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Frequency-Dependent Selection in Bacterial Populations [and Discussion]

B. R. Levin, J. Antonovics and H. Sharma

Phil. Trans. R. Soc. Lond. B 1988 **319**, 459-472
doi: 10.1098/rstb.1988.0059

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Phil. Trans. R. Soc. Lond. B **319**, 459–472 (1988)
Printed in Great Britain

Frequency-dependent selection in bacterial populations

By B. R. Levin

Department of Zoology, University of Massachusetts, Amherst, Massachusetts 01003, *U.S.A.*

There are many situations in which the direction and intensity of natural selection in bacterial populations will depend on the relative frequencies of genotypes. In some cases, this selection will favour rare genotypes and result in the maintenance of genetic variability; this is termed stabilizing frequency-dependent selection. In other cases, selection will only favour genotypes when they are common. Rare types cannot invade and genetic variability will not be maintained; this is known as disruptive frequency-dependent selection. Phage-mediated selection for bacteria with novel restriction-modification systems is frequency-dependent and stabilizing. In mass culture, selection for the production of toxins and allelopathic agents is likely to be frequency-dependent but disruptive. This also occurs in selection favouring genes and transposable elements that cause mutations. Here I review the results of theoretical and experimental studies of stabilizing and disruptive frequency-dependent selection in bacterial populations, and speculate on the importance of this kind of selection in the adaptation and evolution of these organisms and their accessory elements (plasmid, phage and transposons).

Introduction

Frequency-dependent selection can result in either the maintenance of stable polymorphisms or the purging of variation and the persistence of monomorphic populations. In the former case, which I shall call 'stabilizing frequency-dependent selection', genotypes are favoured when they are rare and there is at least one stable internal equilibrium (point or limit cycle). In the latter situation, which I shall call 'disruptive frequency-dependent selection', genotypes are favoured when they are common and all of the internal equilibria are refractory.

Allelopathy: disruptive frequency-dependent selection

Bacteria may produce substances that kill or inhibit the growth of competing bacteria of different genotypes. Included among these allelopathic agents (allelopaths) are the antibiotics and bacteriocins (Reeves 1972). The temperate bacteriophages released by lysogens can also be considered allelopathic substances (Levin & Lenski 1983; Stewart & Levin 1984). In general, bacteria that are capable of producing allelopaths are immune to their action, but for bacteriocins and temperate phages the synthesis of these substances is lethal to the individual induced cells that are producing them. When competing with sensitive bacteria in mass-culture, selection for the allelopathic genotype is frequency-dependent and disruptive. This can be seen from the following variant of the model of the population dynamics of bacteriocins developed by Chao (1979).

Consider a community composed of allelopath-sensitive and allelopath-producing (immune) cells with densities S and P (bacteria per millilitre) respectively and free-allelopath at concentration A (units per millilitre). Producing cells emit the allelopathic agent at a rate

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proportional to their density and a constant, β. In this variant of the Chao model, I assume that the synthesis of the allelopathic substance need not be lethal to the producing cell. Free-allelopath adsorbs to sensitive cells at a rate proportional to the product of its concentration, the density of sensitive bacteria and a rate parameter, δ. Adsorption is lethal to sensitive cells.

The bacteria are maintained in an equable (chemostat) habitat of unit volume (Stewart & Levin 1973) into which resources flow at a rate, ρ (per hour) which is the same at the rate at which excess resources, bacteria and allelopath are removed. Within the habitat, the limiting resource is present at a concentration of r (micrograms per millilitre) and enters the habitat from a reservoir where it is maintained as a concentration C (micrograms per millilitre). Population growth and the uptake of resources occur at rates proportional to the concentration of the limiting resource, $\psi(r)$, and $\psi(r)\,(1-\alpha_P)$ for the S and P populations respectively, where $\psi(r)$ is a monotonic increasing function of r and α_P is the selection coefficient. If α_P is positive, the allelopath-producing cells are at a selective disadvantage. Resource is taken up at a rate proportional to the growth rates of the bacteria, their density and to a 'resource conversion efficiency' parameter e (micrograms). With these definitions and assumptions, the rate of change in population density and the concentrations of resource and the allelopath are given by

$$\dot{r} = \rho(C-r) - \psi(r)\, e[S+P(1-\alpha_P)], \tag{1}$$

$$\dot{P} = \psi(r)\,(1-\alpha_P)\,P - \rho P, \tag{2}$$

$$\dot{S} = \psi(r)\,S - \delta A S - \rho S \tag{3}$$

and

$$\dot{A} = \beta P - \rho A, \tag{4}$$

where a dot ($\dot{}$) denotes differentiation with respect to time.

In a culture of a single clone, as long as the maximum rate of population growth exceeds the rate of loss by flow, $\psi(C) > \rho$ and $\psi(C)\,(1-\alpha_P) > \rho$, each of these populations can be maintained in the habitat and will have a unique equilibrium density (Stewart & Levin 1973). The concentration of resource and bacteriocin in single clone communities would also have a unique equilibrium value. Let $\hat{S}$ and $\hat{P}$, be the equilibrium densities of sensitive cells and of producing cells and $\hat{A}$ be the concentration of allelopath, and $\hat{r}_S$ and $\hat{r}_P$ be the equilibrium concentrations of resources for these two populations when they occur alone.

The S population will only be able to invade an equilibrium community of P when it has a sufficient advantage in growth rate to overcome loss to the allelopath and washout, $\psi(\hat{r}_P) > \delta A + \rho$. The P population can only invade an equilibrium community of S when; (a) it has a growth rate advantage, $\alpha_P < 0$, or (b) its density is sufficient to produce enough of the allelopathic substance to overcome its growth rate disadvantage. This frequency-dependent invasion of P can be seen in the numerical solution to equations (1)–(4), presented in figure 1.

The situation in which the allelopathic agent is a temperate bacteriophage is somewhat more complex, because the allelopathic substance itself can reproduce, either as free phage or as prophage (Lwoff 1953). Although most sensitive bacteria will be killed by lytic infections with free temperate virus, some will become lysogens, and will carry the prophage in a stable state. As a consequence of lysogenic infections, bacteria of the previously non-allelopathic cell line will become allelopathic. The population dynamics of temperate phage and their co-evolution with their hosts have been considered (Campbell 1961; Levin & Lenski 1983; Stewart & Levin

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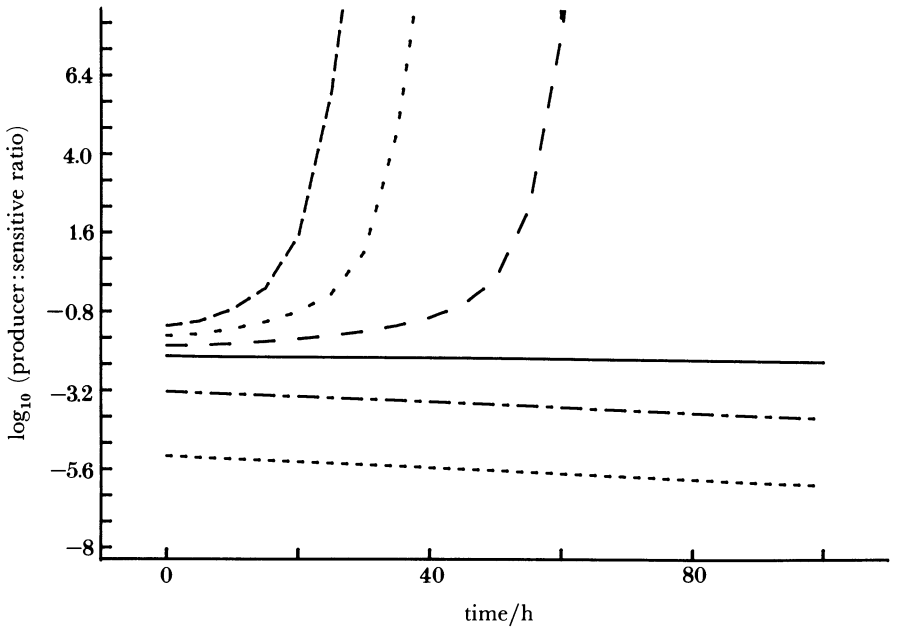

FIGURE 1. Disruptive frequency-dependent selection for allelopathy. Numerical solutions to equations (1)–(4). Log_{10} ratio of producing and sensitive cells. Parameter values: $C = 100$, $\rho = 0.20$, $\psi(r) = 0.70r/(4+r)$, $e = 5 \times 10^{-7}$, $\alpha_P = 0.10$, $\beta = 100$, $\delta = 5 \times 10^{-10}$. Initial values of the variables: $r = 100$, $A = 0$, $P+S = 1.9 \times 10^8$.

1984). Because of constraints in time and space, I shall not consider here this special case of allelopathy.

Empirical observations

Studies of competition between colicin sensitive and colicinogenic *Escherichia coli* in liquid culture are consistent with this hypothesis of disruptive frequency-dependent selection for allelopathy (Zamenhof & Zamenhof 1971; Adams *et al.* 1979; Chao 1979; Chao & Levin 1981). In each of these studies, the unstable equilibrium point above which the colicinogenic population could invade was on the order of 10^{-2}, a 'high' frequency for a bacterial population.

Chao (1979) and Chao & Levin (1981) argued that because of this high unstable equilibrium point, in mass (liquid) culture, bacteriocins (and by extrapolation, other allelopathic agents) that impose a cost in fitness on the producing cells are unlikely to be maintained by selection for allelopathy. Chao (1979) and Chao & Levin (1981) suggest that bacteriocins (and antibiotics) evolved and are maintained by competitive selection in physically structured habitats, where bacteria exist as discrete colonies rather than individual cells.

This 'fixed habitat' hypothesis is consistent with the results of experimental studies. Using *E. coli* with and without a non-conjugative plasmid coding for colicin *E3* (*ColE3*), Chao (1979) and Chao & Levin (1981) presented evidence that in competition with sensitive bacteria in soft agar, colicinogenic cells are able to increase at any initial frequency. This appeared to be due to the killing of sensitive bacteria in the regions around colicinogenic colonies because of the diffusion of colicin, and the consequential formation of zones of inhibition. Hence colicinogenic colonies sequester resources and grow larger (include more cells) than colonies produced by the surviving sensitive bacteria.

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It seems reasonable to assume that the 'colony-level' (group?) selection in these fixed habitats would itself be density- and frequency-dependent. The advantage acquired by the sequestering of resources would be inversely related to the frequency of allelopathic colonies and directly dependent on the absolute density of bacteria, i.e. the extent to which producing colonies compete with sensitive ones. Clearly if the community is so sparse that there is little or no competition between colonies, there would be no advantage in producing the allelopathic agent. Presumably this would also be true in mass (liquid) culture.

At this time I am aware of only one formal (mathematical or numerical) treatment of this type of group-level selection for allelopathy. Chao (1979) developed a computer simulation of the population dynamics of colicins in a two-dimensional habitat. More research on this subject is certainly necessary. It is clear that physically structured habitats and interactions between colonies, rather than individual cells, are important features of the biology of bacterial populations, even if they are inconvenient (difficult) to model.

Detoxification: stabilizing frequency-dependent selection

Bacteria are sometimes capable of detoxifying (neutralizing) compounds that kill or inhibit cell growth and cell division, like antibiotics or heavy metals. In some cases, this detoxification occurs within cells and there is little or no effect on the overall concentration of toxin in the habitat at large. In other cases the denaturing of toxins reduces the effective concentration of these substances in the habitat. Under these conditions, sensitive bacteria that are at a disadvantage, and possibly not even able to maintain populations in the presence of the toxin, can invade and maintain populations in communities where resistant, detoxifying bacteria are present. This can be seen from a variant of the model of competition and detoxification developed by Lenski & Hattingh (1986), given below.

Let S and R be the densities of sensitive and resistant bacteria and t the concentration of the toxin. Assume an equable habitat of unit volume in which toxin and resource are introduced from a reservoir where they are present at concentrations C and T, respectively. These substances enter the habitat at rate ρ, which is the same as the rate at which bacteria, resources and toxin are removed. As in the previous example, the bacteria grow at rates that are monotonic increasing functions of the concentration of a limiting resource, $\psi(r)$ and $\psi(r)(1-\alpha_R)$ for the sensitive and resistant, detoxifying, populations respectively. Resource is taken up at rates proportional to the density of the two bacterial populations, their growth rates and a 'conversion efficiency' parameter, e.

Bacteria sensitive to the toxin are killed at a rate proportional to the concentration of toxin and a parameter, ξ. I assume that the action of toxin is not manifest as a change in its concentration. Resistant bacteria neutralize the toxin at a rate that is a monotonic increasing function of its concentration, $\phi(t)$. With these definitions and assumptions, the rates of change in the concentrations of the different cell populations, resource and toxin are given by

$$\dot{r} = \rho(C-r) - \psi(r)\, e[S + R(1-\alpha_R)], \tag{5}$$

$$\dot{S} = \psi(r)\, S - \xi t S - \rho S, \tag{6}$$

$$\dot{R} = \psi(r)\,(1-\alpha_R)\, R - \rho R \tag{7}$$

and

$$\dot{t} = \rho(T-t) - \phi(t)\, R - \rho T. \tag{8}$$

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In the absence of sensitive cells, as long as the resistant population can maintain itself, $\psi(C)\,(1-\alpha_R) > \rho$, there will be an equilibrium with resistant bacteria, resource and toxin at densities and concentrations, $\hat{R}$, $\hat{r}_R$ and $\hat{t}$ respectively. The sensitive cells can invade and will be maintained as long as, $\psi(r_R) > \xi\hat{t}+\rho$.

In the absence of a resistant population, the sensitive population can be maintained as long as $\psi(C) > \xi T+\rho$. Under these conditions, there would be an equilibrium with sensitive bacteria and resources at densities and concentrations S_T and r_{ST} respectively. Resistant bacteria will be able to invade this equilibrium community of sensitive cells and toxin as long as the resistant bacteria have a growth advantage, $\psi(\hat{r}_{ST})\,(1-\alpha_R) > \rho$. This invasion could result in either the replacement of the sensitive population with a toxin-resistant one or the persistence of a stable community with both sensitive and resistant bacterial populations.

The dynamics of this situation of frequency-dependent selection mediated by detoxification can be seen in the numerical solutions to equations (5)–(8) shown in figure 2. In the absence of a resistant population, the sensitive bacteria are unable to maintain themselves (figure 2*a*). With the same parameters and an established population of resistant detoxifying bacteria, the sensitive bacteria can invade and dominate the community (figure 2*b*). As the sensitive bacteria increase and the relative frequency (density) of resistant bacteria declines, the concentration of free toxin increases. Eventually there is a stable equilibrium with both populations present. At this equilibrium, the intrinsic disadvantage of the resistant bacterial population ($\alpha_R > 0$) is exactly overcome by its resistance to toxin, and the intrinsic advantage of the sensitive cells is exactly offset by loss due to the action of toxin.

A necessary, if not sufficient, condition for this mechanism to maintain stable polymorphism is that the resistant detoxifying cell population has an intrinsic disadvantage relative to the sensitive one. With plasmid-encoded resistance to antibiotics there is likely to be a cost in fitness due to the carriage of the plasmid (Levin 1981; Freter *et al.* 1983). However, with plasmid-determined resistance, the resistant phenotype is also likely to be infectiously transmitted to the sensitive cell population, especially if the resistance is carried by a conjugative plasmid.

Whether this detoxification mechanism will maintain stable polymorphisms, and what may be the level of these equilibria, depend on the absolute density of the community, and not only on the relative frequencies of the sensitive and resistant bacteria. For a more extensive consideration of the theory of 'detoxification-mediated' frequency-dependent selection see Lenski & Hattingh (1986).

Empirical observations

Hattingh (1986) has done experiments on competition between strains of *E. coli* with and without a Cm^{-r} plasmid (Hattingh 1986), in cultures with and without chloremphenicol. The results of her studies are consistent with the hypothesis of stabilizing selection through detoxification. In the absence of chloremphenicol the Cm^{-r} conjugative plasmid (pACYC184) reduced the fitness of the host bacterium relative to a *Cm*-sensitive strain. In the absence of the resistant bacteria, the sensitive cells were unable to maintain populations in chemostats with 2.5 μg ml^{-1} of chloremphenicol. However, when introduced at low frequencies into chemostats with established populations of resistant detoxifying bacteria, the sensitive cells were able to invade and maintain a stable association with the resistant population.

At this time, frequency-dependent selection operating through neutralization of toxins has not been studied adequately to say very much about its importance in natural populations of bacteria. However, laboratory observations suggest that detoxification may be very important

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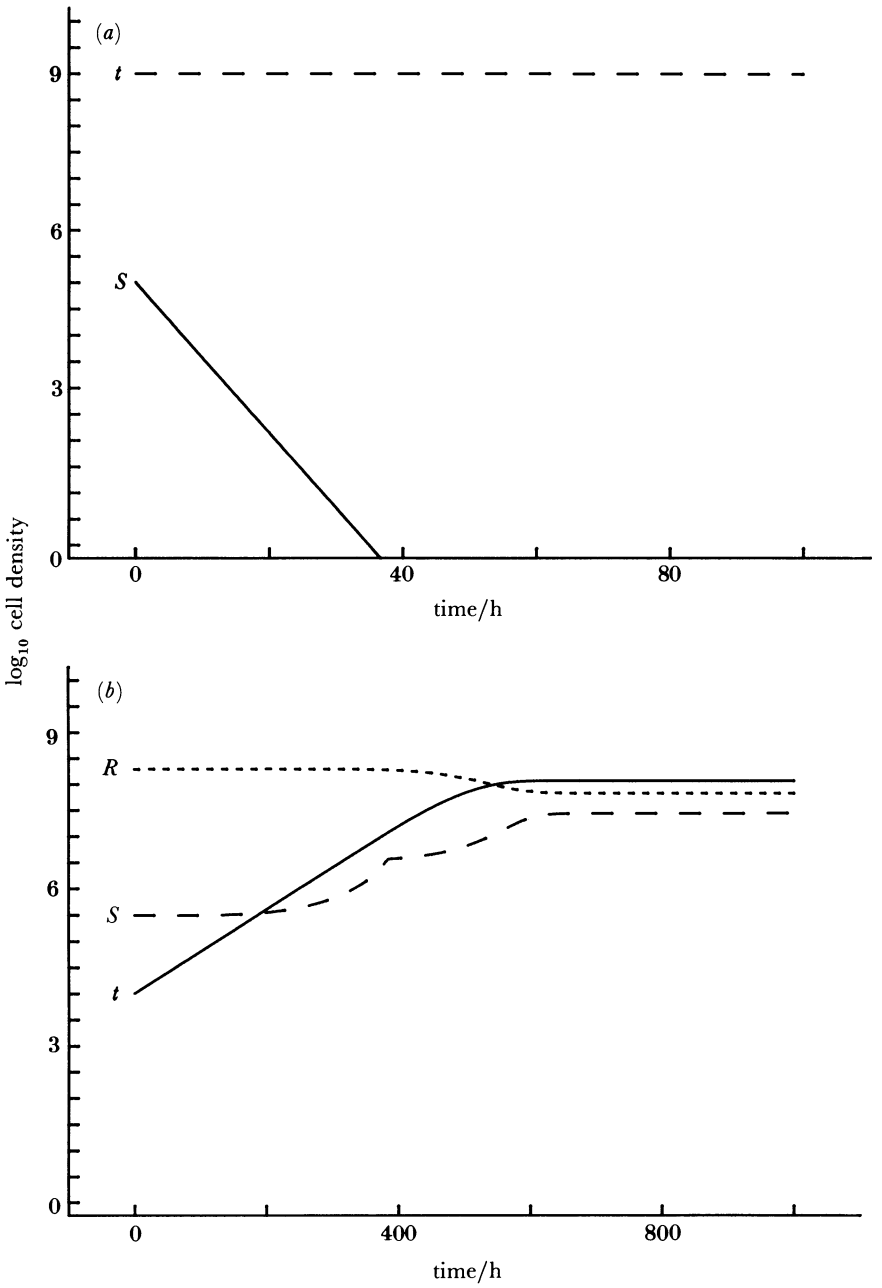

FIGURE 2. Stabilizing frequency-dependent selection for detoxification. Numerical solution to equations (5)–(8). Log_{10} density of cells and concentration of toxin. Parameter values: $C = 100$, $T = 10^9$, $\rho = 0.20$, $\psi(r) = 0.70r/(4+r)$, $e = 5 \times 10^{-7}$, $\alpha_R = 0.10$, $\phi(t) = 0.40t/(10^7+t)$, $\xi = 8 \times 10^{-10}$. Initial values of the variables: (*a*) number of resistant (detoxifying) bacteria present: $r = 100$, $S = 10^5$, $t = 10^9$. (*b*) Invasion of an equilibrium population of resistant (detoxifying) bacteria and toxin: $\hat{r} = 1.86$, $\hat{R} = 1.96 \times 10^8$, $\hat{S} = 10^4$, $\hat{t} = 3.02 \times 10^5$.

for the clinical effectiveness of some antibiotics, such as ampicillin and other penicillins. When mixed cultures of ampicillin sensitive and resistant cells (amp^{-s} and amp^{-r}) are plated on ampicillin-containing agar, there are abundant amp^{-s} colonies in the area around the amp^{-r} colonies.

The detoxification of ampicillin is a significant problem when using amp^{-r} plasmids as vectors

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in recombinant DNA cloning and selecting on ampicillin-containing medium (see, for example, Maniatis *et al.* 1982). We are also finding that it is a problem in experimental studies on the population dynamics of plasmids and transposons under selection (Condit & Levin 1988). As a consequence of detoxification of ampicillin by bacteria carrying the amp^- transposon *Tn3*, we have been unable to maintain situations where the amp^{-s} cells are unable to grow no matter how high a concentration of ampicillin we use.

Based on these observations, it seems reasonable to expect that detoxification by established populations of amp^{-r} bacteria coding for β-lactomases must be important for the treatment of pathogenic bacteria with these antibiotics. Stated another way, the development of resistant strains of pathogens is not the only clinical problem associated with the rise of antibiotic resistance.

Restriction–modification: stabilizing frequency-dependent selection

Restriction–modification can be interpreted as a two-component immune system. Because of the action of enzymes, restriction endonucleases, that cut DNA at specific sequences of bases (restriction sites), bacteria with restriction–modification systems are able to abort (and survive) infections by bacteriophages. To recognize self-DNA, the bacteria also code for modification enzymes which tag their own DNA, usually by methylation of cytosines or adenines in specific regions. This modification of self-DNA prevents the bacteria's restriction endonucleases from destroying it. Occasionally, the DNA of an infecting virus is 'accidentally' modified before being destroyed by the cell's restriction endonuclease. These modified bacterial viruses produce progeny that are modified for growth on cells of the same restriction–modification state as the host from whence they were produced. These host-modified phage can grow with impunity on bacteria of that restriction–modification state. (For a review of the basic biology of restriction–modification see Arber (1965).)

Because phage may be modified for growth on the dominant population of sensitive cells, bacteria of novel restriction states are able to invade established communities of bacteria and phage, even when these invading bacteria have an intrinsic disadvantage in rate of growth relative to the dominant population of sensitive bacteria. However, as their frequency increases so does the frequency of phage modified for growth on bacteria of the previously novel state. The net effect of this frequency-dependent selection is a potential for the stable maintenance of an indefinite number of clones with different restriction–modification states (Levin 1986). This selection for novel restriction–modification systems is, in many ways, analogous to that proposed by Bodmer & Bodmer (1978), for parasite–pathogen mimicry maintaining variation at the histocompatibility (MHC) loci in humans and other mammals. Parasites with antigens that are identical to, or cross react with, those of the host MHC antigens would be more able to invade hosts than those without these antigens. This would select for hosts of novel MHC types, whose ascent would, in turn, favour the parasites with antigens that mimic the new MHC antigens.

The stabilizing, frequency-dependent nature of phage-mediated selection for restriction–modification can be seen from a slightly simplified variant of a model of the process presented by Levin (1986). Let n_i be the density of bacteria of the ith restriction–modification state and p_j the density of phage of the jth modification state. When $i = j$ all adsorptions are lethal and produce phage. When the phages are of the wrong modification state $i \neq j$, only a

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fraction, ω_i, $(0 < \omega_i < 1)$, the modification coefficient, are lethal and produce phage. The progeny phage produced in successful infections are of the same modification state as that of the host on which they reproduce. Phage adsorb at random at a rate proportional to the densities of bacteria and phage and a rate parameter, δ. Productive infections yield β phage particles. All adsorptions result in the loss of the infecting phage particle.

As in the models for allelopathy and detoxification presented earlier, I assume an 'equable' habitat and a single limiting resource. Bacteria of restriction–modification state i grow at a rate $\psi(r)\,(1-\alpha_i)$, where $\psi(r)$ is a monotonic increasing function of r and α_i is the selection coefficient. To be consistent with the model reported by Levin (1986), and unlike the models presented earlier in this report, I assume that resources are taken up at a rate proportional to $\psi(r)$ and not to the growth rates $\psi(r)\,(1-\alpha_i)$. This is an arbitrary modification that does not influence the qualitative conclusions.

With these definitions, and assuming no time delay but allowing for a phage-resistant (refractory) bacterial population of density n_x, the rates of change in the concentration of resource and the density of the component bacterial populations are given by

$$\dot{r} = \rho(C-r) - \psi(r)\,N_T\rho, \tag{9}$$

$$\dot{n}_i = \psi(r)\,(1-\alpha_i)\,n_i + \delta n_i p_i - \delta\omega_i n_i(P_T - p_i) - \rho n_i, \tag{10}$$

$$\dot{p}_j = \delta\beta n_j p_j + \omega_j n_j \delta\beta(P_T - p_j) - \delta N_T p_j - \rho p_j \tag{11}$$

$$\dot{n}_x = \psi(r)\,(1-\alpha_x)\,n_x - \rho n_x, \tag{12}$$

where $N_T = \Sigma_i n_i + n_x$ and $P_T = \Sigma_j p_j$ are the overall densities of the phage and bacterial populations.

As long as the number of discrete phage populations is less than or equal to the sum of the number of distinct resources (one in this case) and the number of host populations, a stable community of phage and bacteria can (but not necessarily will) be maintained (Levin *et al.* 1977). Bacteria of a novel restriction state, i, will be able to invade an established community of bacteria with P_T phage if their growth rate exceeds their rate of loss due to killing by phage and flow through the habitat,

$$\psi(r)\,(1-\alpha_i) > \delta\omega_i P_T + \rho,$$

where r is the resource concentration. If the absolute growth rate $\psi(r)$ is high, as would be anticipated in a phage-limited community, and the modification rate, ω_i, is low, bacteria of novel restriction states will be able to invade, even when they have a disadvantage in growth rate. This is illustrated by the numerical solutions to equations (7)–(10) shown in figure 3.

As long as the relative fitness of resistant (refractory) populations is less than that of the sensitive cells in the community, $\alpha_x > \alpha_i$ for all i, the presence of the resistant population will not upset stable associations between the bacteria and phage (Campbell 1961; Levin *et al.* 1977). However, these resistant clones will be most likely to dominate the community and to be limited by resources (rather than by phage). Consequently, with resistant bacteria present, the concentration of the limiting resource is likely to be low, as is their absolute growth rate $\psi(r)$. Thus the rate of increase of bacteria with novel restriction states will be much reduced. This can be seen in figure 4.

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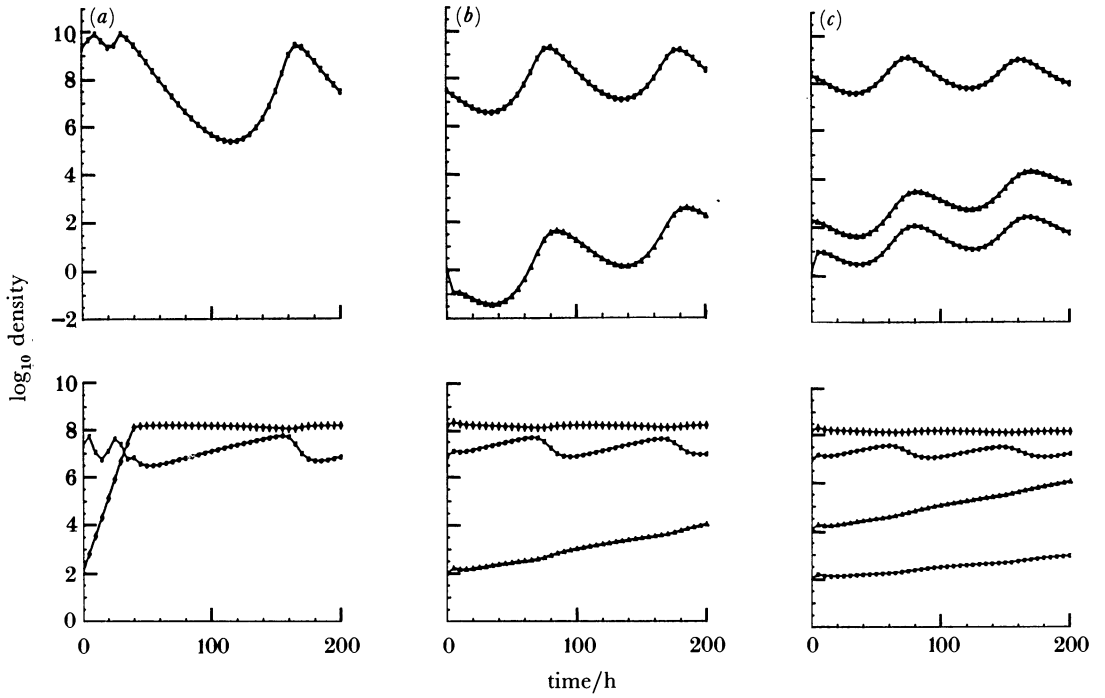

FIGURE 3. Stabilizing frequency-dependent selection for restriction–modification in a phage-limited community. Solution to equations (9)–(12). Standard parameters: $C = 100$, $\psi(r) = 0.7r/(4+r)$, $e = 5 \times 10^{-7}$, $\rho = 0.20$, $\delta = 10^{-10}$, $\beta = 1.00$, $\alpha_1 = 0.00$, $\alpha_2 = 0.05$, $\alpha_3 = 0.10$, $\omega_1 = 0.0001$, $\omega_2 = 0.001$, $\omega_3 = 0.01$. Top graphs, $\log_{10}$ phage density. Bottom graphs, $\log_{10}$ bacterial density. (*a*) Establishment of a phage-limited community. (*b*) Invasion of a phage-sensitive population (2) into the community depicted at $t = 200$ in (*a*). (*c*) Invasion of a phage-sensitive population (3) into the community depicted at $t = 200$ in (*b*). (Figure from Levin (1986).)

Empirical observations

The results of our experimental studies with *E. coli* and a virulent mutant of the phage λ, λ^{vir} (Levin & Laursen 1988), are, with qualifications, consistent with the hypothesis of phage-mediated, stabilizing frequency-dependent selection for restriction–modification. In figure 5, we present the outcome of a single experiment with a low density of a restriction–modification positive, r^+m^+, strain of *E. coli* B invading a high density population of an r^-m^- strain of *E. coli* B in serial transfer culture. In the experimental culture λ^{vir} is present, and in the control this phage is not present.

Initially the r^+m^+ strain does better than the r^-m^- clone in the presence of phage than it does in the phage-free control. However, the originally dominant population of phage-sensitive r^-m^- bacteria was rapidly replaced by phage-resistant mutants. As a result, the initial advantage of the invading population of restricting cells is mollified. Although in later transfers a greater proportion of the phages were modified for growth on the invading bacterial population than they were during early transfer, the overall density of phage declined with the increase of resistant bacteria. Consequently, in the later transfers, phage-mediated selection was less intense than it was initially.

Carol Laursen and I obtained similar results with analogous experiments using λ phage and strains of *E. coli* K-12 with a *K* restriction–modification system and with cells carrying a combination of *K* restriction and a plasmid-borne restriction–modification system (*pDXX1*)

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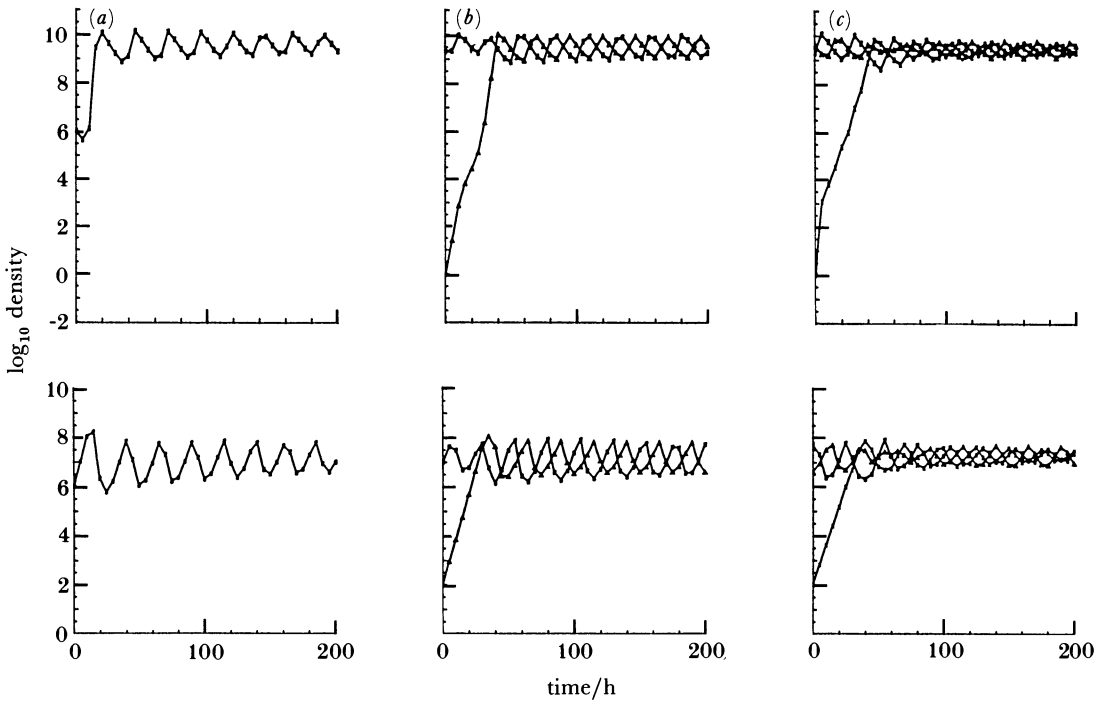

FIGURE 4. Stabilizing frequency-dependent selection for restriction–modification in a resource-limited community with phage. Numerical solutions to equations (9)–(12). Standard parameters identical to those for figure 3. Top graphs, $\log_{10}$ phage density, bottom graphs $\log_{10}$ bacterial density. Resistant cells have a 15% selective disadvantage relative to sensitive population 1, $\alpha_x = 0.15$. (*a*) Invasion of resistant bacteria into the community depicted at $t = 200$ in figure 3*a*. (*b*) Invasion of a phage-sensitive population (2) into the community depicted at $t = 200$ in (*a*). (*c*) Invasion of a phage-sensitive population (3) into the community depicted at $t = 200$ in (*b*). (Figure from Levin (1986).)

(Caugant *et al.* 1981; Piekarowitz *et al.* 1985). Following the increase of resistant bacteria, the relative fitnesses of the λ-resistant mutants of the invading and established populations seemed to be more important in determining the fate of the clones than restriction–modification.

Although these results are approximately consistent with those anticipated from the theory, they also raise the question whether restriction–modification systems have evolved, and are maintained, by phage-mediated selection of the sort considered in this model. Also raising doubts about the importance of phage-mediated, frequency-dependent selection in the maintenance of extant restriction–modification systems is the observation that many phages have mechanisms to avoid the effects of host restriction (see Kruger & Bickle 1983). In addition, mutations in single genes can make bacteria resistant to phages, which the phages may not be able to counter.

One possibility suggested by the experiments that Carol Laursen and I have been doing with λ and *E. coli* (Levin & Laursen 1988) is that the most effective form of selection for restriction–modification is not through the invasion of new variants into established communities of bacteria and phage. From our consideration it seems that the primary advantage of restriction–modification immunity is that it increases the likelihood of bacterial clones becoming established (forming colonies) in habitats in which free-phage are present, and reduces the likelihood that established populations will be eliminated by phage. Because of

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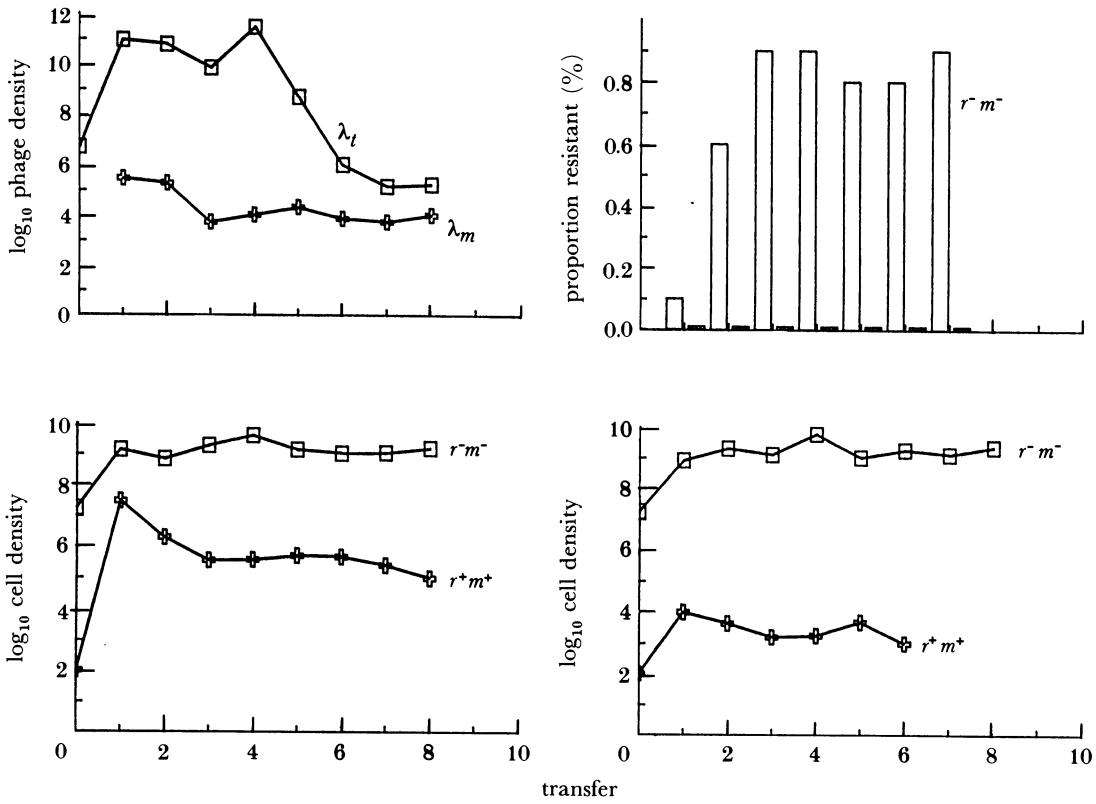

FIGURE 5. Stabilizing frequency-dependent selection for restriction–modification, experimental results. Invasion of a low-density, restriction–modification positive strain of *E. coli* B into a restriction–modification negative strain of *E. coli* B with and without the phage. λ^{vir}. Minimal medium with 300 μg ml^{-1} glucose and serial transfer culture. The recipe for the medium, and the techniques of sampling, are described in Levin *et al.* (1977). Transfers were made daily and at each transfer, 0.1 ml of previous day's culture was added to 9.9 ml of fresh medium. The total phage density was estimated from plaque counts on a r^-m^- lawn and the *B*-modified phage, λ_m, density was estimated from plaque counts on a lawn composed of the r^+m^+ *E. coli* B cells. The densities of resistant bacteria were estimated by streaking colonies of the *lac*$^-$ r^+m^+ cells and *lac*$^+$ r^-m^- cells across high titres of λ^{vir}.

restriction–modification, the minimum density for restriction-sensitive, unmodified phage to become established and possibly to eliminate a clone of bacteria is of the order of the reciprocal of the modification coefficient, 10^3–10^7. Thus bacteria with restriction immunity would be virtually unaffected by phage when these viruses are sparse. Without restriction–modification, infection by a single phage particle may be able to establish a phage population.

It should be noted that this 'group-level', colonization–extinction mechanism is also frequency-dependent and stabilizing. The marauding phage that acts as the selecting agent would be modified for growth on bacteria of the most frequent restriction–modification state. Bacterial clones of rare restriction–modification types would therefore be less liable to extinction due to phage than those of the common type. The net effect would be the same as the scheme of selection based on individuals, as presented in the model.

As can be seen by perusing a catalogue of genetic engineering supplies (e.g. the appendix to the Catalog of New England Biolabs 1986), restriction–modification systems are ubiquitous and diverse in the bacteria. It is tempting to suggest that phage-mediated selection at the group or individual level is responsible for the maintenance and diversity of these 'immune systems'.

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This hypothesis is specially appealing, because I am unable to think of alternatives. It is also tempting to suggest that this type of stabilizing frequency-dependent selection plays a substantial role in the maintenance of the considerable genetic diversity observed for some bacterial species (Selander & Levin 1980; Ochman & Selander 1984; Porras *et al.* 1986). Despite these temptations, I do not believe that at this juncture the empirical support for such hypotheses is strong enough for us to accept them without considerable qualification.

Discussion and implications

The three examples of frequency-dependent selection considered here were chosen for this report primarily because I am familiar with them. They have been studied by me or by people associated with me. The examples were selected also because there were both theoretical and experimental results to report. On the other hand, the topics were originally chosen for investigation because they were interesting and seemed to be important features of the population biology of bacteria, their viruses and plasmids. They were not selected because they were examples of frequency-dependent selection.

Reflecting upon this I would have been more hard-pressed, it seems, to come up with examples of 'absolute' (frequency-independent) selection in populations of bacteria and their accessory elements. The relative fitness of mutator genes and mutation-inducing transposons increases with their frequency (Chao *et al.* 1983, 1985). The intensity of selection for higher rates of infectious transfer in plasmids is a decreasing function of the frequency of cells carrying these elements (Lundquist & Levin 1986). A similar kind of frequency-dependent relation would obtain in selection for prophage with increased rates of induction, lysis of a host cell and release of free temperate viruses (Stewart & Levin 1984). The relative fitness of bacteria that can grow on the metabolic byproducts of competitors declines with their frequency (Levin 1972). I could go on, but I trust my point is made, frequency-dependent selection is likely to be common in bacterial populations.

One of the reasons for its ubiquity in models of the population dynamics of bacteria and their accessory elements is that these models simultaneously treat the ecology as well as the genetics of these organisms. In these clonal (asexual) organisms and replicons 'genotypes' are populations. One need not resort to the analysis of relative frequencies to overcome the inconvenience that recombination produces new 'populations'. Ecological interactions between populations are almost invariably density-dependent, and frequency-dependence is a common hand-maiden of density-dependence. Absolute selection may obtain in situations where the bacteria are responding to aspects of the environment that they cannot influence, such as temperature. But here too one can readily conceive of situations in which selection would still be frequency-dependent, such as competition for microhabitats.

I could suggest that what is true for selection in bacterial populations is also true for selection in populations of eukaryotes. I could also argue that the reason why frequency-dependent selection in eukaryotes may appear to be the exception rather than the rule is an artifact of the way in which the population genetics of sexual eukaryotes is modelled. But such suggestions and arguments would be presumptuous.

I thank Richard E. Lenski for useful commentary and the EcLF Luncheon group for stimulating discussions and occasionally good lunches. This research was supported by a grant from the U.S. National Institutes of Health, GM33782.

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Discussion

J. Antonovics (*Department of Botany, Duke University, North Carolina, U.S.A.*). Dr Levin's remark that what applies to bacteria may apply to elephants is truer than he thinks. It has been argued that it would be difficult for an animal to establish increased territory size simply to prevent other organisms from gaining access to resources (super-territories) unless the population was strongly substructured (see, for example, Lullock 1979) and there is therefore a direct parallel here with spiteful allelopathic effects in bacteria. Has Dr Levin formally extended his models of allelopathic effects in homogeneous cultures to see how conditions for spread of allelopathic effects would be affected by limited dispersal in a two-dimensional habitat? It seems we need a realistic theory of allelopathy.

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B. R. Levin. Although I may have deluded myself for some time with the view that real bacteria live in well-agitated liquid culture of the sort so readily modelled by mass-action equations and chemostats, I do so no longer. We, my students, collaborators and I, are now coming to grips with the realities of physically structured habitats and bacteria living as colonies. We are attempting to do this in our theoretical as well as our experimental studies of the population dynamics of bacteria and their plasmids, phage and transposons.

As I discussed here, in my consideration of the population dynamics of colicins, qualitatively different results obtain in liquid (mass) culture and in the physically structured habitat of soft agar. Chao (1979) developed a simulation model of allelopathy in a two-dimensional habitat. I believe his model has some of the features Dr Antonovics describes.

H. Sharma (71 *Barrack Road, Hounslow, U.K.*). Have bacteria responded through evolution to the phage escape from host restriction?

B. R. Levin. I don't know whether the bacteria have evolved specific mechanisms to counter the various processes phage have developed to escape the effects of host restriction (reviewed by Kruger & Bickle (1983)). Many restriction–modification systems are coded for by plasmids, and the acquisition of a new restriction–modification plasmid would be an easy way for bacteria to respond to a particular phage's mechanisms to avoid its restriction–modification system. If the escape from host restriction is through elimination of restriction sites, the bacteria could respond through selection for a restriction endonuclease with a new restriction site. How that occurs I have no idea. With type II restriction endonucleases, where the restriction and modification are through separate enzymes, it would seem that a successful (non-lethal) change in restriction site would require specific changes to occur simultaneously in two genes.

H. Sharma. In Dr Levin's model, is there any significance of the phage integration into the host DNA, i.e. prophage providing immunity to bacteria?

B. R. Levin. The model of phage-mediated selection for restriction–modification presented here only treats lytic (virulent) bacteriophage. It does not consider temperate bacterial viruses and prophage-encoded, super infection immunity of lysogens. That would require a different model, perhaps a hybrid of the model considered here and that in our 'why be temperate?' study (Stewart & Levin 1984).

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