*J. theor. Biol.* (1984) **108,** 319–325

# Coevolution of Bacteria and Phage: Are There Endless Cycles of Bacterial Defenses and Phage Counterdefenses?

RICHARD E. LENSKI

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

(*Received* 27 *April* 1983)

The assertion that the coevolution of bacteria and bacteriophage leads to an endless arms race between resistant bacterial mutants and corresponding host-range phage mutants is questioned. In general, structural constraints on the highly site-specific phage adsorption process appear more severe than physiological constraints on resource assimilation by bacteria. Several alternative hypotheses are presented that could account for the persistence of phage, despite this fundamental asymmetry in the coevolutionary potential of bacteria and phage.

Rodin & Ratner (1983*a*,*b*) present a model intended to demonstrate that evolutionary processes may be "locally adaptive but globally undirected" owing to a gene-for-gene arms race between exploiter and victim (see also Van Valen, 1973; Maynard Smith, 1976). They have chosen to base their model on the coevolution of bacteria and bacteriophage, for a number of admirable reasons: (1) coevolution of bacterial receptor sites and phage adsorption organelles is experimentally demonstrable and has important consequences to their population dynamics; (2) the modest number of parameters governing their interactions, including mutation rates, are directly estimable; and (3) their population sizes are sufficiently large and their generation times are sufficiently short that evolutionary hypotheses can be experimentally tested.

I wish to address two of their ctitical assumptions: first, that the defenses and counterdefenses of bacteria and phage can be accurately described by a gene-for-gene model (see Flor, 1956; Day, 1974); and second, that a deterministic approach is adequate for dealing with this problem.

According to Rodin & Ratner, there is a multiplicity of more or less equiprobable mutations by which a bacteria evolves resistance; each of these resistant types can be attacked by mutant host-range phage which are incapable of attacking any other bacterial type. Given this extremely simple gene-for-gene relationship (Fig. 1), the authors present a scenario of endless



0022-5193/84/110319+07 $03.00/0 

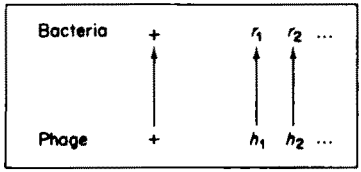


FIG. 1. Schematic diagram of mutations producing resistant bacteria and host-range phage, according to Rodin & Ratner (1983*a*). Arrows indicate the ability of particular phage types to attack particular bacterial types. + denotes wild-type bacteria and phage; $r_1$, $r_2$, ... are bacterial mutations conferring resistance to phage; $h_1$, $h_2$, ..., are corresponding host-range phage mutations.

cycles of resistant bacteria and corresponding host-range phage, perhaps even leading full circle to the original bacterial and phage types (see also Campbell, 1961). While the authors allow for the over-simplification of their model, they assert that it is "without lack of generality." I disagree.

Some of the most well studied interactions between bacteria and phage are those of *Escherichia coli* B and the seven virulent T phage. (It should be noted that T is an arbitrary designation and is not indicative of taxonomic affinities.) It is true that resistance to six of the seven T phage can be readily selected (Demerec & Fano, 1945), and that host-range mutants can be readily isolated in five of the seven T phage (Luria, 1945*a*). However, continuous culture studies suggest that one cannot indefinitely select for new resistant bacteria and new host-range phage. With phage T4 (Horne, 1970) and with phage T5 (unpublished experiments), bacterial resistance readily evolves but no host-range mutants appear, while with phage T2 (Levin, Stewart & Chao, 1977), bacteria and phage can coexist for extended periods without the evolution of either resistant bacteria or host-range phage. With phage T7 (Chao, Levin & Stewart, 1977), and probably also with phage T3 (Horne, 1970), one does observe both resistant bacteria and host-range phage, as well as bacteria that are resistant to the host-range phage. However, once these secondary resistants are obtained, the phage are apparently unable to evolve host-range mutants capable of attacking this cell type.

Moreover, host-range phage are capable of growing not only on bacteria resistant to wild-type phage, but also on wild-type bacteria (Luria, 1945*a*; Hofnung, Jezierska & Braun-Breton, 1976; Chao *et al.*, 1977; Schwartz, 1980). These observations demonstrate that host-range phage have an extended, not a modified, host-range (Hofnung *et al.*, 1976), indicating that there has evolved a reduced selectivity as opposed to an altered specificity (Schwartz, 1980). Associated with the reduced selectivity of host-range phage is a reduced stability (Schwartz, 1980), such that host-range mutants

are likely to be at a disadvantage when competing for wild-type bacteria (Chao *et al.*, 1977).

Figure 2 presents a model of the coevolution between phage and bacteria that is more consistent with the available information. There can exist two broad classes of phage-resistant bacterial mutants: those for which one can

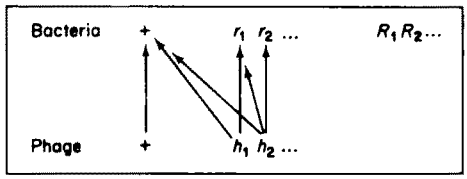


FIG. 2. Schematic diagram of mutations producing resistant bacteria and host-range phage, according to the text. Symbols as in Fig. 1, except $R_1, R_2, \ldots$ are bacterial mutations conferring resistance to phage for which there are no corresponding host-range phage mutations.

select host-range phage mutants and those for which one cannot select host-range phage mutants. The former class comprises mutations in which the phage adsorption sites on the bacterial surface are quantitatively and/or qualitatively altered, while the latter class lacks these sites altogether (Schwartz, 1980). The elegant work of Hofnung *et al.* (1976) has shown that for *E. coli* K12 and phage Lambda, these classes include missense and nonsense mutations, respectively. Since bacterial receptor sites exist, after all, for specific cellular functions which have become exploited only secondarily by bacteriophage, the loss or alteration of these sites is likely to engender some trade-off in the efficiency with which bacteria exploit their environment (Demerec & Fano, 1945; Luria, 1946; Chao *et al.*, 1977; Levin & Lenski, 1983). In general, however, structural constraints on the highly site-specific phage adsorption process are probably more severe than the physiological constraints on resource assimilation by bacteria, so that *there is a fundamental asymmetry in the coevolutionary potential of bacteria and phage.*

Even were the simple gene-for-gene model of bacteria and phage coevolution acceptable, the deterministic approach of Rodin & Ratner is seriously flawed. These authors argue that the "frequencies of mutants" (and not the mutation rates) in bacterial and phage populations are sufficiently high that they "guarantee" the continual appearances of resistant and host-range mutants, even though each host-range mutant completely eliminates, *in their model*, the preceding bacterial population. Again, I must disagree.

First of all, Rodin & Ratner fail to distinguish between cell and phage densities and total numbers (i.e., density × volume). While population

dynamics in mass culture can be adequately described by densities, the likelihood of particular mutations necessarily depends on total numbers. Secondly, Rodin & Ratner assume that the mean frequency of mutants, and not the variation associated with that mean, permits an adequate assessment of the likelihood of particular mutations. The classic experiments of Luria & Delbruck (1943) indicate otherwise; their experiment 23 provides a convenient illustration. Eighty-seven cultures of *E. coli* B were independently grown to about $2{\cdot}4\times10^{8}$ cells. On average, there were 28.6 T1 resistant bacteria per culture, corresponding to a frequency of $1{\cdot}19\times10^{-7}$, hence within the range presented by Rodin and Ratner. Were resistant bacteria randomly distributed through the cultures, each culture would have a probability of only $e^{-28{\cdot}6}=3{\cdot}79\times10^{-13}$ of having no resistant bacteria. Yet 29 of these cultures (33·3%) contained no resistant bacteria; resistant bacteria are not randomly distributed, owing to reproduction subsequent to their origin via spontaneous mutation. That is, cultures containing many resistant bacteria have not experienced many mutations, but rather one or a few mutations which increased through several cycles of binary fission prior to testing of the culture. Luria's (1945*b*) experiment 113 makes a comparable point with respect to host-range phage. 34 cultures of T1 were independently grown to about $7\times10^{8}$ phage. On average, there were 45 host-range phage per culture, corresponding to a frequency of $6{\cdot}43\times10^{-8}$, again within the range presented by Rodin & Ratner. Yet nine of these cultures (26·5%) contained no host-range phage. Thus, even with cell and phage numbers in these experiments roughly two orders of magnitude higher than the "critical" values of Rodin & Ratner (equivalent to scaling their dynamics from 1 ml to 100 ml), one observes a probability of only $(1-0{\cdot}333)\times(1-0{\cdot}265)=0{\cdot}490$ of completing even a single cycle of coevolutionary changes in the bacteria and phage.

Rodin & Ratner also assume that coevolution of bacteria and phage is necessarily antagonistic. While this should generally be the case with virulent phage and their hosts, it may often not be the case with temperate phage and their hosts (Levin & Lenski, 1983). Lysogens, in which the bacterial and temperate phage genomes forge a semi-stable intracellular coexistence, provide an opportunity for mutualistic coevolution. If lysogens have some net growth advantage over non-lysogens (Lin, Bitner & Edlin, 1977), selection acting on the bacterial genome (as well as the phage genome) could favor high efficiency of phage adsorption.

Rodin & Ratner have, in essence, formalized the statement by Stent (1963: 181) that "The coexistence in nature of bacteria and bacterial viruses is thus sustained by a delicate mutational equilibrium that saves both protagonists from total extinction". But biological and probabilistic con-

straints on the mutational processes render unlikely the endless cycling of bacterial defenses (i.e., resistance mutations) and phage counterdefenses (i.e., host-range mutations).

How do phage persist if there exist bacterial resistance mutations that cannot be countered by host-range phage mutations? There are at least four different hypotheses that could account for the existence of phage-sensitive bacterial populations, and consequently could explain the persistence of phage.

According to one hypothesis, "... phage growth might occur on cells which are 'effectively dead' from the genetic point of view ..." (Campbell, 1961: 160). That is, sensitive cells may have refugia which render them insusceptible to phage predation, with phage being sustained by sensitive cells that have lost their refuge. While many exploiters are known to disproportionately prey upon the infirm, phage appear best able to attack healthy bacteria. Therefore, physical refugia seem more likely to be important than physiological refugia.

According to a second hypothesis, "... phages may tend to select binding sites which are essential to the cell" (Schwartz, 1980: 84). While this statement implies choice on the part of bacteriophage, the differential extinction of phage that bind to non-essential sites is plausible. This hypothesis formally requires that for each extant phage, there be at least one host in at least one environment for which the metabolic functions associated with the phage adsorption site are essential to bacterial growth, and hence phage-resistant mutants cannot evolve. As examples, Anderson (1946) found that certain T1-resistant mutants of *E. coli* B cannot grow in the absence of tryptophane, while Williams Smith & Huggins (1980, 1982) found that *E. coli* MW resistant to phage R were unable to infect mice for which the phage-sensitive cells were pathogenic.

According to a third hypothesis, sensitive and resistant bacteria cells can coexist if "... the latter are less fit in the competition for resources than the sensitive ..." (Levin *et al.*, 1977: 22). The sensitive cells are able to grow on resources that have become limiting to the resistant population, while the phage prevent the sensitive cells from competitively excluding the resistant population: hence, predator-mediated coexistence. This hypothesis has one drawback, as noted by Campbell (1961). Both the resistant and sensitive bacterial populations can be expected to evolve towards increased efficiency of exploiting their environment via fixations of mutations conferring competitive advantages (Atwood, Schneider & Ryan, 1951; Kubitschek, 1974; Levin, 1981). This process of periodic selection should occur at a greater absolute rate in the resistant population than in the sensitive population, because of the former's greater size. [Phage-limited

bacterial populations typically occur at densities orders of magnitude below that set by their resources (Paynter & Bungay, 1969; Horne, 1970; Levin *et al.*, 1977; Chao *et al.*, 1977).] Any initial competitive advantage accruing to the sensitive bacteria should be eroded by the faster rate of adaptation in the larger resistant population. As a consequence, the sensitive cells, and with them the phage, could be driven to extinction.

The fourth hypothesis is an extension of the third. Subsequent to the extinction of sensitive cells and phage via periodic selection, the resistant bacteria may revert to sensitivity via mutations which will be favored if they restore the cell function lost as a consequence of phage-resistance. The phage may then reinvade, provided there are other such communities elsewhere in this evolutionary cycle. Because of the reduced stability associated with their reduced selectivity, host-range phage will be outcompeted by wild-type phage for the revertive wild-type bacteria. This hypothesis generates evolutionary changes that are "locally adaptive but globally undirected," but for very different reasons than suggested by Rodin and Ratner. The system may revert to a former state not via the endless cycles of bacterial defenses and phage counterdefenses, but rather as a consequence of constraints limiting the adaptive potential of both bacteria and bacteriophage.

I wish to thank Sue Hattingh and Bruce Levin for providing valuable comments on this manuscript. NIH grant GM19848, to Bruce Levin, supported me during this work.

## REFERENCES

ANDERSON, E. H. (1946). *Proc. natn. Acad. Sci. U.S.A.* **32,** 120.
ATWOOD, K., SCHNEIDER, L. & RYAN, F. (1951). *Cold Spring Harbor Symp. Quant. Biol.* **16,** 345.
CAMPBELL, A. (1961). *Evolution* **15,** 153.
CHAO, L., LEVIN, B. R. & STEWART, F. M. (1977). *Ecology* **58,** 369.
DAY, P. R. (1974), *Genetics of Host–Parasite Interaction.* San Francisco: Freeman.
DEMEREC, M. & FANO, U. (1945). *Genetics* **30**, 119.
FLOR, H. H. (1956). *Adv. Genet.* **8,** 29.
HOFNUNG, M., JEZIERSKA, A. & BRAUN-BRETON, C. (1976). *Mol. gen. Genet.* **145,** 207.
HORNE, M. T. (1970). *Science* **168,** 992.
KUBITSCHEK, H. E. (1974). *Symp. Soc. gen. Microbiol.* **27**, 105.
LEVIN, B. R. (1981). *Genetics* **99,** 1.
LEVIN, B. R. & LENSKI, R. E. (1983). In: *Coevolution*, p. 99. Sunderland, Massachusetts: Sinaeur.
LEVIN, B. R., STEWART, F. M. & CHAO, L. (1977). *Am. Nat.* **111,** 3.
LIN, L., BITNER, R. & EDLIN, G. (1977). *J. Virol.* **21,** 554.
LURIA, S. E. (1945*a*). *Ann. Mo. bot. Gdn* **32,** 235.
LURIA, S. E. (1945*b*). *Genetics* **30,** 84.
LURIA, S. E. (1946). *Cold Spring Harbor Symp. Quant. Biol.* **11,** 130.

LURIA, S. E. & DELBRUCK, M. (1943). *Genetics* **28,** 491.
MAYNARD SMITH, J. (1976). *Am. Nat.* **110,** 325.
PAYNTER, M. J. B. & BUNGAY, H. R. III. (1969). In: *Fermentation Advances,* p. 323. New York: Academic Press.
RODIN, S. N. & RATNER, V. A. (1983*a*). *J. theor. Biol.* **100,** 185.
RODIN, S. N. & RATNER, V. A. (1983*b*). *J. theor. Biol.* **100,** 197.
SCHWARTZ, M. (1980). In: *Virus Receptors, Part I, Bacterial Viruses.* p. 59. London: Chapman and Hall.
STENT, G. S. (1963). *Molecular Biology of Bacterial Viruses.* San Francisco: Freeman.
VAN VALEN, L. (1973). *Evol. Theor.* **1,** 1.
WILLIAMS SMITH, H. & HUGGINS, M. B. (1980). *J. gen. Microbiol.* **121,** 387.
WILLIAMS SMITH, H. & HUGGINS, M. B. (1982). *J. gen. Microbiol.* **128,** 307.