Can phage defence maintain colicin plasmids in *Escherichia coli*?

Michael Feldgarden, Shelley Golden, Hilary Wilson and Margaret A. Riley

Author for correspondence: Margaret A. Riley. Tel: +1 203 432 3875. Fax: +1 203 432 3854. e-mail: riley@beagle.biology.yale.edu

We examined the role of plasmid-based phage defence in maintaining plasmids, using colicin plasmids in *Escherichia coli* as a model system. Experimental data indicated that the possession of a colicin plasmid can confer limited protection against bacteriophages. A continuous culture model, using these experimental values, indicated that the observed limited protection alone could selectively maintain colicin plasmids, without requiring a competitive advantage due to colicinogeny. Phage defence might explain the current maintenance of colicin plasmids, given the naturally occurring high levels of resistance to colicins. This model also suggests that many plasmids might be maintained in natural populations, in part, by phage resistance, including 'cryptic' plasmids for which no phenotype is known.

Keywords: colicin plasmid, ‘cryptic’ plasmid, phage resistance, phage defence, *Escherichia coli*

INTRODUCTION

Colicin plasmids (Col plasmids) are found in 30% of naturally occurring populations of *Escherichia coli* (Riley & Gordon, 1992). Over 20 distinct Col plasmids have been characterized (Pugsley, 1984; Pugsley & Oudega, 1987). Each encodes toxic proteins, known as colicins, which kill *E. coli* and other closely related species. Many Col plasmids also encode two additional proteins: a lysis protein, which functions in releasing colicin from the producing cell; and an immunity protein, which confers specific immunity to its own colicin upon a host cell.

Due to the requirement for specific cell surface receptor recognition of the colicin prior to uptake into the target cell and subsequent killing action (Pugsley, 1984), *E. coli* and other closely related bacteria have been suggested as the primary target of colicins. The importance of colicins in population-level competition has been inferred from the high frequency with which Col plasmids are found in natural populations of *E. coli* (Pugsley, 1984). Several experimental studies have examined the invasion properties of Col plasmids and concluded that there are growth conditions in which possession of a Col plasmid confers a competitive advantage upon the host bacteria against conspecifics (Bradley, 1991; Chao & Levin, 1981). Further, studies on the molecular evolution of Col plasmids argue that there has been significant positive selection acting on colicins and related proteins (Riley, 1993a, b).

Although Col plasmids have been shown in the laboratory to provide an advantage in intraspecific competition, they are often quite large (> 75 kb) and apparently encode additional functions. Studies have indicated that one additional function may be phage defence (Barker, 1988; Duckworth & Pinkerton, 1988). In one instance, a separate genetic element whose specific function appears to be phage defence is carried on the plasmid; however, other phage defence effects might result from specific phage defence mechanisms, pleiotropic effects of colicinogeny, or unknown side effects of plasmid possession. We chose to survey for phage defence in a wide range of characterized colicin plasmids. Given that there are high levels of resistance to colicins in some natural populations (Riley & Gordon, 1992), we wanted to examine if colicin plasmids might also provide defence against phage predation.

Plasmids encode three types of phage defence mechanisms, of which the best known is the restriction/modification system (Arber & Linn, 1969; Boyer, 1971). Restriction/modification systems are encoded by many plasmids in the lactic streptococci (Klaenhammer, 1984; Sanders, 1988) and in *E. coli* (Bannister & Glover, 1968; B. R. Levin, personal communication). Other mechanisms include resistance induction, superinfection exclusion, and abortive infection. Resistance induction is caused by altering the cell receptors, preventing phage adhesion. In *Streptococcus lactis* ME2, plasmid pME0030 prevents many lytic phages from adhering to the cell
(Sanders & Klaenhammer, 1983). Superinfection exclusion mechanisms allow the phage to adhere to the cell, but block phage DNA entry. Such a mechanism might be involved in the resistance of Streptococcus spp. that carry the plasmid pCLP51R to LP10G phage (Dunny et al., 1988). Abortive infection results in the lysis of the host cell, but the phage replication is terminated before completion. In E. coli abortive infections, the early phage proteins are made, but late protein production does not occur (reviewed by Duckworth et al., 1981). The Col plasmid is the best-studied plasmid encoding abortive infection response (Strobel & Nomura, 1966; Nisioka & Ozeki, 1968; Duckworth & Pinkerton, 1988); however, abortive infection genes also are found on plasmids of lactic streptococci (Gautier & Chopin, 1987; Romero & Klaenhammer, 1990; Sing & Klaenhammer, 1990), the F plasmid (Linial & Malamy, 1970; Morrison & Malamy, 1971), and several R plasmids (reviewed by Duckworth et al., 1981). None of these mechanisms is perfect, but they can lower the rate of infection by one to many orders of magnitude.

Since phage defence mechanisms have been found in a wide range of plasmids, it is conceivable that many plasmids, including 'cryptic' ones (those that lack a readily identifiable function), encode phage defence mechanisms. Here, we examine the effects of 16 characterized Col plasmids on phage infection. The data suggest that certain Col plasmids can significantly lower phage predation success. Using a simulation model, we then examine the population dynamic effects of plasmid-encoded phage defence. Specifically, we have addressed three questions: (1) Can plasmids coding solely for phage resistance be retained in a population? (2) Can plasmids coding solely for phage resistance be retained in a population when the alternative strategy of host chromosomal-based resistance is a viable option? (3) Do plasmid-based phage resistance mechanisms invade populations as successfully as chromosomally-based ones? Results from our simulations suggest that Col plasmids can be maintained simply by selection for their role in phage defence.

RESULTS AND DISCUSSION
Survey of Col-plasmid-mediated phage resistance
Sixteen characterized Col plasmids were examined for their ability to effect bacteriophage plaque formation on an E. coli host cell. The relative e.o.p. values for each of five bacteriophages are presented in Table 1. Overall, the data suggest that Col plasmid possession is effective in decreasing phage predation in about half of the cases examined. With phage T2, possession of a Col plasmid always conferred increased resistance to infection by phage (Table 1). In two of these cases (ColE6 and ColE9) the level of resistance to phage T2 predation was increased by two to three orders of magnitude, relative to the plasmid-less strain. In 14 of the 16 Col plasmids, the increase in phage T2 resistance was highly significant (P < 0.001). For phage T4, the reverse seemed to be true: possession of a Col plasmid frequently led to decreased resistance (Table 1). ColE4, ColE7 and ColE9 conferred increased resistance to phage T4, while cells bearing all other Col plasmids examined had significantly decreased levels of T4 phage resistance. For phages T5 and T7, a mixture of increased and decreased resistance was observed (Table 1). Six Col plasmids (ColE1, ColE2, ColE7, ColE9, ColB and Col) conferred significantly increased resistance to phage T5. All other Col plasmids conferred significantly decreased resistance to phage T5. A different subset of Col plasmids conferred significantly increased resistance to phage T7 (8 of 16 Col plasmids).

These data suggest that possession of these Col plasmids can strongly affect phage predation. The nature of this effect depends on both the phage type and the specific Col plasmid. Although Col plasmid possession could either increase or decrease the level of phage resistance, it resulted in an increased resistance rate of one to five
Can phage defence maintain plasmids in *E. coli*?

*Table 1. Effect of Col plasmids on the infection of the T phages*

<table>
<thead>
<tr>
<th>Col plasmid carried</th>
<th>T2</th>
<th>T4</th>
<th>T5</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>2.22</td>
<td>0.003</td>
<td>0.72</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E2</td>
<td>13.40</td>
<td>&lt; 0.001</td>
<td>0.72</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E3</td>
<td>15.11</td>
<td>&lt; 0.001</td>
<td>0.72</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E4</td>
<td>14.87</td>
<td>&lt; 0.001</td>
<td>1.07</td>
<td>0.19</td>
</tr>
<tr>
<td>E5</td>
<td>5.36</td>
<td>&lt; 0.001</td>
<td>0.47</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E6</td>
<td>126.79</td>
<td>&lt; 0.001</td>
<td>0.11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E7</td>
<td>5.50</td>
<td>&lt; 0.001</td>
<td>0.34</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E8</td>
<td>3.26</td>
<td>&lt; 0.001</td>
<td>0.41</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E9</td>
<td>NP</td>
<td>–</td>
<td>NP</td>
<td>–</td>
</tr>
<tr>
<td>Ia</td>
<td>4.18</td>
<td>&lt; 0.001</td>
<td>0.07</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ib</td>
<td>1.76</td>
<td>&lt; 0.001</td>
<td>0.08</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>A</td>
<td>9.97</td>
<td>&lt; 0.001</td>
<td>0.91</td>
<td>0.06</td>
</tr>
<tr>
<td>B</td>
<td>3.43</td>
<td>&lt; 0.001</td>
<td>NP</td>
<td>–</td>
</tr>
<tr>
<td>D</td>
<td>1.69</td>
<td>0.02</td>
<td>0.19</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>K</td>
<td>3.38</td>
<td>&lt; 0.001</td>
<td>0.65</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>N</td>
<td>1.60</td>
<td>0.36</td>
<td>0.71</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

An e.o.p. value greater than one means that the colicinogenic strain is more resistant to phage than the control. A value less than one means that the colicinogenic strain is more susceptible to phage than the control. NP, No plaques.

Possession of a Col plasmid conferred resistance to T2 attack, but decreased resistance to T4. T2 and T4 resemble each other genetically and phenotypically, utilize the same killing function, and possess 85% total DNA sequence similarity (Birge, 1988). Why does the possession of a Col plasmid have such a dramatically different effect on the infection success of these two closely related phages? Possession of ColIb produced a strong inhibitory effect where resistance rates never decreased by more than one order of magnitude.

While this set of Col plasmids clearly has a mixed effect on phage defence, we observed many instances of increased levels of phage defence. In the second half of this study we explored the question whether phage resistance could account for the maintenance of plasmids in natural populations of *E. coli*, in the absence of other functions.

No previous studies have suggested ColE9 involvement in phage resistance. However, we observed a strong inhibitory effect on phage attack by ColE9. Perhaps there exists on the ColE9 plasmid a gene that operates similarly to the *abi* gene of ColIb. ColE9's closest known relative, ColE2 (Lau et al., 1992; Riley, 1993a), however, does not demonstrate a similar inhibitory power. ColE9 is larger than ColE2; part of this size difference is accounted for by an additional portion of the ColE5 genes coding for colicin, immunity and lysis (Curtis et al., 1989). ColE9, however, possesses approximately 4.3 kb of additional DNA, relative to ColE2, whose function is unknown (M.A. Riley, unpublished). It would be of interest to determine whether this region contains the genetic information accounting for the inhibition of plaque formation by ColE9.

Possession of ColIb produced a strong inhibitory effect against attack by the T-odd phages tested here. Duckworth & Pinkerton (1988) previously demonstrated that ColIb inhibited the growth of T5 through a combination of the plasmid gene *abi*, the T5 A3 gene, and two host genes, tentatively assigned as the potassium transport genes, *trkA* and *trkB*. The presence of all of these genes during a T5 phage attack led to abortive infection. They further found that the ability of the Col plasmid to inhibit attack was completely separate from its ability to produce colicin and immunity proteins. The gene responsible for T7 inhibition, *pic*, is not involved in T5 inhibition (Duckworth & Pinkerton, 1988).

Simulation analysis of plasmid-based phage resistance

We explored, through computer simulation, the feasibility of selection for phage resistance alone in maintaining plasmids in natural populations of *E. coli*. As several chromosomally determined mechanisms conferring increased phage resistance are known to exist (Lenski, 1988; Levin, 1986), we included in our model an investigation not only of the ability for plasmid-encoded phage
resistance to invade and be maintained in a bacterial population, but also of the combined condition of chromosomally- and plasmid-based resistance.

The model. The model assumes that lytic phage, chromosomally-based phage-resistant cells, plasmid-based phage-resistant cells, and susceptible plasmid-free cells are growing in a continuous-flow chemostat with flow rate \( \omega \). Plasmid-free (phage-susceptible) cells grow at a rate \( \psi_a \), chromosomally-based phage-resistant cells grow at a rate \( \psi_r \), and plasmid-bearing phage-resistant cells grow at a rate \( \psi_q \). \( K \) is the resource concentration that supports growth at half the maximal rate. \( \epsilon \) is the amount of resource needed to make one cell. Phage absorb at a rate \( \delta \), and give rise to \( \beta \) phage upon lysis. The plasmid conjugation rate is \( y \), and the segregation rate is \( z \). The nutrient source has an initial concentration of \( C_0 \), and the current nutrient concentration is \( C \). The model assumes that there is no latent burst time for the phage. The change in concentrations of phage (\( P \)), chromosomally resistant cells (\( R \)), susceptible cells (\( S \)), plasmid-bearing resistant cells (\( Q \)), and chromosomally- and plasmid-based resistant cells (\( X \)) can be described by the following equations:

\[
\frac{dS}{dt} = \frac{\psi_a S C}{K+C} - \gamma S R - \delta S P + \tau Q - \omega S + \tau Q
\]

(1)

\[
\frac{dR}{dt} = \frac{\psi_a R C}{K+C} + \gamma S R - \delta R P + \tau X - \tau R - \omega R
\]

(2)

\[
\frac{dP}{dt} = \beta \delta \epsilon S P + \beta \delta R P + \beta \delta Q P + \beta \delta \epsilon X P - \omega P
\]

(3)

\[
\frac{dQ}{dt} = \frac{\psi_q Q C}{K+C} + \gamma S (Q + X) - \delta Q P - \tau Q - \omega Q
\]

(4)

\[
\frac{dX}{dt} = \frac{X \psi_x C}{K+C} + \gamma R (Q + X) - \delta Q P X - \epsilon X - \omega X
\]

(5)

\[
\frac{dC}{dt} = (C_0 - C) \omega - \epsilon C (\psi_q R + \psi_x S + \psi_q Q + \psi_x X)
\]

(6)

These equations are similar to those of Levin & Lenski (1983) and Stewart (1984).

The Euler method was used to approximate the differential equations (Ritger & Rose, 1968), with a step size of 0.05. To determine the effects of changing various parameters of the model, the following biologically plausible parameters were used as the base values (experimental data taken from Lenski, 1988): \( \psi_a = 0.7 \) h\(^{-1} \), \( K = 5 \) mg ml\(^{-1} \), \( \gamma = 10^{-9} \) ml cell\(^{-1} \) h\(^{-1} \), \( \delta = 10^{-7} \) ml h\(^{-1} \), \( \delta_q = 0 \), \( \delta_s = 0 \), \( \epsilon = 2 \times 10^{-4} \) mg, \( \psi_f = 0.56 \) h\(^{-1} \), \( \tau = 10^{-3} \) h\(^{-1} \), \( \omega = 0.2 \) h\(^{-1} \), \( \beta = 100 \), \( C_0 = 100 \) mg ml\(^{-1} \), \( \psi_q = 0.56 \) h\(^{-1} \), \( \psi_x = 0.56 \) h\(^{-1} \). The starting phage density was \( 10^8 \) phage ml\(^{-1} \), and the starting densities of the susceptible, chromosomally resistant, and plasmid-bearing populations were \( 10^8 \) cells ml\(^{-1} \). No cells that were both chromosomally and plasmid-based resistant were present initially.

The complexity of the system precluded a formal stability analysis. Instead, we performed invasion-when-rare simulations. The plasmid- and chromosomally-based resistant populations were depressed to levels ranging from 1 to \( 10^6 \) to assess the robustness of the results.

In the descriptions that follow the levels of phage resistance are characterized as unchanged (\( \delta = 10^{-7} \)), slight (\( \delta = 10^{-8} \)), strong but still incomplete (\( \delta = 10^{-10} \)), or complete, allowing no phage infection (\( \delta = 0 \)). Except when noted, all simulations were run for 500 simulation hours. By this time, all populations either maintained stable population densities or changed very slowly, as ascertained by running several simulations for 5000 simulation hours.

Can Col plasmids conferring phage resistance be maintained, in the absence of chromosomally-based resistance? A plasmid-encoded tenfold increase in phage resistance is sufficient to retain Col plasmids in the absence of chromosomally-based resistance (see Fig. 1). There is no quantitative difference in the equilibrium density of the plasmid-bearing strain between the two scenarios of strong incomplete and complete resistance (where \( \delta_q = 0 \) and \( 10^{-11} \)). When \( \delta_q = 10^{-8} \), the equilibrium plasmid-bearing population is smaller (Fig. 1). At the minimum threshold (slight resistance or \( \delta_q = 10^{-8} \)), the plasmid-bearing strain must have a low maximal growth rate for successful maintenance, since high cell densities will lead to overwhelming phage predation followed by extinction of the bacterial population. A high abundance of moderately protected cells with a high growth rate will enable the growth of a large plasmid-bearing population, which in turn can support a very dense plasmage population. As the plasmid-bearing population becomes larger, its rate of increase will slow due to nutrient limitation. At this point, the dense phage population will decrease the plasmid-bearing population to a point where it cannot make up the losses by predation, causing the extinction of the plasmid-bearing population. When \( \delta_q > 10^{-8} \) the
plasmid-bearing strain cannot be maintained by resistance to phage predation. Our simulations suggest, along with those of previous studies, that plasmids cannot be maintained solely by conjugation (in the presence of phage), as long as there is a cost to plasmid carriage (Levin & Lenski, 1983; Simonsen, 1991).

**How do the dynamics of chromosomally-encoded phage resistance differ from those of plasmid-encoded resistance?**

Does chromosomally-based resistance confer a greater selective advantage upon the cell than plasmid-based resistance? One could imagine that the cost of plasmid carriage would result in a higher fitness of cells that have chromosomally-based, rather than plasmid-based resistance. In the situation where resistance is only chromosomally encoded, complete and strong incomplete resistance ($10^{-10}$ to $10^{-11}$) are effective in maintaining the resistant population relative to the sensitive state (data not shown). When $\delta = 10^{-5}$, the equilibrium density of the plasmid-based resistant population is greater than the chromosomally-based resistant population (data not shown). Finally, chromosomally-encoded phage resistance has a higher threshold than plasmid-encoded resistance. This is because conjugation between the sensitive population and the plasmid-bearing population will offset losses due to predation, allowing $\delta_o$ to be lower (these results are robust for a wide range of parameters; data not shown).

**When plasmid-encoded and chromosomally-encoded phage resistance mechanisms co-occur, which type predominates?**

When both chromosomally- and plasmid-borne phage resistance are complete, or very strong, the highest frequency of resistant cells has both types of phage resistance (see Fig. 2a). Even when plasmid carriage lowers the maximal growth of the cell more than chromosomally-based resistance does, plasmids are still retained at high frequencies. When $\delta_o = 0$ and $\delta_s = 10^{-8}$, the largest class of cells at equilibrium is again the dually resistant population (Fig. 2b), except when plasmid carriage is substantially more costly than chromosomally-based resistance, in which case the dually resistant and chromosomally-resistant populations are present in approximately equal numbers. In this latter case, the plasmid-based phage resistance is more likely maintained by conjugation, as lowering the conjugation rate to levels many orders of magnitude lower than those seen in laboratory and natural populations (Simonsen, 1991; Smit et al., 1993) allows dominance of the chromosomally resistant condition (Fig. 2b). When plasmid-based resistance is complete and chromosomal resistance provides moderate protection ($\delta_s = 10^{-8}$), the plasmid overwhelms the population (Fig. 2c). When both resistance systems provide moderate protection ($\delta_o = \delta_s = 10^{-9}$; $\delta_o = 10^{-11}$), the dually resistant combination dominates (data not shown). Interestingly, even when the conjugation rate is lowered to levels rarely seen in natural populations, the dual resistant state still predominates in this case. These results indicate that plasmid-based phage resistance can maintain plasmids under a wide range of parameters, even when chromosomally-based resistance is a viable option.

**Invasibility when rare: chromosomally-based phage resistance alone**

To determine the effect of chromosomal resistance on the ability of resistant cells to invade a population, the initial starting density of the chromosomally resistant population was varied from $1$ to $10^6$ cells ml$^{-1}$ using the same base parameters (see above). For any density equal to or greater than $2$ cells ml$^{-1}$, the population reached the same equilibrium as when the population was started with $10^6$ cells ml$^{-1}$. The only influence that starting density had was on the approach time: higher initial densities caused equilibrium to be reached more quickly.

**Invasibility when rare: plasmid-based resistant cells alone**

The identical procedure was followed for the plasmid-based resistant population as for the chromosomally-based resistant population. Here too, a minimum density of $2$ cells ml$^{-1}$ was required for the population to reach equilibrium. Likewise, the initial starting density only influenced the time to equilibrium.

**Invasibility when rare: chromosomally-based and plasmid-based phage resistance co-occur**

Using the base model parameters with $\delta_o = \delta_s = 0$, the starting densities of both the chromosomally-based resistant population and the plasmid-based resistant population were singly and together varied from $1$ to $10^6$ cells ml$^{-1}$. When the initial density of the chromosomally resistant cells remained at $10^6$ cells ml$^{-1}$, low densities ($n = 2$ to $10$) of the plasmid-bearing cells resulted in an equilibrium density of plasmid-bearing cells that was lower than the base model (Fig. 2d). When the initial density of plasmid-bearing cells was less than $10^5$, the final density of plasmid-bearing cells was lower; however, the density of the dually resistant cells was also higher (Fig. 2d). In instances where the initial plasmid-based resistant population was more dense than the chromosomally resistant population, the plasmid-bearing population dominated both the dually resistant population and the chromosomally resistant population (Fig. 2e). Regardless of the total densities, when the chromosomally resistant strain and the plasmid-bearing strain were inoculated at equal densities, the result was the same as when the densities were both $10^6$.

**Conclusions**

The results of the model suggest that plasmids might be maintained successfully by selection only for phage resistance. In the absence of chromosomal resistance, even weak plasmid-based resistance will be retained. In fact, plasmid-based resistance can be weaker than chromosomally-based resistance and still be retained. When chromosomally- and plasmid-based resistance co-occur, in most cases the dual possession of chromosomally- and plasmid-based mechanisms is predominant, except when plasmid carriage is very costly relative to the cost of chromosomal resistance. Even when
M. FELDGARDEN and OTHERS

Fig. 2. Final densities of plasmid-bearing resistant (PB), chromosomally resistant (Chr), chromosomally- and plasmid-bearing resistant (Chr+PB) cells, and sensitive (S) cells, under various conditions. (a) When $\delta_1 = 10^{-11}$, and the growth rate of the sensitive strain (0.7 h$^{-1}$; base value) is greater than the growth rate of the chromosomally resistant strain, which in turn is greater than the growth rate of the plasmid-bearing strain. (b) When $\delta_r = 0$ and $\delta_q = 10^{-9}$. The effects of differences in growth rate between chromosomally resistant and plasmid-bearing strains are shown, as is the effect of lowering the conjugation rate ($\gamma = 10^{-12}$). (c) When $\delta_q = 0$ and $\delta_r = 10^{-9}$. Note that the plasmid-bearing and sensitive populations become extinct when the conjugation rate is lowered ($\gamma = 10^{-12}$). (d) When $\delta_r = \delta_q = 10^{-11}$. $R$ indicates the initial density of chromosomally resistant cells and $Q$ indicates the initial density of plasmid-bearing resistant cells. (e) When $\delta_r = \delta_q = 10^{-11}$. $R$ indicates the initial density of chromosomally resistant cells and $Q$ indicates the initial density of plasmid-bearing resistant cells.

plasmid carriage greatly lowers the maximal growth rate, conjugation will prevent plasmids from disappearing. The invasion-when-rare simulations indicate that the results presented here are fairly robust, and are not sensitive to initial starting conditions. However, these results were generated by a deterministic, albeit complex, set of equations. Were stochastic death processes modelled, the initial conditions might play a stronger role in influencing the system.

One interesting result from these simulations is that possession of both phage resistance systems, chromosomally- and plasmid-based, was often the most prevalent outcome, despite the apparent redundancy of the two systems. This situation is analogous to observations on antibiotic resistance, where antibiotic-resistance genes (R genes) have been found to occur on both plasmids and the chromosome (Rubens et al., 1979; Levy, 1986; Murphy, 1989). Previous studies have suggested
that plasmid carriage of R genes may result from their intermittent selection (i.e., since they are not housekeeping genes there is no benefit to their being maintained on the chromosome). However, our study suggests that an alternative explanation may simply be that once R genes moved onto already existing plasmids, by well-explored mechanisms such as transposition (Rubens et al., 1979; Murphy, 1989), they might easily be maintained in this condition.

As described above, many plasmids are known to alter the ability of phage to infect a bacterial cell, through either specified or unspecified mechanisms (Duckworth et al., 1981; Sanders, 1988). Our survey of strains bearing Col plasmids suggests that certain Col plasmids confer increased resistance to phage infection. Considering that E. coli carries an average of four plasmids per strain (Ochman & Selander, 1984), our simulations suggest that Col plasmids, either alone or perhaps in tandem with other plasmids in the cell, might provide sufficient resistance to maintain these plasmids. Perhaps cryptic plasmids in fact function as part of a phage resistance defence array.

ACKNOWLEDGEMENTS

The authors would like to thank Y. Tan, R. R. Wagner and J. Wernegreen for comments on versions of this manuscript. They would also like to thank J. P. Wang and S. Parker for technical assistance. This work was supported by NSF DEB-9458247, NIH GM47471, and a General Reinsurance Award to M. A. R.

REFERENCES


Received 3 April 1995; revised 12 June 1995; accepted 20 June 1995.