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SELECTION OF BENEVOLENCE IN A HOST–PARASITE SYSTEM

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Abstract.—A paradigm for the evolution of cooperation between parasites and their hosts argues that the mode of parasite transmission is critical to the long-term maintenance of cooperation. Cooperation is not expected to be maintained whenever the chief mode of transmission is horizontal: a parasite's progeny infect hosts unrelated to their parent's host. Cooperation is expected to be maintained if the chief mode of transmission is vertical: a parasite's progeny infect only the parent's host or descendants of that host. This paradigm was tested using bacteria and filamentous bacteriophage (f1). When cells harboring different variants of these phage were cultured so that no infectious spread was allowed, ensuring that all parasite transmission was vertical, selection favored the variants that were most benevolent to the host—those that least harmed host growth rate. By changing the culture conditions so that horizontal spread of the phage was allowed, the selective advantage of the benevolent forms was lost. These experiments thus support the theoretical arguments that mode of transmission is a major determinant in the evolution of cooperation between a parasite and its host.

Key words.—Bacteriophage, benevolence, cooperation, evolution, f_1, mode of transmission.

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The existence of cooperation between species poses an interesting challenge to current evolutionary theory. The difficulty lies, not in identifying mutual benefits of interspecific cooperation, but in explaining the evolutionary stability of cooperation—why individuals of one species do not benefit by exploiting the other species and evolving toward parasitism. One factor that promotes evolutionary stability of cooperation is "partner fidelity," or the pairing of individuals over a long series of exchanges (Axelrod and Hamilton, 1981; the term "partner fidelity" was introduced by Bull and Rice, 1991). The benefit of cooperation is thus gained gradually, and any selfish individual who exploits its partner realizes a corresponding future loss when that partner no longer reciprocates.

A main determinant of partner fidelity is the availability of alternative partners. An abundance of potential partners provides the setting for low partner fidelity and the instability of cooperation, whereas a scarcity of potential partners enforces partner fidelity and promotes cooperation (Axelrod and Hamilton, 1981; May and Anderson, 1982; Ewald, 1983, 1987; Lenski, 1988; Maynard Smith, 1989). Phage–bacterial systems provide an easy means of manipulating partner fidelity and thus of empirically testing hypotheses on the evolution of cooperation. Many phages are typically propagated with low partner fidelity by providing them with a superabundance of bacteria for infection. With an abundance of available partners, there is no benefit gained from benevolence to the host bacterium longer than is necessary to produce phage progeny. Yet if phage were to be denied the opportunity for infectious or "horizontal" transmission, such as by eliminating phage-sensitive bacteria from the culture, high partner fidelity would be enforced so that benevolent phage should evolve.

This paper describes an experimental system to study the evolution of benevolence. Despite the various models indicating that selection for cooperation is effected by enforcing high partner fidelity, experimental studies of this process are scarce: evidence for the short-term evolution of myxoma virus virulence in Australia may be consistent with the model, but information is not

available on the magnitude of partner fidelity between the virus and the host: rabbit (Dobzhansky, 1970; Lewontin, 1970). Bouma and Lenski (1989) investigated the evolution of cooperation between a noninfectious plasmid and its host over the course of several hundred generations of propagation in the laboratory, observing that the bacterium evolved to tolerate the plasmid. In this latter study, therefore, absolute partner fidelity was enforced. The present study was undertaken to carry the empirical manipulation of cooperation one step further.

Filamentous, Single-Stranded DNA Phages

The phages of particular utility in this context include M13, f1, and fd—the filamentous phages (Model and Russell, 1988). These phages exist as single-stranded DNA molecules covered with protein. They infect F-piliated (male) bacteria, but not female bacteria, the pilus of the male being the initial site of attachment. On entering the bacterium, the single-stranded DNA becomes double-stranded (denoted as the replicative form, or RF) and proceeds to utilize the bacterial metabolic machinery to produce other RF and progeny single-stranded molecules. Five properties of this phage life history lend themselves to empirical studies on the evolution of cooperation:

1. Phage production occurs continuously and without killing the bacterium. The phage DNA molecule is packaged into new particles as it passes through the bacterial membrane, leaving the latter intact.
2. Infected bacteria are resistant to further infection.
3. Cell division continues after infection; both daughter cells usually inherit viral (RF) molecules and thus retain the state of infection.
4. Infected bacteria divide at a slower rate than uninfected bacteria, hence a deleterious but nonlethal effect of wild-type phage can be demonstrated. This reduced division rate of infected cells enables plaque assays.
5. There are no severe packaging requirements on the length of the phage DNA molecule, so that a variety of selectable markers can be incorporated into the phage genome without grossly affecting phage viability.

METHODS AND MATERIALS

Bacteria and Phage.—Three strains of *Escherichia coli* were used in this study: KI345: *E. coli* K12 Hfr Δ*traDIZ* Δ*lac galE* Gal^R (K. Ihler, Texas A&M University), an F-piliated strain that is conjugation defective due to a deletion of some *tra* genes; UT481 *E. coli* K12 Δ(*pro-lac*) *sup*D Tn*10* *hsdS*/F′*traD*36 *proA*$^+$*B*$^+$ *lacZ*ΔM15 (C. Lark, University of Utah), another F-piliated, conjugation defective strain; and *E. coli* C, a wild-type strain that is F^- and thus does not adsorb to filamentous phages. A chimeric phage-plasmid, R208, was obtained from M. Russell (Rockefeller University). The chimera had been constructed from wild-type phage f1 by converting the *Hae*III restriction site at nucleotides 5725–5728 into an *Eco*RI site and ligating *Eco*RI-linearized pBR322 into this site. Due to the presence of pBR322 DNA, cells infected with R208 express resistance to both ampicillin (Ap^R) and tetracycline (Tc^R). A second phage, R386 (also from M. Russell), consisted of a wild type f1 with a chloramphenicol resistance (Cm^R) gene cassette cloned at nucleotide 5614 (Terwilliger et al., 1988).

Bacteriological and Molecular Methods.—Recipes for LB and M9 galactose (0.2% w/v) media followed those given in Maniatis et al. (1982) or Sambrook et al. (1989). Ampicillin was routinely used at 50 μg/ml and chloramphenicol at 30 μg/ml. Isolation of RF DNA, restriction digestions, and agarose gel electrophoresis (0.8% agarose) were also as described in Maniatis et al. (1982).

Assays of Infectivity, Phage Sensitivity, and Cell Growth Rate.—Cells expressing Ap^R were tested for the production of infectious phage in either of two ways. Method (a): the supernatant from a culture was incubated at 65°C for 20 min to kill remaining cells and was added to a culture of growing phage-sensitive cells for at least 2 hr before plating on LB agar containing Ap. Method (b): Colonies of infected KI345 growing on LB agar containing Ap were gridded to M9 galactose

TABLE 1. Summary of key notation.

Notation	Description
Ap	The antibiotic ampicillin
Cm	The antibiotic chloramphenicol
RF	Replicative form of a phage in its host, consisting of double-stranded DNA
KI345	Male strain used as host for phages
R208	Chimera of phage fl+ and plasmid pBR322, carrying resistance to Ap
HPF	High partner fidelity line, initiated from R208-infected KI345 and propagated by growing infected cells in LB broth containing Ap
LPF	Low partner fidelity line, initiated from R208-infected KI345 and propagated by using the supernatant of a culture to infect new KI345
R208.H	The descendant of R208 that evolved in the HPF line
R208.L	The descendant of R208 that evolved in the LPF line

plates seeded with UT481. KI345 does not grow on M9 galactose; however, viable phage that are secreted from infected KI345 can be detected by a halo around the site of innoculum. The phage sensitivity of ampicillin-resistant, infected KI345 (i.e., cells with a history of exposure to R208) was tested by growing the cells in the presence of phage R386 for at least 2 hr and plating on LB agar containing Cm. Cell densities were measured in LB broth from side-arm flasks inserted into a Klett-Summerson colorimeter (filter #54, approximately 550 nm wavelength). The growth rate of infected cells grown with aeration was measured as the time interval required for a culture in exponential growth to advance from a Klett value of 25 to a value of 75 (approximately 1.3×10^8 to 4.7×10^8 cells/ml, assayed as colony-forming units).

Preliminary Selection.—The overall objective of our study was to determine how the means of phage transmission influenced selection of phage benevolence. Consequently, a preliminary experiment was conducted to generate phage variants that could then be used to study selection. KI345 was infected with R208, and two lines were propagated, selecting for Ap^R. One line, designated the HPF line (for "high partner fidelity"), was propagated by serial transfer of cells into LB broth, usually containing Ap. A typical cycle in the HPF propagation consisted of the inoculation of fresh media with a small aliquot of cells from the previous cycle followed by 9–10 generations of cell growth. A typical cycle in the second line, designated the LPF line (for "low partner fidelity"), employed a different design: infected cells were plated on LB agar containing Ap; 30 colonies from one plate were inoculated into 0.5 ml media and grown for approximately 1 hr; cells were removed by centrifugation, and the supernatant was incubated at 65°C for 20 min; the supernatant was added to a culture of uninfected KI345 for at least 2 hr before replating on LB agar containing Ap. Early in the history of each line, cells were occasionally grown in the presence of nitrosoguanidine (10 μg/ml) to enhance mutation rates. Both lines were passaged for more than 15 cycles, at which time sufficient divergence had occurred to initiate the selection experiments described below.

Notation and Terminology.—As will be described below, the preliminary selection experiments generated two distinct forms derived from R208 so that it is convenient to use separate notation to distinguish them. R208.H will designate the derivative of R208 that evolved in the HPF line; R208.L will designate the LPF line derivative. As will also be described, R208.H is no longer a phage, so the DNAs R208.H and R208.L will be referred to collectively as "parasites" of their hosts. Table 1 summarizes some of this notation for convenient reference.

RESULTS

Differences between the HPF and LPF Lines

The goal of the preliminary selection was to generate phage variants that differed in their benevolence to the host. Infected cells and phage derivatives from the HPF and LPF lines therefore were compared for several phenotypes to evaluate differences in the extent and nature of phage benevolence between the two lines. Cells expressing Ap^R from the HPF line grew significantly faster than Ap^R cells from the LPF line: in independent experiments, the times to grow from a density of 25 Klett units to 75 units were:

HPF:	56, 56, 54, 51 min (mean 54.25)
LPF:	85, 85, 81, 78 min (mean 82.25)

This difference is highly significant in a t test ($t = 13.5$, $P \ll 10^{-4}$). To obtain a more useful interpretation of these differences in growth rates, assume that bacterial growth was continuous and strictly exponential during the interval monitored:

$$\frac{dN}{dt} = rN$$

where N is the number of bacteria in the culture, and r is the per capita growth rate; t is measured in minutes. Supposing that bacterial density increased 3-fold between Klett value 25 and 75, the means for the two groups correspond to growth rates of $r = 20 \times 10^{-3}$/min and 13×10^{-3}/min. A second suggestion of greater benevolence in the HPF line is that R208.H was not infectious, although infectivity per se is not strictly a measure of benevolence to the host.

In principle, the enhanced growth rate and other properties of the HPF line could have stemmed from R208.H or from the host: in each host–parasite combination, the phage and bacterial genomes were transmitted together, so that changes in either genome that enhanced host growth rate would have been favored. Several comparisons were therefore instituted to analyze more specifically any differences between R208.H and R208.L. Restriction analysis of R208.L RF DNA revealed that it was of similar size to that of the original R208, but the R208.H RF DNA was considerably smaller. The latter appeared to consist of pBR322 with an insertion of 100–300 bp at the original *Eco*RI site. This observation indicates that the divergence between HPF and LPF lines resulted at least in part from changes in the phage. Second, KI345 was transformed with R208.H RF DNA, and the growth rate of Ap^R transformed cells was compared to the growth rates of Ap^R HPF cells and Ap^R LPF cells: growth times for three independent cultures of transformed cells were 63, 62, and 58 min (mean 61, corresponding to $r = 18 \times 10^{-3}$/min). Comparing growth rates of the HPF, LPF, and transformed lines indicates highly significant heterogeneity in an analysis of variance [$F(2,8) = 102$, $P \ll 10^{-4}$]. Furthermore, pairwise t tests involving transformed cells with the HPF line and with the LPF line both indicate highly significant differences. (A one-tailed test was used, as our a priori expectation was that the transformed cells would be intermediate between the HPF and LPF values.) Thus, most but not all of the increase in growth rate observed in the HPF line appears to stem from changes in the parasite rather than in the host.

An additional difference was noted between KI345 cells transformed with R208.H and HPF cells themselves. HPF cells were resistant to infection by phage R386, whereas KI345 transformed by R208.H were sensitive. Therefore, resistance of the HPF cells to infection by f1 was likely due to changes in the host, not in R208.H. This result is therefore the second line of evidence that both the host bacterium and the phage underwent changes during the evolution of the HPF line.

Competition Experiments

From the preceding analyses, sufficient divergence existed between R208.H and R208.L that they could be used in short-term experiments to address the evolution of benevolence. The objective was to expose a mixture of Ap^R LPF and HPF cells to conditions providing different opportunities for infectious transmission and to observe changes in the proportion of each cell type, and therefore of R208.H and R208.L. The basic design involved three steps: (1) inoculate a defined mixture of HPF and LPF cells into three types of cultures: (α) LB broth, (β) LB broth containing *E. coli* C (resistant to f1), and (γ) LB broth containing KI345 (sensitive to f1), (2) grow for 4–6 hr, and (3) monitor the change in proportion of R208.H between the onset and end of the experiment. The experiment was conducted twice: a 6-hr experiment designated A, and a 4-hr experiment designated B. At the onset of each experiment, the ratio of ampicillin-resistant cells (HPF and LPF cells) to ampicillin-sensitive cells in β and γ (*E. coli* C and KI345, respectively) was approximately 1:4 (range 1:3.7 to 1:5.9); the combined density of HPF and LPF cells at the onset was $4–6 \times 10^5$/ml.

The partner-fidelity model makes several predictions about the change in frequency of R208.H relative to R208.L over the course of the experiment. First, the benev-

olent parasite (R208.H) should be favored in the absence of opportunities for horizontal transmission, i.e., conditions α and β. Second, when opportunities for horizontal transmission are allowed (condition γ), the deleterious parasite (R208.L) should experience less of a disadvantage than in α and β. Although R208.L might actually increase in frequency over the course of the experiment in γ, we do not predict that it will necessarily increase, because there is no basis for knowing how much horizontal transmission is sufficient to offset the disadvantage resulting from debilitating growth of the host.

To formalize these predictions, consider the following model. For each $i \in \{\alpha, \beta, \gamma\}$, write

$$p'_i = \frac{p_i w_i}{p_i w_i + 1 - p_i} \tag{1}$$

hence

$$w_i = \frac{p'_i(1 - p_i)}{p_i(1 - p'_i)} \tag{2}$$

where

p_i = true proportion of R208.H in culture i at the outset of the experiment

p'_i = true proportion of R208.H in culture i at the end of the experiment

w_i = fitness of R208.H relative to R208.L over the course of the experiment.

Carats will indicate sample values. Note that w_i is not a per-generation fitness, rather it is a fitness measure applied over the duration of the experiment, encompassing several host–parasite generations. This measure is used for statistical convenience, and aside from this consideration, the hypotheses as well as the assessment of compatibility between observations and hypotheses are unaffected by use of it instead of a per-generation fitness.

Employing these symbols, the hypotheses from the partner-fidelity model are

I. $w_\alpha > 1$
II. $w_\beta > 1$
III. $w_\alpha > w_\gamma$
IV. $w_\beta > w_\gamma$

The corresponding null hypotheses substitute equalities for these inequalities.

The partner-fidelity model addresses one further aspect of this system. Culture conditions (α) and (β)—the LB and LB plus *E. coli* C conditions—both enforce conditions of high partner fidelity and so should reveal similar values of w. Note that, because KI345 is conjugation defective, it cannot transfer F (maleness) to *E. coli* C. A slight effect of *E. coli* C on the relative growth of HPF cells would not be at variance with the partner-fidelity model (e.g., the competitor *E. coli* C might reduce competition between HPF and LPF cells by reducing their growth rates during the experiment), but there is no formal basis for anticipating the magnitude of such an effect. We will thus treat

V. $w_\alpha - w_\beta = 0$

as a null hypothesis consistent with the partner-fidelity model, rejecting it if the magnitude of the observed difference is either too large or too small to be explained by chance alone.

From plaque assays of 150 Ap^R colonies plated at the onset and 150 colonies plated at the end of the experiment per culture condition, the sample values $\hat{p}_i$ and $\hat{p}'_i$ and corresponding $\hat{w}_i$ were as shown in the table at the bottom of the page. All observed $\hat{w}_i$ are consistent with (I)–(IV). As $\hat{p}_i$ and $\hat{p}'_i$ are subject to sampling error, however, it is necessary to demonstrate that sampling alone would not likely have generated such extreme values of $\hat{w}_i$. (In view of the large number of parasites and bacteria used per culture condition, any random sampling in the form of genetic drift that might have occurred during the course of the experiment can be neglected.) Tests of (I) and (II) are straightforward: hypotheses (I) and (II) are supported over their corresponding null hypotheses in both experiments because $\hat{p}'_i$

Experiment	$\hat{p}_\alpha, \hat{p}'_\alpha$	$\hat{p}_\beta, \hat{p}'_\beta$	$\hat{p}_\gamma, \hat{p}'_\gamma$	$\hat{w}_\alpha$	$\hat{w}_\beta$	$\hat{w}_\gamma$
A	0.38, 0.94	0.45, 0.97	0.54, 0.54	25.6	39.5	1.0
B	0.58, 0.96	0.52, 0.88	0.59, 0.27	17.4	6.8	0.3

is significantly larger than $\hat{p}_i$ (Fisher's exact test, $P < 10^{-6}$ in all four tests; CBE test, $P < 10^{-7}$ in all tests; Rice, 1988). That is, the observed increase in R208.H during the experiments is too great to be explained by sampling variance alone, indicating that w_α and w_β exceeded unity. Tests of (III) and (IV) are also straightforward in experiment B: not only is $\hat{p}'_i$ significantly greater than $\hat{p}_i$ for (α) and (β), but $\hat{p}'_\gamma$ is significantly less than $\hat{p}_\gamma$, indicating that $w_\alpha > 1 > w_\gamma$ and $w_\beta > 1 > w_\gamma$ (Fisher's exact test, $P < 10^{-6}$; CBE test, $P < 10^{-7}$).

Tests of (III) and (IV) in experiment A are slightly more complicated, because the observed frequency of R208.L did not change in (γ). The question to be tested, then, is, if we had drawn additional samples from the cultures, would $\hat{w}_\alpha - \hat{w}_\gamma$ and $\hat{w}_\beta - \hat{w}_\gamma$ have consistently exceeded zero, as they did in the observed samples? To answer this question, the following bootstrap-like procedure was conducted. A hypothetical frequency, $\hat{P}_i(\hat{P}'_i)$ was calculated by drawing 150 random variables from a binomial distribution whose mean was $\hat{p}_i(\hat{p}'_i)$. This process was repeated for all $i = \alpha, \beta, \gamma$. These hypothetical $\hat{P}_i$ and $\hat{P}'_i$ were used to calculate $\hat{W}_i$ from (2), and the quantities $\hat{W}_\alpha - \hat{W}_\gamma$ and $\hat{W}_\beta - \hat{W}_\gamma$ were recorded. This procedure was repeated at least 10,000 times, yielding the distribution of these differences that would have resulted from sampling alone. In this way, it was found that the observed $\hat{w}_\alpha - \hat{w}_\gamma$ and $\hat{w}_\beta - \hat{w}_\gamma$ were both significantly greater than zero for experiment A ($P < 10^{-4}$ and $P < 10^{-3}$, respectively), supporting (III) and (IV) over their corresponding null hypotheses. In contrast, null hypothesis (V) was not rejected under this bootstrap-like procedure [$P \geq 0.2$ when the observations were tested separately against the two alternatives $w_\alpha - w_\beta > 0$ and $w_\alpha - w_\beta < 0$; probabilities for replicates A and B were incorporated into the consensus combined probability test (Rice, 1990) in calculating these overall significance levels]. Thus, (I)–(V) are supported by the data.

These analyses have addressed the significance of results within the context of each experiment, in that all sampling error is assumed to arise during the drawing of individual cells from a common pool for each culture condition. It is also of interest to evaluate the significance of these results when accounting for between-replicate errors, and we offer two forms of such tests. (1) Under the null hypothesis that all changes in frequency are purely random, and ignoring the magnitudes of any changes, the probability of obtaining results consistent with (I)–(IV) is $^1/_{12}$. [The probability is one-half for each of (I) and (II) and is one-third for the joint combination of (III) and (IV).] Incorporating the results of the two (independent) experiments A and B into a single test, the null hypothesis is rejected with $P < 0.007$. (2) Student's t tests of the (logged) fitnesses combined for replicates A and B call for rejection of the respective null hypothesis in favor of the alternative for (I) ($P < 0.025$), for (III) ($P < 0.025$), and for (IV) ($P < 0.05$); the significance level for (II) is ($0.05 < P < 0.1$). Overall, therefore, we observe substantial collective support for hypotheses (I)–(IV). Again, there is no basis for rejecting hypothesis (V) with either test (1) or (2).

Discussion

This study fully supports the mathematical and conceptual arguments suggesting that cooperation between a parasite and its host evolves according to the opportunities for horizontal versus vertical transmission, i.e., according to partner fidelity (Axelrod and Hamilton, 1981; May and Anderson, 1982; Ewald, 1983, 1987; Lenski, 1988; Bull and Rice, 1991). In the system developed here, the host was a bacterium and the parasite was a phage or its derivative. Two forms of the parasite were used, a benevolent form that permitted a relatively high host growth rate but was incapable of infection, and a deleterious form that hindered host growth rate but was capable of infecting other bacteria. When hosts were propagated so that infectious spread of the parasite was prevented, thus ensuring high partner fidelity, the benevolent form of the parasite prevailed. This selective advantage of the benevolent form was observed whether the parasitized hosts were propagated in isolation of other bacteria or were propagated in the presence of bacteria resistant to infection; in both cases there was no opportunity for horizontal transmission.

In contrast, when horizontal transmission of the parasite was allowed by providing an abundance of alternative hosts—permitting low partner fidelity—the strong advantage of the benevolent form was lost.

Although this study provides the first experimental demonstration of the importance of mode of transmission in the evolution of parasite benevolence, the life history of temperate phages provides a remarkable parallel. The temperate phages have evolved a mechanism that allows them to switch between benevolent and deleterious modes of parasitism in response to the opportunities for horizontal and vertical transmission. These phage can grow lytically, producing a burst of 100–200 progeny per cell, or they can enter into the lysogenic pathway, where they are passively replicated by the host without producing any phage progeny. Two environmental factors determine the lysis–lysogeny decision: a high multiplicity of infection (indicating a paucity of alternative hosts) leads to lysogeny, whereas a low multiplicity of infection promotes lytic growth. In addition, nutritional deprivation of the host cells promotes the lysogenic response. However, if the viability of the lysogenic cell is threatened, the phage is induced into the lytic pathway (Friedman et al., 1984).

The objective of this study was to demonstrate natural selection of cooperation between a host and its parasite. In the particular system used (bacteria infected with phage R208), maintenance of the parasite was ensured by exposure of cells to ampicillin, resistance to this drug being carried by a plasmid cloned into the phage. Because a plasmid can function as an independent replicon, i.e., it can replicate and segregate as an entity separate from the phage, mere excision of the plasmid replicon from the chimera provided a simple means of evolving benevolence while maintaining ampicillin resistance—as indeed evolved in the HPF line. A major advantage of this system, therefore, was that it facilitated assay of the competition experiments, because the benevolent form that evolved in the HPF line was easily distinguished from the infectious form that prevailed in the LPF line. Many aspects of the experimental design here might not have been possible if the original parasite had not been so prone to mutate to such a highly benevolent state.

An interesting topic for future work is the molecular basis of benevolence, and for this question, the pair of replicons in R208 does not constitute a special advantage. This is not to say that the results obtained are irrelevant to more natural systems: (1) many naturally occurring plasmids and phages also contain multiple, independent replicons; (2) the benevolent plasmid that evolved from R208 did not result from precise excision of pBR322 DNA; it likely retains part of the f1 genome; and (3) a similar outcome—evolution of a noninfectious phage genome retaining expression of antibiotic resistance—has been observed in one of four repetitions of the HPF design using phage R386, which carries only the f1 replicon (unpublished). Rather, despite the applicability of our results to certain natural systems, it is apparent that the evolution of benevolence may follow various genetic pathways, and R208 or any other phage–plasmid chimera likely provides a limited repertoire of possible outcomes. For example, published work on filamentous phages describes several kinds of mutations that appear to increase phage benevolence without eliminating infectivity (as assayed by plaque turbidity: Salivar et al., 1964; Model and Russell, 1988). Current work of ours suggest yet additional pathways by which f1 phage may evolve benevolence. Understanding the manifold genetic bases for the evolution of benevolence will thus benefit from experimentation with various phages, hosts, and multiple replications of each design. Such an experimental system promises to yield insights to many basic problems in evolution that far transcend cooperation.

The evolution of bacteria resistant to the parasite was not specifically addressed in this study, because exposure of cells to ampicillin killed all cells lacking a parasite. An important extension of this work is therefore to allow the evolution of phage-resistant bacteria. The partner-fidelity model provides insight only to the *relative* level of benevolence expected to evolve. For example, although high partner fidelity selects benevolence, if even the most benevolent parasites debilitate their hosts somewhat,

the evolution of host resistance could result in the loss of the parasite. The maintenance of stable cooperation thus encompasses a broader scope of problems than addressed here.

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