac{1}{2}}$) is given by

$$t_{\frac{1}{2}} = -\log_e 2 / \log_e \lambda. \tag{A 6}$$

Expansion of $\log_e \lambda$ in powers of $1/N$ showed that $t_{\frac{1}{2}} \sim N \log_e 2$ as $N \to \infty$.

(ii) *Random segregation*

We also considered a random segregation model in which the $2N$ plasmid copies in a dividing cell are distributed at random between the two daughter cells. The probability of obtaining a daughter cell with s copies of plasmid 1 and t copies of plasmid 2 if the parent has k copies of plasmid 1 and $2N-k$ copies of plasmid 2 at

division is

$$P(s,t|k,2N-k) = \left\{\binom{2N}{s+t}(\tfrac{1}{2})^{2N}\right\}\left\{\frac{\binom{k}{s}\binom{2N-k}{t}}{\binom{2N}{s+t}}\right\},$$

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 in the daughter cell at birth given that the daughter cell receives a total of $s+t$ plasmid copies. This simplifies to

$$P(s,t|k,2N-k) = (\tfrac{1}{2})^{2N}\binom{k}{s}\binom{2N-k}{t} \quad \text{for} \quad \begin{cases} 0 \leqslant s \leqslant k \\ 0 \leqslant t \leqslant 2N-k. \end{cases} \tag{A 7}$$

This segregation model produces plasmid-free cells at a rate of $(\frac{1}{2})^{2N}$ per generation. Taking these cells into account in the calculations is inconvenient so we restricted attention to cells that carried plasmids. This accounts for the normalizing term $2^{2N}-1$ that appears in the equations below. Combining equations (A 1) and (A 7) shows that the probability that a plasmid-carrying cell at division contains j copies of plasmid 1 given that the generation before there were i copies of plasmid 1 at division is

$$q_{i,j} = \begin{cases} \dfrac{1}{2^{2N}-1} \displaystyle\sum_{s=1}^{\min(i,j)} \sum_{t=1}^{\min(2N-i,2N-j)} \dfrac{\binom{j-1}{s-1}\binom{i}{s}\binom{2N-j-1}{t-1}\binom{2N-i}{t}}{\binom{2N-1}{s+t-1}} \\ \qquad \text{for} \quad 1 \leqslant i \leqslant 2N-1, \quad 0 < j < 2N \\ 0 \quad \text{for} \quad i = 0, \quad j > 0 \quad \text{or} \quad i = 2N, \quad j < 2N \\ 1 \quad \text{for} \quad i = 0, \quad j = 0 \quad \text{or} \quad i = 2N, \quad j = 2N \\ \dfrac{2^{2N-i}-1}{2^{2N}-1} \quad \text{for} \quad 1 \leqslant i \leqslant 2N-1, \quad j = 0 \\ \dfrac{2^{i}-1}{2^{2N}-1} \quad \text{for} \quad 1 \leqslant i \leqslant 2N-1, \quad j = 2N. \end{cases} \tag{A 8}$$

We used a computer to calculate $q_{i,j}$ for N in the range 2–20 and used this to find the segregation rates from a variety of starting distributions of the two plasmids.

(iii) *Differential growth rates*

We set up differential equations for the number of cells carrying only plasmid 1 (x), only plasmid 2 (y) and both plasmids (m). We assumed that cells carrying both plasmids grew exponentially with rate constant k and segregated symmetrically into cells containing only one type of plasmid at a rate s. We assumed that cells carrying only plasmid 1 grew at a rate $k-e$ and that cells carrying only plasmid 2 grew at a rate $k+d$. These assumptions gave the equations:

$$\begin{aligned} \dot{x} &= (k-e).x+s.m/2, \\ \dot{y} &= (k+d).y+s.m/2, \\ \dot{m} &= k.m-s.m. \end{aligned} \tag{A 9}$$

https://doi.org/10.1017/S0016672300018176 Published online by Cambridge University Press

The set of equations (A 9) is linear and easily solved analytically. We inserted the measured parameters of the initial values of x, y and m into the equations. We used measured values of k and used three choices of the values of d and e to obtain the desired value for s.

(a) No correction: $d = e = 0$.

(b) Cells carrying both plasmids grew at the same rate as cells carrying plasmid 1 alone: $e = 0$ and the value of $d\ (> 0)$ was chosen to give the observed 5% difference in growth rates.

(c) Cells carrying both plasmids grew at the same rate as cells carrying plasmid 2 alone: $d = 0$ and the value of $e\ (> 0)$ was chosen to give the observed 5% difference in growth rates.

(iv) *Democratic replication*

We considered a 'democratic' replication model in which each plasmid copy is replicated once per generation. We used the equal number segregation model (equation (A 2)). This gave the probability that there were j copies of plasmid 1 in a cell at birth given that there were i copies of plasmid 1 at birth one generation before as

$$r_{i,j} = \begin{cases} \dfrac{\binom{2i}{j}\binom{2N-2i}{N-j}}{\binom{2N}{N}} \\ \quad \text{for} \quad 1 \leqslant i \leqslant N-1 \quad \text{and} \quad \max(0,\ 2i-N) \leqslant j \leqslant \min(2i,\ N) \\ 1 \quad \text{for} \quad i = 0, \quad j = 0 \quad \text{or} \quad i = N, \quad j = N \\ 0 \quad \text{otherwise.} \end{cases} \tag{A 10}$$

We used computer programs to calculate $(r_{i,j})$ for N in the range 2–20. We calculated the steady state segregation rate by computer modelling of segregation using the transition probabilities $(r_{i,j})$ and also modelled the situation where cells start with 1 copy of plasmid 1 and $N-1$ copies of plasmid 2.

(v) *Random pool replication with unequal chances of replication*

To obtain equation (A 1) we assumed that the probability of replication was the same for every plasmid copy in a cell. Here we consider a model in which replication is biased in favour of one plasmid so that the probability of replicating plasmid 1 is α times greater than that of replicating plasmid 2. Thus if there were s copies of plasmid 1 in a cell and t copies of plasmid 2, the probability that plasmid 1 would be replicated next is $\alpha s/(\alpha s+t)$ and the probability that plasmid 2 is replicated next is $t/(\alpha s+t)$. We could not obtain an equation analogous to equation (A 1) because each order of replicating the two plasmids has a different probability. We were, however, able to calculate the transition probabilities by using a computer program that summed over all the possible orders of replication. We used this to study the effect of different values of α on the degree of bias in segregation after starting from an equal number of copies of the two plasmids.

We wish to thank the Medical Research Council for a Training Grant (to J.C.) and a Project Grant (to P.B.).

REFERENCES

ABE, M. (1974). The replication of prophage P1 DNA. *Molecular and general Genetics* **132**, 63–72.

ACHTMAN, M., WILLETTS, N. & CLARK, A. J. (1971). Beginning a genetic analysis of conjugational transfer determined by the F factor in *Escherichia coli* by isolation and characterisation of transfer-deficient mutants. *Journal of Bacteriology* **106**, 529–538.

ALFARO, G. & WILLETTS, N. (1972). The relationship between the transfer systems of some bacterial plasmids. *Genetical Research* **20**, 279–289.

ANDRESDOTTIR, V. & MASTERS, M. (1978). Evidence that F'*lac* replicates asynchronously during the cell cycle of *Escherichia coli* B/r. *Molecular and general Genetics* **163**, 205–212.

BARTH, P. T., DATTA, N., HEDGES, R. W. & GRINTER, N. J. (1976). Transposition of a deoxyribonucleic acid sequence encoding trimethoprim and streptomycin resistances from R483 to other replicons. *Journal of Bacteriology* **125**, 800–810.

BAZARAL, M. & HELINSKI, D. R. (1970). Replication of a bacterial plasmid and an episome in *Escherichia coli*. *Biochemistry* **9**, 399–406.

CABELLO, F., TIMMIS, K. & COHEN, S. N. (1976). Replication control in a composite plasmid constructed by *in vitro* linkage of two distinct replicons. *Nature* **259**, 285–290.

COLLINS, J. & PRITCHARD, R. H. (1973). Relationship between chromosome replication and F'*lac* episome replication in *Escherichia coli*. *Journal of Molecular Biology* **78**, 143–155.

COOPER, S. & HELMSTETTER, C. E. (1968). Chromosome replication and the division cycle of *Escherichia coli* B/r. *Journal of Molecular Biology* **31**, 519–540.

CULLUM, J., COLLINS, J. F. & BRODA, P. (1978). Factors affecting the kinetics of progeny formation with F'*lac* in *Escherichia coli* K12. *Plasmid* **1**, 536–544.

DATTA, N. & BARTH, P. T. (1976). Compatibility properties of R483, a member of the I plasmid complex. *Journal of Bacteriology* **125**, 796–799.

DE VRIES, J. & MAAS, W. K. (1973). Description of an incompatibility mutant of *Escherichia coli*. *Journal of Bacteriology* **115**, 213–220.

DE VRIES, J. K., PFISTER, A., HAENNI, C., PALCHAUDHURI, S. & MAAS, W. (1975). F incompatibility. In *Microbiology 1974* (ed. D. Schlessinger), pp. 166–170. Washington: ASM.

DONACHIE, W. D., BEGG, K. J. & VICENTE, M. (1976). Cell length, cell growth and cell division. *Nature* **264**, 328–333.

DOWMAN, J. E. (1973). Implications of stochastic inheritance. *Journal of theoretical Biology* **39**, 55–72.

DURKACZ, B. W. & SHERRATT, D. J. (1973). Segregation kinetics of colicinogenic factor ColE1 from a bacterial population temperature-sensitive for DNA polymerase 1. *Molecular and general Genetics* **121**, 71–75.

ECHOLS, H. (1963). Properties of F′ strains of *Escherichia coli* superinfected with F-lactose and F-galactose episomes. *Journal of Bacteriology* **85**, 262–268.

ENGBERG, B., HJALMARSSON, K. & NORDSTRÖM, K. (1975). Inhibition of cell division in *Escherichia coli* K12 by the R-factor R1 and copy mutants of R1. *Journal of Bacteriology* **124**, 633–640.

FINKELSTEIN, M. & HELMSTETTER, C. E. (1977). Cell cycle analysis of F'*lac* replication in *Escherichia coli* B/r. *Journal of Bacteriology* **132**, 884–895.

GUSTAFSSON, P. & NORDSTRÖM, K. (1975). Random replication of the stringent plasmid R1 in *Escherichia coli* K12. *Journal of Bacteriology* **123**, 443–448.

GUSTAFSSON, P., NORDSTRÖM, K. & PERRAM, J. W. (1978). Selection and timing of replication of plasmids R1*drd-19* and F'*lac* in *Escherichia coli*. *Plasmid* **1**, 187–203.

HASHIMOTO-GOTOH, T. & SEKIGUCHI, M. (1977). Mutations to temperature-sensitivity in R plasmid pSC101. *Journal of Bacteriology* **131**, 405–412.

ISHII, K., HASHIMOTO-GOTOH, T. & MATSUBARA, K. (1978). Random replication and random assortment model for plasmid incompatibility. *Plasmid* **1**, 435–446.

JACOB, F., BRENNER, S. & CUZIN, F. (1963). On the regulation of DNA replication in bacteria. *Cold Spring Harbor Symposia on Quantitative Biology* **28**, 329–347.

JAMIESON, A. F. & BERGQUIST, P. L. (1977). Role of *dnaB43* in F′-plasmid incompatibility. *Molecular and general Genetics* **150**, 161–170.

KLINE, B. C. (1974). Mechanism and biosynthetic requirements for F plasmid replication in *Escherichia coli*. *Biochemistry* **13**, 139–146.

MACFARREN, A. C. & CLOWES, R. C. (1967). A comparative study of two F-like colicin factors, ColV2 and ColV3, in *Escherichia coli* K12. *Journal of Bacteriology* **94**, 365–377.

MAY, J. W., HOUGHTON, R. H. & PERRET, C. J. (1964). The effect of growth at elevated temperatures on some heritable properties of *Staphylococcus aureus*. *Journal of general Microbiology* **37**, 157–169.

NOVICK, R. P. & BRODSKY, R. (1972). Studies on plasmid replication. I. Plasmid incompatibility and establishment in *Staphylococcus aureus*. *Journal of Molecular Biology* **68**, 285–302.

NOVICK, R. P. & HOPPENSTEADT, F. C. (1978). On plasmid incompatibility. *Plasmid* **1**, 421–434.

NOVICK, R. P. & SCHWESINGER, M. (1976). Independence of plasmid incompatibility and replication control functions in *Staphylococcus aureus*. *Nature* **262**, 623–626.

NOVICK, R., WYMAN, L., BOUANCHAUD, D. & MURPHY, E. (1975). Plasmid life cycles in *Staphylococcus aureus*. In *Microbiology 1974* (ed. D. Schlessinger), pp. 115–129. Washington: ASM.

PRITCHARD, R. H., BARTH, P. T. & COLLINS, J. (1969). Control of DNA synthesis in bacteria. *Symposia of the Society for general Microbiology* **19**, 263–297.

ROWND, R. (1969). Replication of a bacterial episome under relaxed control. *Journal of Molecular Biology* **44**, 387–402.

SAN BLAS, F., THOMPSON, R. & BRODA, P. (1974). An *Escherichia coli* K12 mutant apparently carrying two autonomous F-prime factors. *Molecular and general Genetics* **130**, 153–163.

TIMMIS, K. N., ANDRES, I. & SLOCOMBE, P. M. (1978). Plasmid incompatibility: cloning analysis of an *incFII* determinant of R6–5. *Nature* **273**, 27–32.

UHLIN, B. E. & NORDSTRÖM, K. (1975). Plasmid incompatibility and control of replication: copy mutants of the R-factor R1 in *Escherichia coli* K12. *Journal of Bacteriology* **124**, 641–649.

WHITTLE, P. (1970). *Probability*. Penguin Books Ltd.