COEVOLUTION

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COEVOLUTION IN BACTERIA AND THEIR VIRUSES AND PLASMIDS

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INTRODUCTION

Populations of bacteria can be parasitized by a variety of independently replicating genetic molecules. Based on functional rather than phylogenetic considerations, these replicons are classified as *plasmids* or *viruses*. Plasmids are extrachromosomal molecules of DNA that are present in one or more copies per cell. They replicate at the same average rate as the host chromosome and, in the course of cell division, are transmitted to descendants of infected cells with high frequency. In addition to this capacity for vertical transmission, some plasmids have specific adaptations for horizontal (i.e., infectious) transmission. By processes that require cell-cell contact, copies of these *conjugative* plasmids may be transmitted from *donor* cells to *recipient* cells. For reviews of the basic biology of plasmids, see Meynell (1973), Falkow (1975), and Broda (1979).

Bacterial viruses (*bacteriophage*, commonly contracted to *phage*) differ from plasmids in that they can exist outside the cell, encapsulated in a protein coat that both augments their extracellular term of survival and enables them to attack sensitive cells. Phage infection commences with the adsorption of the virus to specific receptor sites on the bacterium and the passage of its genetic material (DNA or RNA) into that cell. For *virulent* bacteriophage, replication is necessarily by a *lytic* cycle, which terminates with the death of the

host and the release of large numbers of phage particles. *Temperate* bacteriophage may also go through this lytic replication cycle, but there is a certain probability that following adsorption the viral DNA will be maintained as a *prophage* that is stably inherited by the descendants of the originally infected cell. This prophage may be integrated into the host chromosome or, in the case of some temperate bacteriophage, exist as a plasmid-like extrachromosomal element. At rates that depend on environmental conditions, individual bacteria carrying prophage—*lysogens*—will be *induced* and go through a lytic cycle that terminates with the death of the host cell and the release of phage particles. For reviews of the basic biology of bacterial viruses, see Adams (1959), Stent (1963), and Stent and Calendar (1979).

Bacterial viruses and plasmids are parasites in the sense that they have no host-free mode of reproduction and do not unconditionally increase the likelihood of their hosts surviving and reproducing (a definition of parasite somewhat broader than that in Chapter 9 by May and Anderson). Indeed, in the case of virulent bacteriophage, the cost of infection for individual cells is quite dear, and even the carriage of seemingly innocuous plasmids may impose a burden on bacteria. On the other hand, for many plasmids and some phage, the association between these autonomous replicons and their hosts is more that of mutualists. Resistance to antibiotics and heavy metals; the capacity to produce restriction enzymes, toxins, bacteriocins, and antibiotics; the ability to ferment certain carbon sources; and the production of structures for the invasion of specific habitats are characteristics that are often determined by plasmid-borne, rather than chromosomal, genes. (For reviews of the various kinds of plasmid-determined bacterial phenotypes, see Novick, 1974; Chakrabarty, 1976; Broda, 1979; and Davey and Reanney, 1980.) Although the abundance and diversity of phage-determined bacterial phenotypes is less than that of plasmid-determined phenotypes, there are some cases where antibiotic resistance and toxin production are due to genes borne on the prophage of temperate viruses.

Conjugative plasmids and temperate and sometimes virulent bacteriophage also play a role in bacterial adaptation and evolution by serving as vehicles for the exchange of genetic material. In the course of their infectious transmission, these autonomous replicons may pick up chromosomal genes or non-self-transmissible plasmids from one bacterium and may transmit them to another. Because the host ranges of bacterial plasmids and viruses often exceed "species" bounds and because there are mechanisms for recombination in the absence of close genetic homology (insertion sequences and transposable genetic elements; see Calos and Miller, 1980), the range of gene exchange mediated by plasmids and phage can encompass very phylogenetically diverse groups of bacteria.

It seems clear that the association between bacteria and their viruses and plasmids is a very ancient one. Phage and/or plasmids have been found in virtually every species of bacterium that has been examined for their presence. More than 90% of genetically distinct clones of *Escherichia coli* carry at least one plasmid, and the majority of these carry more than one (see, for example, Caugant et al., 1981). It has been estimated that nearly 100% of naturally occurring members of the genus *Pseudomonas* are lysogenic for some temperate virus (Holloway, 1979). For *E. coli* and closely related Enterobacteriacea, more than 200 plasmids and more than 80 species of phage have already been described (Novick, 1974; Reanney, 1976).

Plasmids and phage (as well as their bacterial hosts) can accumulate genetic variability through mutation, recombination, and the acquisition or loss of transposable elements. Because plasmids and phage cannot reproduce outside their bacterial hosts and because these replicons influence their hosts' survival and reproduction, coevolution must be very significant to the overall evolution of bacteria and their plasmids and phage. In this chapter, we will consider the nature and consequences of this coevolution. Using simple models tailored to the specifics of the interactions (Slatkin and Maynard Smith, 1979; Chapter 3 by Roughgarden) between these replicons and their hosts, we will predict the direction of selection on the parameters governing the systems. We will compare these a priori considerations with empirical results obtained from experimental and natural populations of bacteria and their plasmids and phage.

COEVOLUTION IN VIRULENT PHAGE AND THEIR HOSTS: A PRIORI CONSIDERATIONS

A model

A schematic representation of the association between populations of virulent phage and host bacteria is presented in Figure 1. The mathematical model, also presented in the figure, is a modified version of one employed by Levin, Stewart, and Chao (1977) and is analogous to that developed by Campbell (1961). The model assumes that bacteria and phage are thoroughly mixed in a liquid habitat of constant volume; bacterial resources enter and populations are washed out at a constant rate ϱ.

In the absence of phage infection, the bacteria multiply (via binary fission) at a rate ψ, the intrinsic rate of increase of the bacterium under specified environmental conditions. Environmental conditions critical to bacterial growth include temperature and resource concentrations

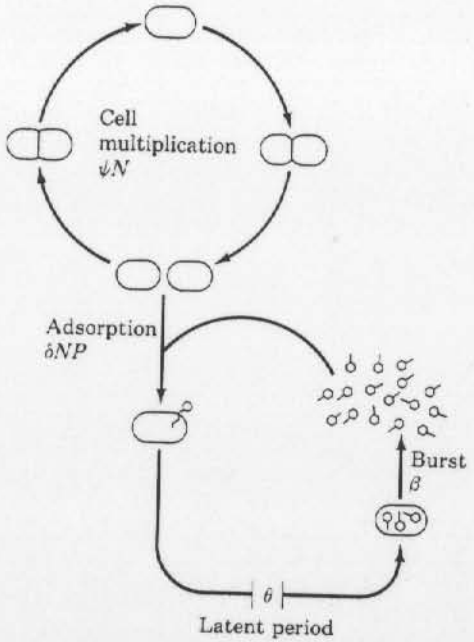

FIGURE 1. Schematic representation of the dynamics of a virulent phage and its bacterial host. N, Density of uninfected host cells; P, density of free phage; ψ, rate of cell multiplication; δ, phage adsorption rate parameter; θ, time between adsorption and burst of infected cells; β, number of phage produced per infected cell; ϱ (not shown in figure), rate of flow through the habitat and concomitant dilution of cell and phage populations. This model can be expressed as time-delay differential equations:

$$dN/dt = \psi N - \delta NP - \varrho N$$
$$dP/dt = \beta e^{-\varrho\theta}\delta N'P' - \delta NP - \varrho P$$

where N' and P' refer to the densities of cells and phage θ time units before the present.

(see Monod, 1949). Phage adsorb to sites on bacterial membranes at a rate that is proportional to cell density N and to the adsorption rate parameter δ. Infected bacteria are removed from the growing cell population because, as a result of infection, they cease multiplication and are fated to die. After a latent period of duration θ (during which the phage multiply in the host cell), a fated cell lyses and bursts, releasing β phage particles. Infected and uninfected cells and free phage are washed out from their populations at a rate ϱ that is independent of their densities.

Heritable variation in the parameters governing the growth and phage infection properties of the bacterial and phage populations can

result in differential rates of survival and reproduction, that is, natural selection. Therefore, by considering the effects of changes in the various parameters of the model, we can make inferences concerning the direction and intensity of selection in these populations.

Selection in the bacterial population

From the perspective of the host bacteria, selection should act to increase the rate of cell multiplication ψ, regardless of the density of phage. Because adsorption leads to cell death, selection acting on the cell population should reduce the rate of adsorption δ; the intensity of this selection will, however, be dependent on phage density. Thus, at low phage density, a mutation rendering a cell resistant to the phage may be disadvantageous if that mutation engenders a significant reduction in the cell's intrinsic rate of increase. The same mutation will likely be advantageous, however, when phage are abundant.

Because neither the burst size β nor the lag time θ directly enters the equation for bacterial growth in the mass-action model presented above, changes in these parameters should not be the direct result of selection acting on the bacterial population.

Selection in the phage population

The growth rate of the phage population is directly related to the burst size β and to the rate of adsorption to uninfected cells δ, and hence any increase in either of these parameters should be favored by selection acting on the phage.

Selection acting on the phage should reduce the latent period θ, although an examination of the time-delay differential equation for phage population growth does not immediately reveal this. An increase in the latent period effectively reduces the burst size, as a result of the washout of infected cells. Because "progeny" phage are subject to the same rate of washout ϱ, whether they are in infected cells or free, the advantage of reducing the latent period is *not* related to ϱ. Instead, the advantage of shortening the latent period lies in the earlier opportunity it provides for progeny phage to infect new host cells and further multiply.

Antagonistic selection and persistence

Unilateral selection in the bacterial population could result in the elimination of the phage from the habitat. If more resistant cells (those

with lower δ) replace the more sensitive and if the phage are unable to increase when rare in a population of these more resistant cells, the phage would be eliminated. In cases where resistance is complete (i.e., $\delta = 0$), the resistant cells could completely displace the sensitive cells *only* if the former have an equal or greater rate of increase ψ. Thus, *if resistance engenders some cost in the competitive performance of the bacteria and if the sensitive cells are able to maintain a stable association with the phage, the evolution of resistance will not lead to the elimination of the phage from the habitat* (Campbell, 1961; Levin et al., 1977).

Unilateral selection in the bacterial population can actually augment the density of the phage population and possibly stabilize its association with the phage. This can be seen most readily by inspection of the equations for the equilibrium density of bacteria and phage. As long as the bacteria and phage can maintain their populations in the habitat, there will be an equilibrium with

$$\hat{P} = \frac{\psi - \varrho}{\partial}$$

$$\hat{N} = \frac{\varrho}{\partial(\beta e^{-\varrho\theta} - 1)}$$

(Levin et al., 1977). Thus, partially resistant bacteria are likely to have a selective advantage in cultures with phage and sensitive bacteria, and their evolution would result in an increase in the equilibrium density of the phage. The net effect of this would be a community that is further from the inelastic boundaries of $N = 0$, $P = 0$.

Selection in the phage population is necessarily antagonistic to the bacteria and can lead to the demise of the phage population. This, too, can be seen by an examination of the above equilibrium equations. Selection in the phage population would favor increases in the adsorption parameter δ and burst size β and reductions in the latent period θ. The effect of these changes in the infection parameters is a reduction in the equilibrium density of the bacteria. An increase in the adsorption rate parameter δ would also reduce the equilibrium density of the phage. The interested reader may wish to contrast the expectations derived from this model with those based on the predator–prey model presented in Chapter 3 by Roughgarden.

COEVOLUTION IN VIRULENT PHAGE AND THEIR HOSTS: EMPIRICAL CONSIDERATIONS AND EXTENSIONS

Resistance and persistence

From a priori considerations, we anticipate that selection in the bacterial and phage populations is antagonistic. The bacteria would be selected for resistance (reductions in δ), whereas the phage would be

selected for higher levels of virulence (increases in δ and β and reductions in θ). It is clear that the potential for this type of antagonistic coevolution exists. One can readily isolate bacterial mutants that are fully resistant to phage to which other members of their clone are sensitive, and it is frequently possible to isolate *host range* phage that can attack these resistant bacteria as well as the sensitive cells. Indeed, bacterial resistance to phage was the phenotype used in the original demonstration of the randomness of mutation (Luria and Delbruck, 1943), and host range phage mutants played a very significant role in the early studies of bacteriophage genetics (Luria, 1945; Hershey, 1946).

This antagonistic selection in bacteria and their virulent phage has been observed in the various studies that have been done with experimental populations (Paynter and Bungay, 1969; Horne, 1970; Levin et al., 1977; Chao et al., 1977). These studies used a variety of different strains of *E. coli* and species of T phage, but in most cases phage-resistant mutants evolved and became the dominant clones. The evolution of these resistant mutants changes the continuous culture populations from a phage-limited to a resource-limited state. In the study by Chao et al. (1977) with *E. coli* B and the phage T7, there were at least three bacterial clones: the original sensitive, one mutant resistant to the original phage, and another mutant resistant to the original phage and to a host range mutant of that phage. At least two phage clones were present in these cultures: the original clone and a host range mutant of that clone. In spite of the antagonistic coevolutionary changes in these experimental populations, they persisted for extended periods of time (more than 80 weeks in the Horne, 1970, study).

It is of particular interest to ask why these "predator-prey" systems are stable (*sensu* persistence). If the bacteria are selected for a resistant mutant and if that mutation is not countered by a host range phage, why would the phage population not be eliminated? In the absence of mutants that are resistant to the phage and with continuous selection for higher levels of virulence, why would the phage not eliminate all of the bacteria? There are a variety of mechanisms that can account for the observed stability of the phage/host system. As demonstrated in a theoretical study by Levin et al. (1977), there are parameter values that specify stable states of co-existence for sensitive bacteria and virulent phage in the absence of genetic changes in their populations. Once one allows for evolutionary changes in these populations, there are at least three ways the association can continue to persist: (1) continuous selection for resistant hosts and counterselection for host range phage; (2) lower competitive performance of the

resistant bacteria (relative to sensitive ones), and lower competitive performance of host range phage (relative to wild type) on sensitive hosts; and (3) the evolution of partially resistant bacteria with rates of adsorption that are still high enough to support the population of virulent phage. We believe that the second and third mechanisms are the primary ones accounting for both the short-term and long-term persistence of the associations between virulent phage and bacteria.

As demonstrated in theoretical studies by Campbell (1961) and Levin et al. (1977), sensitive bacteria and virulent phage can maintain a stable association as long as the phage-resistant cells are at a competitive disadvantage to sensitive cells in the absence of phage. This was observed in the experimental portion of the study by Levin et al. (1977). The introduction of T2-resistant clones of *E. coli* K-12 into chemostat populations of T2-limited *E. coli* B resulted in the ascent of the K-12 clone, with the persistence of the phage and of the sensitive *E. coli* B population. In phage-free competition, the T2-resistant K-12 clone was at a selective disadvantage relative to the T2-sensitive *E. coli* B. A similar result was obtained by Chao et al. (1977), but in that case T7-resistant clones of *E. coli* B evolved in that culture. Both the first-order resistant and second-order resistant *E. coli* in these experiments were, in the absence of phage, at a competitive disadvantage to the sensitive cell population from which they were derived. Chao et al. also found that the host range phage had a selective disadvantage when competing with the wild-type T7 for sensitive hosts.

Constraints on antagonistic selection

From these theoretical and empirical considerations, we postulate that to a great extent the evolutionary stability of phage/host associations can be accounted for by costs associated with resistance and host range shifts. Based on physiological considerations, one would expect that phage resistance imposes a cost on the bacteria (see discussions of constraints in Chapter 2 by Slatkin and in Chapter 3 by Roughgarden). The receptor sites to which the phage adsorb are membrane organelles that are likely to have other functions. Changes in their structure associated with resistance could impair these functions. In addition to the study by Chao et al. (1977), other evidence supports the view that mutations to phage resistance impose a cost. Demerec and Fano (1945) performed 50 pairwise competition experiments with a phage-sensitive clone of *E. coli* B and various mutants that were resistant to one or more T phage. In 34 of these phage-free experiments, the ratio of sensitives to resistants at the end of the experiment exceeded that at the beginning ($p < 0.05$). An analogous physiological argument could be put forth for the anticipated competitive disadvantage of host range phage relative to wild type, but perhaps more appealing is an a posteriori

argument. If host range phage were as fit as or fitter than wild-type phage when competing for sensitive clones, there would be no wild-type phage. Unfortunately, save for the limited study of Chao et al. (1977), we are unaware of any experimental analyses of the relative competitive abilities of wild-type and host range phage. If there is generality to the observations of lower competitive ability for resistant bacteria and for host range phage and if physiological constraints prevent these disadvantages from being readily overcome, then a continuous progression of resistant and host range mutations is not only unnecessary for stability, but unlikely.

In addition to mutations that render bacteria absolutely resistant to phage, there are also those that result in quantitative reductions in the rate of phage adsorption. Although little consideration has been given to these "partially resistant" mutants, both theoretical considerations and informal results suggest they may play an important role in the evolution of stable host/virulent phage associations. One class of partially resistant mutants of *E. coli* excretes a mucilaginous substance (giving their colonies an aesthetically unappealing character). When cultures of *E. coli* K-12 (but not *E. coli* B) are challenged by a variety of different virulent and temperate phage, these mucoid colony types appear in high frequency among the surviving cells.

Cultures of these mucoid cells are able to maintain high density populations in the presence of phage and at the same time support a high density of the phage (B. R. Levin and P. Gidez, unpublished observations). We attribute this to a reduction in the rate parameter of phage adsorption associated with the mucoid phenotype. If these partially resistant mutations have only a small effect on cell growth rate, then their presence could preclude the evolution of fully resistant types. In that way, they might also restrict the evolution of host range mutants.

COEVOLUTION IN TEMPERATE PHAGE AND THEIR HOSTS: A PRIORI CONSIDERATIONS

A model

A schematic representation of the association between populations of temperate phage and host bacteria is shown in Figure 2. Uninfected cells are multiplying at a rate ψ_N, exclusive of losses to the phage and washout. As with virulent phage, temperate phage adsorb to host cells at a rate that is the product of cell density N and to the adsorption parameter δ. In contrast to virulent phage, however, not all infected cells are subject to cessation of growth and lysis. Instead, some frac-

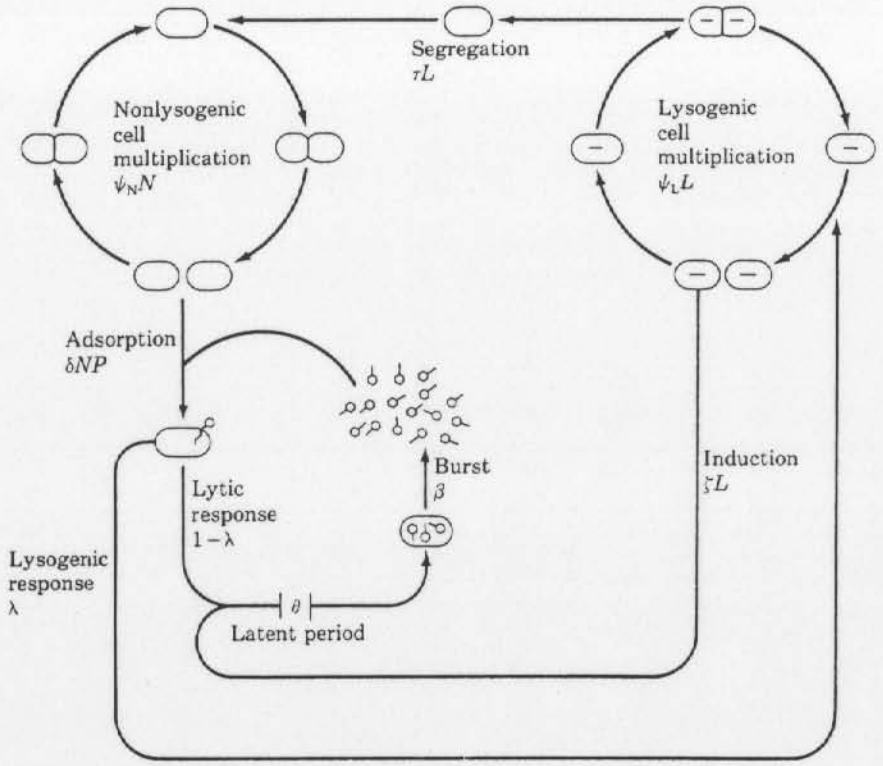

FIGURE 2. Schematic representation of the dynamics of a temperate phage and its bacterial host. N, L, Densities of nonlysogenic and lysogenic cells, respectively; ψ_N, ψ_L, rates of cell multiplication; λ, probability of lysogenic response given adsorption; ζ, rate of induction; τ, rate of segregation; P, δ, θ, β, ϱ, see Figure 1. This model can be expressed as time-delay differential equations:

$$dN/dt = \psi_N N + \tau L - \delta NP - \varrho N$$
$$dL/dt = \psi_L L + \delta\lambda NP - \tau L - \zeta L - \varrho L$$
$$dP/dt = \beta e^{-\varrho\theta}[\delta N' P'(1-\lambda) + \zeta L'] - \delta NP - \varrho P$$

where N', L', and P' refer to population densities θ time units before the present. (After Lwoff, 1953.)

tion λ of the infected cells incorporate the phage genome as a *prophage*. These *lysogenic* cells of density L continue to multiply at a rate ψ_L, which may differ from the growth rate of the nonlysogenic cells. The remainder $(1 - \lambda)$ of the infected cells exhibit the lytic response, just as if the adsorbed phage were virulent.

The lysogenic cells are immune to subsequent infection by phage particles of the same type. (At high levels of superinfection, this immunity may break down. This effect is not present in our model but has been considered in a model by Noack, 1968.) However, at a rate ζ, lysogenic cells are induced to exhibit the lytic response and thus enter the fated cell population. In addition, some lysogenic cells lose the phage genome through segregation at a rate τ, thereby entering the nonlysogenic cell population. As in the previous model, cells and phage are washed out of the habitat at a constant rate ϱ.

Let us again consider the consequences of changes in the various parameters of this model on the growth rates of cell and phage populations. In this case, however, we cannot view selection as acting independently on the sensitive cell, lysogenic cell, and free phage populations. Both the bacterial and phage genomes exist in two states, the former as sensitive cells and lysogens and the latter as prophage and free phage. Thus, in examining selection in this system, it is necessary to consider the phage and bacterial genomes at large rather than separately treat the different states in which they exist.

Selection on the bacterial genome

As in the previous model, any increase in the rate of cell multiplication is favored, for both lysogenic and nonlysogenic cells. Selection acting on the bacterial genome is expected to increase the likelihood of lysogeny (given adsorption), because any infected cell that does not become a lysogen is fated to death. Similarly, selection acting on the bacterial genome should minimize the rate of induction ζ, because induced cells are also fated to lysis.

Selection acting on the bacterial genome is somewhat more complex with respect to the parameters δ and τ. The direction of selection on the segregation rate will depend on the relative growth rates of lysogenic and nonlysogenic cells and on the relative death rates due to lysis of the two cell populations. If $\psi_L - \zeta > \psi_N - \delta P(1 - \lambda)$, then the phage genome is an advantage to its host and selection acting on the bacterial genome should minimize τ. Conversely, if the expected net growth of the nonlysogenic population is greater, then τ should be increased by selection acting on the bacterial genome. As with virulent phage, selection on the host genome should tend to reduce the rate of phage adsorption δ, *unless* the net rate of lysogenic cell multiplication is sufficiently greater than that of nonlysogens to offset the risks of lysis.

Selection on the phage genome

As with virulent phage, selection in temperate phage will be intense for increased rates of adsorption δ, because phage cannot reproduce outside their hosts. Similarly, the burst size β will be maximized and the latent period θ minimized.

The nature of selection acting on the phage genome for the likelihood of the lysogenic response λ is clearly critical to our understanding of the adaptive value of a temperate mode of existence (a $\lambda = 0$ renders a phage virulent). Therefore, it is important to examine the relative contributions of adsorbed phage that exhibit the lysogenic and the lytic responses. After time θ, the lytic response yields β free phage, whereas the lysogenic response nets $e^{\psi_L \theta}$ prophage (cells growing at rate ψ_L for time θ, assuming ζ and τ are near zero). Given realistic values for β (e.g., 100), θ (e.g., 0.5 hours), and ψ_L (e.g., 0.7/hour), the *short-term* dynamics are such that the lytic contribution will be far greater than the lysogenic contribution. However, if we compare the fates of the *progeny* free phage and the *progeny* prophage, we obtain a different conclusion. If nonlysogenic host cells are very rare, then free phage produced via the lytic response will have very low rates of subsequent reproduction, because adsorption to a new host is infrequent. In contrast, the progeny prophage resulting from lysogenic cell multiplication do not need to find a new host and can continue to multiply at the modest cellular rate indefinitely. If the product δN is sufficiently small to offset the short-term advantage of the lytic response, then selection acting on the phage genome should increase the probability of lysogeny (see also Campbell, 1961). *Thus, from the perspective of the phage, temperance appears to be an adaptation to low host-cell densities.*

Selection acting on the phage genome with respect to the rate of lysogen induction ζ will be of opposite direction and similar intensity to selection on λ, because induction is essentially a reversal of lysogeny. Under all conditions, selection in the phage population would be to minimize the rate of prophage loss by vegetative segregation, that is, to minimize τ.

If conditions are such that selection has favored phage temperance, an opportunity exists for the development of a mutualistic relationship with its host. As long as the cost in lower β or δ or higher θ is small, it would be to the advantage of the phage to carry genes that enhance the growth rate of lysogenic cells.

COEVOLUTION IN TEMPERATE PHAGE AND THEIR HOSTS: EMPIRICAL CONSIDERATIONS AND EXTENSIONS

Resistance and immunity

As with virulent phage, selection in temperate phage and their hosts *could* be antagonistic, that is, for more resistant hosts and more virulent phage. In accord with the model, selection would favor hosts that are resistant to the phage; but as long as the rate of mutation to resistant types is less than the probability of lysogeny, immune lysogens are likely to precede resistant clones. When one challenges sensitive *E. coli* with the temperate phage Lambda, the vast majority of surviving cells are Lambda lysogens rather than Lambda-resistant mutants. Indeed, Lambda-resistant clones are most readily isolated by challenging the sensitive bacteria with virulent mutants of Lambda. Thus, although resistance can evolve, the primary evolutionary response to infection with temperate phage should be the rise of the lysogenic population in which selection on the phage and bacterial genomes could be complementary.

At this time, we are aware of only one experimental study that has been directed at the evolutionary response of populations of sensitive bacteria to infection by temperate phage (J. Arraj and B. R. Levin, unpublished). The results of that study, with *E. coli* K-12 and Lambda in chemostats, support the hypothesis that the primary response of the bacteria is the rise of a lysogenic population. However, these results also suggest that the situation is somewhat more complex. Following the rise of Lambda lysogens, clones that are both lysogenic *and resistant* to Lambda appear and achieve substantial frequencies. It may seem redundant to be resistant as well as immune; however, immunity becomes ineffective at high levels of superinfection, whereas resistance apparently does not. The evolutionary importance of resistant lysogens has not yet been explored.

Selection for prophage-determined host phenotypes

In accord with the view that the direction of evolution in temperate phage is toward a mutualistic association with their host, there are a variety of prophage-determined characters that augment the growth rate of their hosts. The most obvious of these is, of course, immunity to subsequent infection by phage of that type, that is, *superinfection* immunity. There are also restriction enzymes coded for by prophage (Arber and Linn, 1969) and prophage-borne resistance to antibiotics (Williams Smith, 1972). Although the mechanism is not immediately apparent, we would anticipate that the diphtheria toxin coded for by the Beta phage of *Corynebacterium diphtheriae* (Uchida et al., 1971) enhances the fitness of the bacterium.

There is some evidence suggesting that prophage genes that augment host fitness are quite general. In a series of pairwise competition studies with *E. coli* in chemostats, Edlin and his colleagues have shown that under some culture conditions lysogens have a competitive advantage over nonlysogenic, resistant cells. They have obtained this result for a variety of different phage: Lambda, P1, P2, and Mu (Edlin et al., 1977; Lin et al., 1977). At this time, it is not clear how these prophage enhance the competitive performance of their hosts.

The advantages of temperance

To us, the most intriguing problem concerning coevolution of bacterial viruses and their hosts is the conditions under which selection favors a temperate rather than a virulent mode of replication. We see three distinct hypotheses: (1) lysogeny enhances the stability of the phage–host association; (2) lysogeny enhances the fitness of the bacteria; and (3) lysogeny is an adaptation to low host densities. Although these hypotheses are not mutually exclusive, we consider the last mechanism to be the most important. We expand on this below.

It seems likely that the associations between temperate phage and their hosts are more stable than those of virulent phage and their hosts, that is, temperate phage are unlikely to drive their hosts to extinction. A number of authors have suggested differential extinction as a mechanism to account for the evolution and maintenance of temperance (e.g., Dove, 1971; Echols, 1972). This mechanism requires that selection operate at the level of the group (interdemic selection), the more stable phage–host associations having a group advantage. However, if virulent phage have an advantage *within* these groups, then the conditions for interdemic selection to favor temperate phage are likely to be very restrictive (Levin and Kilmer, 1974). If temperate phage have an advantage over virulent phage within these groups, then interdemic selection is not necessary.

If the prophage code for characters that enhance the fitness of their hosts, then selection could favor higher probabilities of lysogeny and lower rates of induction, that is, evolution in the direction of greater temperance. However, it seems unlikely that such "niceness" could be the *primary* selective pressure leading to lysogeny. For phage to express genes that enhance the fitness of their hosts, the phage genome would have to be maintained by host cells, that is, form some sort of prophage. Thus, from the perspective of the phage, they would already have to *be* somewhat temperate to *become* temperate. From the perspective of the host, selection would, of course, favor any mechanism that reduces the likelihood of a lytic infection by phage, even if that mechanism results in the maintenance of the phage genome. However, it is unlikely that the evolution of the temperate phage was through unilateral selection in the host population; although there is variation in the probability of lysogeny and rate of induction among bacteria, the proteins involved in insertion and excision (lysogeny and induction) are coded for by phage genes and *not* host genes.

The hypothesis that the temperate mode of phage existence evolved as an adaptation to low densities of sensitive cells seems, to us, the most parsimonious of the three. When sensitive hosts are rare, free phage produced via lytic infections would have low rates of subsequent reproduction, because adsorption to a new host is infrequent. On the other hand, prophage replication does not require a quest for new hosts. Although we know of no evidence to either support or refute this hypothesis, it should be amenable to direct experimental tests.

COEVOLUTION IN PLASMIDS AND THEIR HOSTS: A PRIORI CONSIDERATIONS

A model

In Figure 3 we present a schematic representation of the association between populations of conjugative (i.e., self-transmissible) plasmids and their host bacteria. The model presented in the figure is identical to that employed by Stewart and Levin (1977). The plasmid-free (P-F) and plasmid-bearing (P-B) cells grow at rates ψ and ψ_+, respectively.

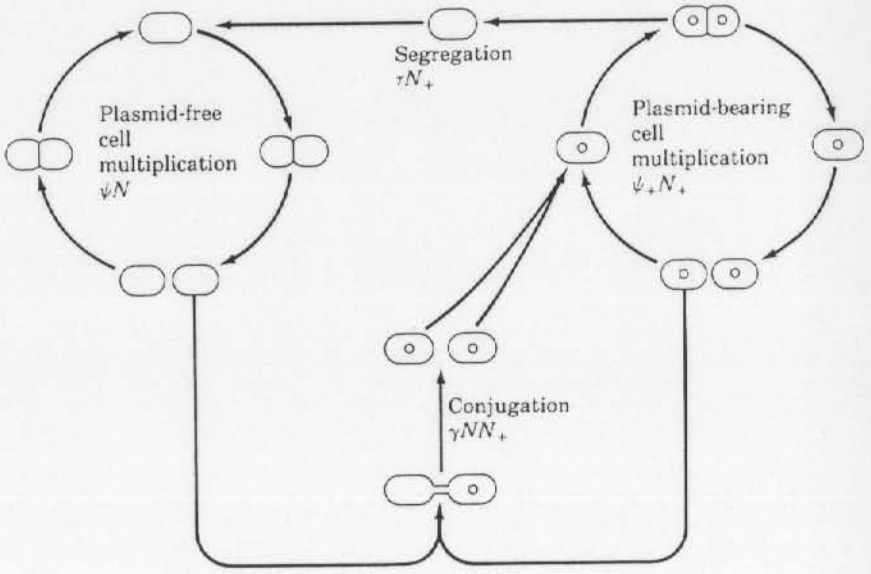

FIGURE 3. Schematic representation of the dynamics of a conjugative plasmid and its bacterial host. N, N_+, Densities of plasmid-free and plasmid-bearing cells, respectively; ψ, ψ_+, rates of cell multiplication; γ, conjugational transfer rate parameter; ϱ, τ, see Figures 1 and 2. This model can be expressed as differential equations:

$$dN/dt = \psi N + \tau N_+ - \gamma N N_+ - \varrho N$$
$$dN_+/dt = \psi_+ N_+ + \gamma N N_+ - \tau N_+ - \varrho N_+$$

P-B and P-F cells encounter one another at random with a frequency that is proportional to the product of their densities. The proportion of encounters resulting in the transfer of a copy of the plasmid from a P-B cell to a P-F cell is governed by the conjugational transfer rate parameter γ. P-B cells can lose the plasmid, and thereby enter the P-F population, via vegetative segregation, at a rate τ.

Selection on the host genome

For both P-B and P-F cells, selection should favor an increase in the exponential growth rates ψ and ψ_+. The intensity of selection on the two growth rates will be independent of densities and relative frequencies of the component populations. Selection acting on the host genome will favor an increase in the segregation rate parameter τ if and only if the growth rate of the P-F cells exceeds that of the P-B cells.

With respect to the conjugative rate parameter γ, selection acting on the host genome is somewhat more complex. Any mutation arising in the P-B cell genome that increases *donor ability* would probably be selected against, because there is likely to be some cost associated with the mechanics of conjugative transfer (e.g., plasmid replication and the synthesis of structures called conjugative pili that are required for conjugation). This prediction is independent of the relative magnitudes of the growth rates of the P-B and P-F cells. However, a mutation that increases the *receptivity* of P-F cells will be favored if the plasmid augments the growth rate of P-B cells. The intensity of this selection for the recipient's contribution to γ will depend on the density of P-B cells.

Selection on the conjugative plasmid genome

Plasmid-borne genes that increase the growth rate or decrease the death rate of cells carrying that plasmid (those determining a higher ψ_+) would be favored by selection. In this model, the intensity of the selection for plasmid-borne genes that enhance host fitness would be independent of the frequency of P-B cells and the density of the population. Under all conditions, selection would favor plasmid-borne genes that augment the stability of the plasmid in the host, that is, that reduce the segregation rate τ. The intensity of this selection would be independent of the density of the population or the frequency of P-B cells.

Selection would favor plasmid-borne genes that increase the rate of infectious transfer of the plasmid. The intensity of this selection for higher γ would be directly proportional to the population density and the frequency of P-F cells. At very low densities of P-F cells, infectious transfer would make a negligible contribution to the rate of increase of the plasmid. Thus, if there were a significant cost associated with conju-

gative ability, selection in the plasmid could actually favor reductions in the rate of infectious transmission when recipients are rare.

Selection on the nonconjugative plasmid genome

From one perspective, nonconjugative (non-self-transmissible) plasmids can be considered as a limiting case of the conjugative factors (where the rate constant of transfer $\gamma = 0$). However, this simple interpretation is not really sufficient. Nonconjugative plasmids, like segments of the host chromosome, can be infectiously transmitted by being "picked up" by either conjugative plasmids or phage, a process known as *mobilization*. In some cases mobilization can be quite effective (e.g., Levin and Rice, 1980).

In modeling the population biology of nonconjugative plasmids, one must consider the population dynamics of the mobilizing replicon(s), as well as that of the nonconjugative plasmid. This is an exercise we shall refrain from in this forum (but see Levin and Stewart, 1980). The primary issue of concern here is that even for non-self-transmissible plasmids, there could be selection for changes in the rates of infectious transfer by mobilization. The nature and direction of this selection would be similar to that considered for the rate constant of conjugative plasmid transfer.

Plasmids coding for allelopathic substances

In the model presented in Figure 3, the plasmids may increase the fitness of cells by augmenting the growth rate of their immediate hosts. There is, however, an important class of plasmid-determined characters for which the plasmid is to the *disadvantage* of the individual host carrying it but which enhance the fitness of the P-B population at large. The most extensively studied plasmid-determined characters of this type are the *bacteriocins* (Reeves, 1972). These are proteins that kill sensitive bacteria of the same or closely related species. In addition to coding for the production of these *allelopathic* molecules, bacteriocinogenic plasmids also confer immunity to these agents. The individual cells carrying these plasmids are at a disadvantage because bacteriocin synthesis and release is lethal to the host cell. At any given time, however, only a small minority of the bacteriocinogenic population is induced to synthesize and release bacteriocin. If there is a cost associated with the carriage of plasmids coding for other antibiotics (such as those produced by the streptomycetes; Hopwood and Merrick, 1977), then these too fall into this class of plasmid-determined phenotypes.

Although the model presented in Figure 3 is not an accurate analog of the population biology of allelopathic plasmids, the major components of the population dynamics of these types of replicons have been mimicked by simple models (Chao, 1979). The primary conclusion drawn from Chao's model of nonconjugative bacteriocinogenic plasmids is that selection for these elements would be frequency-dependent. Cells carrying them could only increase when they are relatively common. The reasons for this are rather straightforward. Due to the lethal synthesis and other costs associated with these factors, bacteriocinogenic cells would be at a disadvantage when competing with sensitive P-F cells, *unless* the concentration of that allelopathic agent is high enough to kill sufficient numbers of P-F (i.e., sensitive) cells to make up for that competitive disadvantage. The latter will occur only when the P-B cells are at relatively high densities.

COEVOLUTION IN PLASMIDS AND THEIR HOSTS: EMPIRICAL CONSIDERATIONS AND EXTENSIONS

Selection and persistence: plasmids are not just "selfish DNA"

Plasmids, unlike phage, have no free state; they are parts of cells, like chromosomes. Thus, one might conclude that their evolution would be toward an increasingly mutualistic relationship with their hosts. There are, however, possible exceptions to this mutualistic form of coevolution. If the plasmids impose a cost on their hosts (i.e., reduce their rates of growth), selection in the plasmid and host populations would be antagonistic. The plasmids would be selected for higher rates of infectious transmission and the hosts for resistance to infection by plasmids and higher segregation rates. Thus, in considering the nature of coevolution in plasmids and their hosts, it is first necessary to ask (1) whether plasmids are likely to impose a fitness cost on their hosts and (2) whether plasmids can be maintained by infectious transfer alone.

In the absence of selection favoring plasmid-borne genes, cells carrying plasmids have a competitive disadvantage relative to identical cells without those extrachromosomal genetic elements. This is, in fact, what one would anticipate on physiological grounds. The additional DNA and protein synthesis associated with the carriage of plasmids must impose some cost on a bacterium. The magnitude of this cost for conjugative plasmids is surprisingly high. In competition experiments between P-B and P-F *E. coli* in conditions where there was no selection for plasmid-borne genes, Levin (1980) reported a 10% lower growth rate for cells carrying the conjugative plasmid R1. Whether plasmids generally impose this high a cost on their hosts remains to be seen.

Using a mathematical model like the one presented here for conjugative plasmids, Stewart and Levin (1977) demonstrated the existence of a broad set of conditions under which infectious transfer can overcome segregational loss and substantial levels of selection against P-B cells and thereby lead to the maintenance of the plasmid in high frequency. Although these conditions for the maintenance of plasmids deleterious to their hosts can be met under laboratory conditions (with high density cultures and mutant plasmids with very high transfer rates), Levin et al. (1979) and Levin (1980) suggest that these conditions are unlikely to obtain in natural populations of bacteria. This idea can be stated as a hypothesis: *for plasmids to be maintained in natural populations of enteric bacteria, they must carry genes that (under at least some conditions) enhance the fitness of their immediate hosts or that of cells carrying that plasmid in the population at large.* "There are no neutrals there" (Reece, 1932), that is, plasmids are not just "selfish DNA" (Doolittle and Sapienza, 1980).

If the above hypothesis is valid and general, then for all plasmids and their hosts, coevolution would necessarily be mutualistic. The existence of a diverse array of plasmid-determined phenotypes is clear evidence in support of this interpretation. Most characters coded for by plasmid-borne genes can enhance the fitness of the bacteria carrying that element. Just how significant plasmid-determined characters are for bacterial adaptation is very dramatically (and from a clinical perspective, frighteningly) demonstrated by the rise of antibiotic resistance. Most clinically important antibiotic resistance in bacteria is determined by plasmid-borne genes; and since the start of the antibiotic era in the late 1940s, the frequency of bacteria carrying antibiotic resistance (R) plasmids has increased enormously. At present, the majority of clinical isolates for some pathogenic bacteria like *Salmonella typhimurium* (gastric enteritis) and various species of *Shigella* (bacterial dysentery) carry at least one R-plasmid (see reviews by Anderson, 1968; Mitsuhashi, 1971; Falkow, 1975).

In addition to the increase in the frequency of bacteria carrying R-plasmids, the number of antibiotic resistance genes carried by single R-plasmids also increased during this period. When these plasmids were first discovered in the late 1950s (Watanabe, 1963), the majority isolated from enteric bacteria conferred resistance to one or two antibiotics. The average number of resistances determined by single R-plasmids increased rapidly during the early 1960s (Anderson, 1968). Currently, it is not uncommon to isolate from enteric bacteria R-plasmids that have four or five resistance genes.

The rapid rate by which plasmids acquire additional antibiotic resistance genes can be attributed to the fact that these genes are often

present as parts of transposable genetic elements (i.e., transposons; Falkow, 1975; Broda, 1979; Campbell, 1981). Genes on transposons do not require sequence homology for recombination and insertion into chromosomes and plasmids. In the course of their travels among different hosts, conjugative plasmids pick up transposons with different resistance genes. As long as bacteria are confronted with a variety of different antibiotics, plasmids that confer resistance to more antibiotics would have a selective advantage.

In accord with this hypothesis of mutualistic coevolution, we would anticipate that if plasmids augment the fitness of their hosts, there would be selection on the host to increase the receptivity to plasmids and reduce the rate of segregational loss. At this juncture, we know of no evidence to either support or refute this interpretation. However, because one can select hosts that are refractory to conjugative plasmids (Reiner, 1974), it should be possible to test this hypothesis.

The allelopathic plasmids

In our a priori considerations, we suggested that selection acting on plasmids that code for allelopathic substances would be frequency dependent. The results of studies that have been done with colicinogenic plasmids in experimental populations support this interpretation (colicins are bacteriocins that affect *E. coli* and closely related species). Using a variety of different Col plasmids, Zamenhof and Zamenhof (1971), Adams et al. (1979), Chao (1979), and Chao and Levin (1981) did competition experiments with colicinogenic and sensitive *E. coli* in chemostats. In all of these experiments, selection favored the colicinogenic cells only when they had initial frequencies in excess of 1%.

Because the sensitive bacteria are being killed by the colicin, it seems reasonable to assume that in these experiments, there would be selection for colicin-resistant cells. This was, in fact, observed in the colicin E1 study of Adams et al. (1979). Chao and Levin (1981) also reported the existence of mutants that were resistant to the colicin E3 they were studying, but in their cultures, these resistant cells did not achieve substantial frequencies. They attributed this to a marked reduction in competitive performance associated with the Col E3 resistance mutation.

The importance of physically structured habitats

Frequency-dependent selection for allelopathic plasmids raises a question about the evolution and maintenance of these types of plasmids. If they cannot increase in frequency *until* they are relatively common, how does one account for their evolution and persistence? One possible explanation is "hitchhiking," that is, bacteriocin-determining genes

are on plasmids that code for characters that enhance the competitive performance of the individual cells carrying them (e.g., antibiotic resistance). An alternate hypothesis, which we favor, was suggested by Reeves (1972) and expanded upon and tested by Chao (1979) and Chao and Levin (1981). According to this hypothesis, bacteriocins (and, by extension, other allelopathic substances like antibiotics) are adaptations for interference competition (Gill, 1974) in *physically structured* habitats. Although cells carrying the Col E3 plasmid could not increase when rare and when competing with P-F sensitive cells in liquid (mass) culture, they could increase when competing in a soft agar matrix.

In a physically structured habitat, the bacteria grow as colonies rather than as individual cells (as they do in mass culture). The allelopathic agents synthesized by induced cells of bacteriocinogenic colonies diffuse out into the environment and kill sensitive cells in the vicinity of that colony. The net effect is reduced competition for limiting resources for colonies of cells carrying plasmids coding for allelopathic substances, and the production of more P-B cells. As a result of this *resource sequestering*, cells carrying allelopathic plasmids have an advantage over sensitive cells at all *frequencies* (although only at high *densities*, where competition is important). Presumably, this same type of mechanism would also favor colonies that are lysogenic for temperate phage.

Constraints on infectious transmission

Because it is copies of plasmids rather than the plasmids themselves that are transmitted by conjugation, it is reasonable to assume that selection would always favor plasmids with higher rates of infectious transmission. But empirical considerations suggest that this is not the case. There are a vast number of apparently viable plasmids that are non-self-transmissible, and among these there are some that are extraordinarily difficult to mobilize with conjugative plasmids. Moreover, most conjugative plasmids isolated from natural populations are repressed for conjugative pili synthesis (Meynell, 1973). At any given time, only a minority of the P-B cell population produces conjugative pili and is capable of transmitting the plasmid. Shortly after the receipt of a plasmid, a cell produces conjugative pili and is capable of transmitting that element. As time proceeds, a plasmid-coded repressor protein accumulates in that cell and its descendants and competence for transfer of that plasmid declines. For the wild-type (repressed) plasmid R1 in steady-state P-B populations, the rate constant

of plasmid transfer γ is three orders of magnitude lower than that for its permanently derepressed mutant, R1-drd-19 (see Levin et al., 1979).

We see two (not mutually exclusive) hypotheses for the evolution of repressible conjugative pili synthesis and the generally lower-than-possible fertility of wild-type plasmids. Anderson (1968) suggested that repression of conjugative pili synthesis is a mechanism to avoid infection by donor-specific bacteriophage that adsorb to conjugative pili. The second hypothesis, which we prefer on grounds of parsimony and generality, asserts that repressible conjugative pili synthesis and the existence of non-self-transmissible plasmids is a consequence of the limited opportunities for transfer in natural populations and the high cost of self-transmissibility. As we pointed out in our a priori considerations, the intensity of selection for infectious transmission is dependent on the density of potential recipients. If the latter is low, as a result of a low overall population density or a low relative frequency of possible recipients, and if there is a cost associated with infectious transmission, selection would favor lower rates of infectious transmission.

The mechanisms for conjugative pili synthesis require a substantial number of genes: approximately 15 megadaltons of DNA or approximately ¼ of the genome of a plasmid like R1 (Willetts, 1972). As a result of this additional DNA and the synthesis of conjugative pili, the burden imposed by conjugative plasmids would even be greater than that for nonconjugative elements. The results of the limited number of experiments we have done on this problem are consistent with this view. In competition experiments with the nonconjugative plasmid pCR1, P-B cells had a disadvantage of less than 5% (relative to P-F cells) as compared to 10% for the conjugative plasmid R1 (Levin et al., 1979). Moreover, cells carrying the permanently derepressed mutant, R1-drd-19, had a disadvantage of between 15 and 20% relative to P-F cells of their type (Levin, 1980). Thus, unless the density of recipients is substantial, one would not anticipate selection to favor higher rates of infectious transmission.

DEFENSE MECHANISMS AND MEASURES TO COUNTER THEM: A POSTERIORI EVIDENCE FOR COEVOLUTION

Compelling evidence for the long-term coevolution of plasmids, phage, and their hosts is the existence of specific systems to prevent and/or limit infections by parasitic DNAs and mechanisms to counter these barriers to their replication. Host defense mechanisms operate in three basic ways: (1) preventing novel DNAs from entering cells, or *exclusion*; (2) destroying novel DNAs that do enter, or *restriction*; and (3) preventing foreign DNA from replicating at a rate sufficient for persistence, or *incompatibility* (Bennett and Richmond, 1978). In the following section, we briefly consider the nature of these defense mechanisms and how they serve as evidence of coevolution among these replicons and their hosts or, in some cases, among different replicons.

Exclusion

The cell envelope is probably the most important barrier to infections by plasmids and phage. To some extent this may be coincidental, perhaps like our "resistance" to Dutch elm disease. However, in other cases it is clear that an exclusion mechanism evolved for the specific purpose of limiting invasions by parasitic replicons. The existence of mutations conferring resistance to bacteriophage surely stands as evidence for the potential for the evolution of exclusion. Analogously, the existence of host range mutations serves as evidence for the potential of phage to evolve mechanisms to overcome cell envelope defense. Some exclusion mechanisms are determined by replicons themselves. Many conjugative plasmids code for mechanisms that prevent entry by closely related plasmids (Novick, 1969). It seems reasonable to assume that these evolved for plasmids to prevent competition for replication within individual bacteria.

Restriction

As is the case with the microparasites of multicellular organisms, once past their host envelope the parasitic DNAs of bacteria have to contend with "immune" systems of their hosts. Some of these intracellular mechanisms to prevent replication of invading DNAs are rather limited in the range of DNAs upon which they can operate, for example, the repressors responsible for superinfection immunity for temperate phage. In other cases, the range of foreign DNAs acted upon is quite broad. The *restriction modification* systems are perhaps the prime example of "immune" systems of the latter type (Arber and Linn, 1969; Stent and Calendar, 1978).

Novel DNAs entering a cell are cut at specific sequences of bases by *restriction endonucleases*. As is the case for the generalized "immune" systems of higher organisms, there is a need to distinguish "self" from "nonself." This is accomplished by modifying bases within the cleaving sequences recognized by the restriction endonuclease, by adding methyl groups to the cytosines or adenines in these cleaving regions. The modification methylases that catalyze the latter reactions are coded for by genes that are closely linked to those for the restriction endonucleases.

The effectiveness of restriction varies considerably among the vari-

ous phage and plasmid DNAs penetrating the cell envelope. In some cases, it is clear that restriction systems are very effective, reducing the likelihood of cells succumbing to a mortal infection by a phage by four or five orders of magnitude. However, as one might anticipate, the modification system necessary for recognizing self limits the effectiveness of restriction. Phage or plasmid DNA that evade restriction are modified and recognized as self DNA. These modified replicons are fully effective against bacteria with that restriction modification system. Some phage, such as T-even coliphage, have 5-hydroxymethylcytosine instead of cytosine as part of their normal genome and are therefore relatively immune to most restriction enzymes. Could it be that the atypical bases of these phage evolved as a mechanism to overcome host restriction?

Those investigators using specific endonucleases for DNA manipulations (e.g., gene splicing) have shown that there are many different restriction enzymes with different cleavage sites. These enzymes are coded for by host, plasmid, and temperate phage genes and are present in a very phylogenetically and ecologically diverse array of bacterial groups. Included among these is *Thermoplasma acidophilium* (McConnell et al., 1978), an Archaebacterium that lives in coal piles and is most readily cultured between pH 1 and 2 at 59°C (Searcy et al., 1981). If restriction modification systems are, as they appear to be, defense against novel DNAs, the *Thermoplasma* situation clearly indicates just how universal the problem of coping with parasitic DNA is to bacteria.

Incompatibility

The successful passage through the gauntlet of exclusion and restriction defenses does not ensure maintenance of a parasitic DNA in a bacterial lineage. For stable inheritance, it is necessary for that DNA to replicate at a rate at least as great as that of the host chromosome and, upon cell division, to be transmitted to both daughter cells. Temperate phage and plasmids have evolved a variety of mechanisms to ensure this vertical transmission. The incorporation into the host chromosome by prophage and the production of multiple copies by plasmids clearly augment the likelihood of vertical transmission. Many autonomous single-copy replicons are also very stable (with vegetative segregation rates of 10^{-5} per generation or less) and some rather extraordinary mechanisms have evolved to ensure their stability (see Austin et al., 1981).

Operating against the stable inheritance of replicons are a variety of incompatibility systems. In some cases, the invading DNA remains intact and expresses its genes but fails to replicate at all (Stocker, 1956; Hayes, 1968). In other cases, replication does occur, but the segregation rate is high and the replicon, usually a plasmid, can only be maintained in a lineage by continuous selection for the genes carried by that element. In some cases these incompatibilities may be coincidental; there is no specific mechanism to preclude replication, but the replicon simply has not evolved a mechanism to allow for its stable inheritance. This may well be the case where the plasmid is transferred to a species that is phylogenetically very distant from the donor. There are, however, incompatibility systems that have clearly evolved as mechanisms to preclude the stable inheritance of invading replicons. The best studied of these are coded by plasmid rather than host genes and operate most effectively against plasmids of the same or closely related types (Falkow, 1975). Why do these and other replicon-determined defense mechanisms fit the cliché of competition being most intense among closely related species?

COEVOLUTION AND BACTERIAL SEXUALITY: MUCH ADO ABOUT VERY LITTLE

Save for the incorporation of free DNA (a process known as *transformation*), plasmids and phage are the sole vectors for the exchange of genetic material between bacteria. Thus, it might seem that a good deal of the coevolution of these organisms would be directed toward the role of plasmids and phage as vehicles of recombination. We suggest that this has not been the case. Although gene exchange mediated by these vectors is unquestionably important to bacterial adaptation and evolution (Reanney, 1976; Bennett and Richmond, 1978; Davey and Reanny, 1980), it is unlikely that natural selection has acted *directly* to increase the effectiveness of these replicons as vectors for recombination.

In natural populations of bacteria, recombination appears to be an extremely rare event from the perspective of an individual bacterium. To be sure, high rates of recombination can be obtained with laboratory strains such as *E. coli* K-12 and permanently derepressed F plasmids that incorporate into the host chromosome (*Hfr*: see Hayes, 1968). However, most naturally occurring plasmids are repressed for conjugative pili synthesis (Meynell, 1973) and do not readily incorporate into the chromosomes of their hosts (Holloway, 1979). Based on estimates of the rate constants of plasmid transfer and phage adsorption, on the likelihood that these vectors will pick up and transfer host genes, and on the densities of natural populations, Levin (1981) suggests that for *E. coli* in their natural habitat the per capita rate of gene

exchange by plasmid- and phage-mediated recombination is as low as or lower than by mutation (10^{-6} per cell per generation or less). The results of electrophoretic studies of structural gene diversity in natural populations of *E. coli* (Selander and Levin, 1980; Caugant et al., 1981) are consistent with this interpretation. Gene complexes are maintained for extended periods of time without being broken down by recombination, and natural populations are far from linkage equilibrium.

The fact that in laboratory culture one can select for plasmid and phage that are effective vehicles for host gene recombination and that vectors of this type do not exist in natural populations is, of course, a posteriori evidence for the absence of selection for high rates of recombination in natural populations. This, however, begs the question of *why* high rates of phage- and plasmid-mediated recombination have not been selected for. We believe that part of this answer lies in the fact that being a vehicle for the transfer of host genes is likely to be a disadvantage for a plasmid or phage. This is clearly the case for some of the plasmids and phage used for recombination analyses in genetic studies. In the case of the F plasmid incorporated into the chromosomes of *Hfr*s, the F replicon is not transmitted in entirety until the whole host chromosome is transmitted (Hayes, 1968). Thus, that plasmid would usually gain neither from the advantages of being in the recombinant nor from the mobility of infectious transfer. For many general transducing phage, the individual virus responsible for recombination contains few or possibly none of its own genes. In the course of replication in the donor cell, it "accidentally" picks up a headful of host genes. Whether all high frequency recombination plasmids and phage used in laboratories are less fit than the native replicons from which they were derived remains to be seen.

A more general explanation for the low frequency of plasmid- and phage-mediated recombination arises from the limitations of natural selection for altering the frequency of occurrence of intrinsically rare events. This can be seen if we take the rather extreme view that the receipt of random genes necessarily augments the fitness of the recombinant (a view we would not want to defend), for example, increasing its growth rate by 10%. If the basal probability of recombination is 10^{-6} and if a mutation doubles the probability of a cell becoming a recombinant (or doubles the probability of a vector transmitting host genes), then for cells of the mutant type the expected growth rate is $\psi(1+2\times10^{-7})$ as compared to $\psi(1+1\times10^{-7})$ for the nonmutant type; this is a very low selective differential and one that is likely to be overridden by stochastic factors or periodic selection (Atwood et al., 1951; Levin, 1981).

In discussing the evolution of sex in bacteria, we have intentionally neglected group or interdemic selection. In fact, we do not believe *any*

selection is necessary to account for plasmid- and phage-mediated recombination in bacteria. It is most parsimonious to assume that these low rates of recombination are the results of errors in replication and infectious transfer for the vector plasmids and phage. Although these errors are not products of natural selection, they play an important role in the adaptation and evolution of bacteria, perhaps approaching the significance of the errors responsible for mutation.

AN OVERVIEW

We have attempted to portray the various types of plasmids and phage as functionally similar genetic elements, that is, as parasitic replicons. Our intent was to deemphasize the differences in their modes of vertical and horizontal (infectious) transmission and offer a more unifying view of this phenomenon. However, because we succumbed to the convenience of separate treatment, we fear the reader may have failed to appreciate a more comprehensive interpretation. For this reason, some emphasis, explanation, and expansion seems warranted.

By suggesting that the various kinds of plasmids and phage are a single type of genetic element, we *do not mean* to imply that they have a common ancestry. This is clearly not the case for bacteriophage and unlikely to be so for plasmids. Bacteriophage have a variety of different genetic molecules: some single-stranded DNA, some double-stranded DNA, and some RNA; and these molecules replicate in a number of fundamentally distinct ways (Stent and Calendar, 1978). Although all known bacterial plasmids appear to be covalently closed circles of double-stranded DNA, this may be the result of convergent evolution due to the constraints of vertical transmission. There are at least two mechanisms of control for the replication of plasmid DNA (Falkow, 1975), and it may well be that the assay methods used may fail to detect plasmids of other types of genetic molecules. Because of these differences, we conclude that extant bacterial plasmids and phage are polyphyletic. Furthermore, it is likely that many plasmids and phage do not have unique ancestries but are chimeras composed of components from a variety of plasmid, phage, and host lineages.

It seems reasonable to suppose that all lineages of bacterial plasmids and phage were originally derived from the DNA or RNA of bacteria or possibly that of higher organisms and that incipient plasmids and phage are continually being generated from these sources. It also seems likely that some plasmids and phage evolved from each other. It is clear that the lines separating the different modes of replication and horizontal transmission are neither sharp nor insurmountable. Some

prophage, such as P1, replicate as plasmids (Ikeda and Tomizawa, 1968). With modest genetic changes, temperate phage can be made virulent (Lwoff, 1953; Ptashne, 1971), conjugative plasmids can be made nonconjugative (Willetts, 1972), and temperate phage can be made into plasmids (Signer, 1969).

Viewing the various types of plasmids and phage as a single type of genetic element is useful for evolutionary and ecological considerations. In their nascent phase, as they emerge from cellular DNA or RNA, all of these elements would be autonomous replicons without specific mechanisms to assure either their maintenance in the descendants of their original host cell or their infectious transmission. They are likely to be confronted with some physiological mechanism of selection by the host to rid itself of the burden of foreign DNA or RNA. To survive in this hostile climate, the incipient plasmid or phage would have to evolve some mechanism for "over-replication" (Campbell, 1981). We see two nonexclusive ways for this to occur: (1) "niceness," that is, acquiring genes that enhance host fitness; and (2) infectious transmission. As long as there is a finite rate of segregation, becoming innocuous (selectively neutral) and replicating with high fidelity would not be sufficient for maintenance.

The route that is taken for over-replication will depend on the genetic and physiological constraints on the replicon and its host and on the environment of the bacterial population. With respect to the latter, we believe that *population density of the host is the primary factor in determining the form of over-replication.* In high density populations of bacteria, replicons with effective mechanisms of horizontal transmission would be favored. The extreme of this would be virulent phage and the resulting antagonistic coevolution. As the density of the host population declined, so would the intensity of selection for infectious transmission. Niceness would become increasingly important for the persistence of the autonomous replicon, and mutualistic coevolution would result. In bacterial populations of intermediate density or in populations with high amplitude oscillations in density, temperate phage and conjugative plasmids would flourish. In populations with sustained low densities, the costs associated with the mechanisms for infectious transmission could not be overridden, and niceness would serve as the only means available for the over-replication of parasitic genetic molecules; under these conditions, nonconjugative plasmids would be favored.

We have made a number of general and specific statements about the nature and direction of coevolution in bacteria and their viruses and plasmids. Although somewhat legitimatized by mathematical modeling and selected facts, most of these statements about how things came to be are no more than microbial "just so stories." As is the case with other evolutionary phenomena, there is no way to formally demonstrate that the suggested pathways are indeed the actual ways things came to be. However, in the case of bacteria and their plasmids and phage, these evolutionary hypotheses can be readily tested with experimental and natural populations. We hope researchers will find some worthy of testing.