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E-Article

Optimal Foraging by Bacteriophages through Host Avoidance

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abstract: Optimal foraging theory explains diet restriction as an adaptation to best utilize an array of foods differing in quality, the poorest items not worth the lost opportunity of finding better ones. Although optimal foraging has traditionally been applied to animal behavior, the model is easily applied to viral host range, which is genetically determined. The usual perspective for bacteriophages (bacterial viruses) is that expanding host range is always advantageous if fitness on former hosts is not compromised. However, foraging theory identifies conditions favoring avoidance of poor hosts even if larger host ranges have no intrinsic costs. Bacteriophage T7 rapidly evolved to discriminate among different *Escherichia coli* strains when one host strain was engineered to kill infecting phages but the other remained productive. After modifying bacteria to yield more subtle fitness effects on T7, we tested qualitative predictions of optimal foraging theory by competing broad and narrow host range phages against each other. Consistent with the foraging model, diet restriction was favored when good hosts were common or there was a large difference in host quality. Contrary to the model, the direction of selection was affected by the density of poor hosts because being able to discriminate was costly.

Keywords: optimal foraging theory, host avoidance, adsorption, bacteriophage T7, experimental evolution.

All organisms eat. Yet many consume only a narrow range of items within the full spectrum of possible foods they encounter. At face value, the failure to utilize potential food items is enigmatic, and a common rationale for the evolution of narrow diets is a trade-off in efficiency due to antagonistic pleiotropy: specialization on few food types enables more effective exploitation than generalization across a wide range of food types (the specialist-generalist model; Wilson and Yoshimura 1994). A forager that evolves to use one resource better might then use other resources less efficiently. Yet another explanation for diet restriction comes from optimal foraging theory: not all food types are equally valuable, and the optimal diet consists of only the best types, down to some threshold below which all other types are avoided (Emlen 1966; MacArthur and Pianka 1966; Pyke et al. 1977). Here, the downside of diet breadth is a reduced rate of food acquisition; the net gain from a poor food item is not worth the lost opportunity of finding a good item. In contrast to the specialist-generalist model, the optimal foraging result imputes no cost or benefit to specialization other than these opportunity costs—the poor hosts are intrinsically poor, and the only option is whether to utilize them. Another difference is that optimal foraging is generally applied to plastic behaviors (Werner and Hall 1974), while the specialist-generalist model applies to fixed differences. Optimal diet notions are also typically intended for animals, although they have been applied to bacterial dispersal (K. L. Hillesland, personal communication), and trade-off models incorporating diminishing returns have been used to explain bacterial metabolic specialization (Pfeiffer et al. 2005). Also, a somewhat related model of optimal phage lysis time has been interpreted from the perspective of optimal foraging in a patchy environment (Abedon et al. 2003; Wang 2006; Heineman and Bull 2007).

In this article, we address the optimal host range of T7 bacteriophage, a virus that infects *Escherichia coli*. Phages typically infect only some strains of a bacterial species. However, some phages do have broad host ranges, indicating that there is no intrinsic restriction to few hosts. The evolutionary reasons for these restricted host ranges are thus unclear, though many justifications focus on the specialist-generalist model (Bohannan and Lenski 2000; Duffy et al. 2006). In any case, the overwhelming number of phages in the environment means that phage host range is likely to affect microbial diversity, which, in turn, may be important in shaping ecology at larger scales (Weinbauer 2004). Understanding host range evolution in

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phages also has implications for eukaryotic viruses. Host shifts in mammalian viruses have caused devastating epidemics (Sharp et al. 2001; Truyen 2006), and the evolution of new tissue tropisms within a host (which can be considered a form of host range evolution) is important in some diseases (Hueffer and Parrish 2003). Understanding viral evolution to avoid/infect certain tissues is thus important to understanding the nature of within-host viral adaptation and the balance between adaptation for maximum transmission and adaptation within the host (Zhang et al. 1993).

Phage Biology

There are two issues in testing whether phages evolve optimal host ranges. The first is mechanistic: can the phage easily evolve to discriminate among strains of possible bacterial hosts? If host range does not easily evolve, then the phage is not expected to achieve optimality. Second, if host range can evolve easily, do models of optimal diet successfully predict how it evolves? Formal tests of both issues have not been presented previously, and only such tests can provide the ultimate answers to these questions. However, there is a considerable background of phage biology that should be considered in deciding whether such tests are feasible and reasonable.

In agitated liquid environments (as here), phages encounter their hosts randomly, moving both by Brownian motion and by the externally driven dynamical mixing, until they accidentally collide with a bacterium (Adams 1959). To infect a bacterium, a phage must stick to it when this collision occurs (phage attachment is referred to as adsorption), and this stickiness depends on the phage as well as on the bacterial physiological state and genotype. If a collision does not result in adsorption of the phage to the bacterium, the phage is unaltered and, with virtually no lost opportunity, is free to encounter other bacteria to which it may adsorb.

As the stickiness, or adsorption rate, of the phage is decreased, progressively fewer of the collisions with bacteria will lead to adsorptions. Infection involves additional steps beyond adsorption, but in the context of our study, adsorption is the step by which the phage increases or decreases its infection rate of a bacterial strain. Whether a bacterial strain is part of a phage's host range is therefore often quantitative, not absolute, yet optimal foraging theory can be tested from these quantitative differences.

Host range/adsorption rate is generally considered to be genetically determined, not plastic, in phages (Adams 1959). There is ample evidence that phages can evolve to expand their host range or switch to a new host, although previous work has not specifically addressed whether phages routinely evolve to discriminate among hosts. In tailed phages, such as the one used in this study, tail fiber proteins effect adsorption by attaching to specific sites on the host's surface, and simple changes in those genes have been demonstrated to alter host range (Drexler et al. 1989, 1991; Hashemolhosseini et al. 1994; Cerritelli et al. 2003). Furthermore, sequence comparisons of phage genomes often reveal rapid changes in tail fiber sequences, suggesting that changes in host range are common (Montag et al. 1987). Thus, phage host range appears to be a versatile trait capable of evolving through changes in single genes, suggesting that phages have no intrinsic impediments to evolving an optimal diet.

The second issue is whether phages with different host ranges evolve according to models of optimal diet. Although phages do not exhibit behavior in the usual sense, optimal foraging theory can be applied to the evolution of phage host range (Bull 2006). Known cellular mechanisms that destroy infecting phage genomes and abort all phage progeny (Chopin et al. 2005) may impart strong selection for phages to be choosy. In addition, host metabolic quality is important to the phage life cycle (Weinbauer 2004), so there may be selection to avoid hosts that are relatively poor, even when infections yield progeny. There is wide variation between the host ranges of different phages isolated from the wild (Weinbauer 2004), so it might be imagined that discrimination among hosts routinely evolves. However, the natural history of phages in nature is largely unknown, so it is not feasible to test whether natural isolates are optimal for their environment.

One benefit of phages that overcomes this drawback of unknown ecology is that tests of evolutionary models can often be done experimentally. With respect to host range, phages have been directly selected to adopt new hosts to test for specialist-generalist trade-offs (Drexler et al. 1989, 1991; Duffy et al. 2006), but those direct-selection methods did not provide phages with a choice and thus did not address the most interesting of the optimal diet predictions. Here we employ an experimental evolution approach to study phage host range optimality. Following the logic outlined above, our approach applies tests in two steps. (i) Does T7 evolve discrimination when strongly selected to do so? (ii) Does the optimal foraging model accurately predict when discrimination will be favored under more subtle conditions?

Methods

Phage Lines and Cell Strains

The wild-type T7 ($T7^+$) bacteriophage (GenBank AY264774) has a 40-kb dsDNA genome encoding around 60 proteins and infects several strains of *Escherichia coli* (Molineux 1999). Derivatives of three *E. coli* strains were

used as hosts here: C, B, and K12. All three strains are infected by $T7^+$. Yet these strains are known to differ in their lipopolysaccharide (Wright et al. 1980), surface molecules that serve as the receptors for T7 (Molineux 1999). They are thus plausible candidates for the evolution of discrimination by T7. *Escherichia coli* C was used as a permissive host (supporting phage replication) in all adaptations. An *E. coli* B strain IJ1958 trxA::Kn was the nonpermissive cell in the $T7\_B^-$ adaptation to avoid B, while IJ1517 trxA::Kn, a K12 strain, played that role in the $T7\_K^-$ adaptation to avoid K12. Both nonpermissive strains allow infection but abort all T7 progeny because they lack the host factor thioredoxin. Adsorption assays used *E. coli* C, BL21 (a permissive B strain), and IJ113 (a permissive K12 strain). For optimal foraging assays, a strain designated *E. coli* $C_{tet\text{-}R}$ (*E. coli* C carrying a tetracycline-resistant pBR322 plasmid) was used as the good host, while IJ1142 (a tetracycline-sensitive K12 otherwise nearly identical to IJ1133) acted as the poor host.

Selection for Host Discrimination Mutants

Wild-type $T7^+$ was transferred in a mix of permissive C and either nonpermissive K12 or nonpermissive B in Luria-Bertani (LB) broth (10 g NaCl, 10 g Bacto tryptone, 5 g Bacto yeast extract/L) at 37°C. In general, a large excess of nonpermissive cells was used, with enough permissive cells to maintain the phage population. A somewhat heuristic approach was used to select avoidance of K12, in which a culture of phage was often challenged with pure nonpermissive cells before adding permissive ones; a more standardized protocol of serial transfer into flasks with 95% nonpermissive cells was used to select avoidance of B. The stringency of the negative selection likely affects the rate of adaptation to avoid a host but is not expected to alter the outcome.

Construction of $T7_{choosy}$ Phage

The phage that evolved to avoid K12 ($T7\_K^-$) carried a substitution in its tail fiber gene (*17*) but also carried at least one other new substitution in gene *17.5*. To separate the effects of the *17* substitution from the effects of other changes, site-directed recombination was used to create a wild-type phage carrying just the *17* substitution. This protocol involved creating plasmid pRS36255, a pUC18 vector carrying the gene *17* fragment of $T7\_K^-$ spanning nucleotides 36054–36360. Phage $T7^+$ was plated on Topo10 cells (Invitrogen) bearing pRS36255 and was incubated at 37°C. Several plaques were suspended in LB broth, and the suspension of phage was enriched for recombinants bearing the substitution by a 2-h incubation with IJ1517 trxA::Kn (which preferentially destroyed phages that adsorbed to K12). The culture was then treated with chloroform to kill cells and was plated on *E. coli* C. Plaques were isolated and sequenced to check for the presence of the desired substitution in gene *17* and the lack of the substitution in *17.5*. This phage, in which the only genetic change should be one that decreases adsorption to K12 (although it may have epistatic effects), is called $T7_{choosy}$.

Adsorption Rate Assays

Cells were grown from frozen aliquots for 1 h to a density of approximately 10^8 cells/mL. The culture was infected with 10^6 plaque-forming units of the requisite phage from a lysate less than 5 days old. At 5 min, the culture was plated from centrifuged and uncentrifuged aliquots to determine the density of unadsorbed phages (N_u) and total phages (N_t). Adsorption rate α (mL/min) was calculated from $N_u = N_t e^{-5C\alpha}$, where C is the density of cells per milliliter, also determined by plating (Bull et al. 2004).

Optimal Foraging Assays

A 50 : 50 mix of $T7_{choosy}$ (infects C, avoids K12) and $T7^+$ (an indiscriminate phage that infects C and K12) was added to a flask containing, in 10 mL of LB broth, a mix of good-quality hosts (tetracycline-resistant $C_{tet\text{-}R}$) and poor hosts (tetracycline-sensitive K12, IJ1142). Flasks were placed in a water bath with continual shaking (200 rpm) to maintain cell suspension and aeration. Cells were added from frozen aliquots at volumes expected to yield the cell densities reported at 1 h, but cell densities were measured by plating in each set of trials to confirm densities. Tetracycline at varying concentrations was added 10 min before phage addition to decrease the productivity of K12 infection. After phage growth for 30 min and before phage densities exceeded cell densities, a portion of this flask was added to another flask with the requisite cell densities. This was repeated so that each phage mixture was adapted for a combined 2 h.

Sequencing

The DNA for sequences was obtained from polymerase chain reaction (PCR) products, either of isolates or of populations. Genomes of the two phages adapted to avoid a cell type were sequenced over genes *17* (tail fiber; Dunn and Studier 1983) and *17.5* (a holin thought to affect lysis; Vukov et al. 2000; Wang et al. 2000). Phage $T7\_K^-$ was also sequenced over genes *11* and *12*, which encode other tail proteins. All reported nucleotide numbers presented are from $T7^+$ (GenBank V01146; Dunn and Studier 1983). The population at the end of each optimal foraging assay,

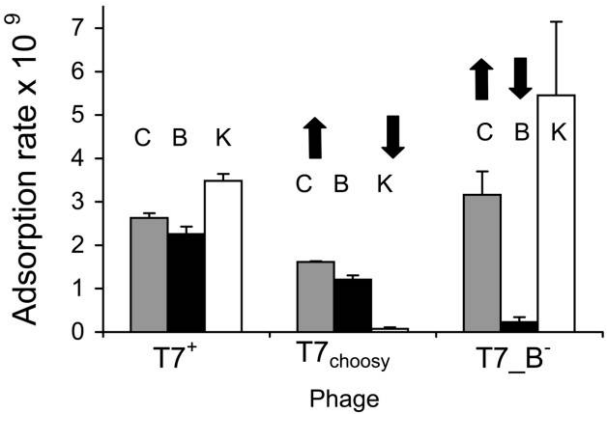

Figure 1: Adsorption rates are shown for different phage isolates ($T7^+$, $T7_{choosy}$, and $T7\_B^-$) on three hosts (C, B, and K12, indicated by letters above each bar). Wild-type $T7^+$ adsorbed well to all cell types. Up (down) arrows indicate selection to grow on (avoid) a particular host strain. Adapted phages' adsorption was low on hosts they were selected to avoid, while there were varying (but less extreme) effects on adsorption to other cell strains. Phage $T7_{choosy}$ carries the mutation in the tail fiber gene that caused adsorption changes during the adaptation to avoid K12 and is statistically indistinguishable from $T7\_K^-$. All error bars represent 1 SE. An upper limit for adsorption rate, based on physical properties, is considered to be 10^{-8} (Adams 1959), so values greater than 10^{-9} are considered to indicate moderately high adsorption rates.

as well as the original 50 : 50 mix of $T7^+$ and $T7_{choosy}$, was sequenced across base 36254 in the tail fiber gene, at which the phages differ. The relative peak height of the two bases was used to estimate frequencies by dividing the height of one peak by the total combined height of both peaks. The known 50 : 50 mix was found to yield peak heights of very close to 50% each (50.3% $T7_{choosy}$), validating the accuracy of this method. Sequences were obtained from PCR products with ABI Big Dye mix (ver. 3.1) and an ABI3100, analyzed with AB Sequencing Analysis Software, version 5.1.1, and DNA Star, version 4.05.

Results

Evolution of Discrimination

Wild-type $T7^+$ was independently adapted in two mixes of *Escherichia coli* strains: C with either B or K12. In both adaptations, C was the permissive host, while the other (B or K12, depending on the adaptation) aborted T7 infections due to deletion of a host gene needed for viral replication. Both adapted phages evolved to largely avoid the nonpermissive host but maintained a high adsorption rate to C; the evolved phages are designated $T7\_B^-$ and $T7\_K^-$, respectively (fig. 1; the adsorption of $T7\_K^-$ is represented with $T7_{choosy}$, a recombinant that carries the relevant adsorption mutation). The selective environment in both treatments was confined to just two of the three possible hosts (C, B, and K12). However, avoidance of the nonpermissive host evolved without greatly compromising adsorption to either the permissive host or the unselected host (a slight reduction on the permissive host was observed for TK_K^-).

The adsorption rates observed here are small, and if one is not accustomed to quantitative aspects of phage dynamics, the implications just claimed may not be obvious. We thus offer some basics. The maximum attainable adsorption rate for a generic phage, in which nearly all phage-bacterial collisions result in adsorption, is approximately 10^{-8} mL/min (Adams 1959). The low magnitude of this value is due to the small sizes of the phage and bacterium and the consequent improbability of a random collision per minute between the two individuals in a milliliter of media. Adsorption rates of most lab-adapted phages on their lab hosts are within the range of $2–6 \times 10^{-9}$ and are hence close to this limit. A drop of even 10-fold, while appearing to be small relative to 10^{-8}, has a profound effect on infection rates. For example, under the conditions used in this design (10^8 cells/mL), a change in the adsorption rate from 3×10^{-9} to 3×10^{-10} to 3×10^{-11} would mean that after 10 min, the fraction of phage adsorbed would drop from 95% to 25% to 3%. Thus, the evolved declines in adsorption rates have a substantial effect in reducing the phage's use of that host.

Expansion of host range in tailed phages often stems from changes in tail fiber genes (Drexler et al. 1989, 1991; Hashemolhosseini et al. 1994), so it was anticipated that the T7 discrimination would also stem from changes in the tail fiber gene (gene *17*). Sequencing of *17* indeed revealed one coding substitution in each adaptation, at nine and 33 residues from the carboxyl (distal) end of the 533 amino acid protein (table 1); this end of the protein is involved in interaction with the host, so changes in this

Table 1: Genetic evolution of $T7\_K^-$ (adapted to avoid K12) and $T7\_B^-$ (adapted to avoid B)

Phage and base	Gene	Change
$T7\_K^-$:		
36254	*17*	T → C V544A
36389	*17.5*	A → G I16V
$T7\_B^-$:		
36183	*17*	T → G D520E
36405	*17.5*	T → C V21A

Note: $T7\_K^-$ was sequenced over bases 24097–27659 and 34554–36676; $T7\_B^-$ was sequenced over bases 35046–37179. Gene *17* encodes the tail fiber protein, essential for adsorption; gene *17.5* encodes a holin, whose function is in lysis timing.

region plausibly affect adsorption (Steven et al. 1988). Site-directed mutagenesis/recombination was used to introduce the *17* change from T7_K$^-$ to T7$^+$, and this phage (T7$_{choosy}$) was found to have adsorption statistically indistinguishable from that of T7_K$^-$ on both C and K12. Thus, the discrimination between C and K12 can be attributed to the single substitution in the tail fiber gene. It is likely that the (different) gene *17* change in T7_B$^-$ caused the discrimination between C and B, but the effect of this substitution alone was not tested.

An amino acid change was also detected in the adjacent gene *17.5* of each evolved phage. This gene has a role in lysis timing. Lysis time was measured in one of the evolved phages, T7_K$^-$, and was significantly earlier than in T7$_{choosy}$, which was wild-type, except for the gene *17* substitution (methods as in Heineman et al. 2005; data not shown). Because only part of that phage was sequenced, it cannot be ruled out that unidentified changes were responsible for the difference in lysis time, but the change in lysis time is compatible with an effect of the *17.5* change on lysis.

Predictions of Optimal Foraging Theory

The conditions used to select discrimination were extreme and thus merely tested whether T7 could easily evolve to discriminate between a pair of host strains. Given that host discrimination mutants exist, optimal foraging theory makes three testable predictions (Emlen 1966; MacArthur and Pianka 1966; Pyke et al. 1977; Bull 2006). For simplicity, we consider optimal foraging theory as it applies to a phage growing on a population of two hosts of different qualities (one host is good, the other poor for phage reproduction). (1) Dependence on relative host quality: holding host densities constant, discrimination will be increasingly favored as the difference in quality of the two hosts is amplified. (2) Dependence on good-host density: discrimination will be selected against at low densities of the good host but will be favored at high densities of the good host (holding density of the poor host constant). (3) Lack of dependence on poor-host density: the density of poor hosts affects the strength of selection on avoidance but not the direction.

These predictions rest on several assumptions: (i) the two host types are encountered randomly, in proportion to their abundances; (ii) hosts outnumber phages; and (iii) avoiding one host type has no effect on the rate of encountering another. The first two assumptions are easily met by the experimental conditions: a liquid culture in which the two hosts are continually mixed by shaking and from which phages are transferred before their densities outnumber cell densities. The third assumption is met by virtue of the kinetics of phage-bacterial encounters, as reviewed above (Adams 1959).

Tests of Optimal Foraging Theory

The predictions were tested by competing phages with different host ranges on mixed populations of two hosts. The two hosts were engineered to present different benefits to the phage by supplementing the media with low levels of the antibiotic tetracycline and endowing one of the hosts with a tetracycline-resistant plasmid; impairing bacterial physiology and protein synthesis with this drug directly impairs phage physiology because the phage depends on many bacterial functions. Specifically, 1 : 1 mixes of T7$^+$ and T7$_{choosy}$ were introduced to different densities of *E. coli* C (C$_{tet\text{-}R}$, tetracycline resistant) mixed with K12 bacteria (tetracycline sensitive), and changes in phage frequencies were monitored to assess relative fitness. These tests could also have employed T7_B$^-$ as the discriminating phage (on a mix of *E. coli* C and B hosts), but the apparently similar molecular bases of host discrimination by T7_K$^-$ and T7_B$^-$ led us to focus on just one phage. Furthermore, when the engineered strain T7$_{choosy}$ was utilized, the difference in host range was known to be the single amino acid difference in the tail fiber gene, and confounding effects of other substitutions were thereby avoided.

Phage T7$_{choosy}$ infects C and avoids K12, whereas T7$^+$ infects both at high rates (fig. 1). Prediction 1, that larger differences in quality between good and poor hosts will increasingly favor discrimination, was tested by competing the two phages under conditions imposing different disadvantages to infecting the poor host (K12, tetracycline sensitive) by using different concentrations of tetracycline. Increasing levels of tetracycline may have also affected fitness of C$_{tet\text{-}R}$ cells somewhat, but for the model, it is the increasing disparity between the two host types that is important. The same density of cells was used in all experiments (approximately 5% C$_{tet\text{-}R}$ and 95% K12, with a total density of 10^8 cells/mL).

Results of 2-h competitions were consistent with the optimal foraging model. The proportions of the two phages were calculated by comparing the height of sequence peaks at the nucleotide in the tail fiber gene that differed between the phage lines (the nucleotide known to be the basis of host discrimination). Avoidance of K12 was favored at the higher levels of tetracycline (1.5 μg/mL and above) when K12 was most impaired but was selected against at the lower levels of tetracycline (0.5 μg/mL; fig. 2). For all but one tetracycline concentration, only one phage base (and, hence, only one phage type) was detectable in the sequence profile from the 2-h endpoint culture. At one intermediate tetracycline concentration (1 μg/mL), selection was weak enough that both phage types

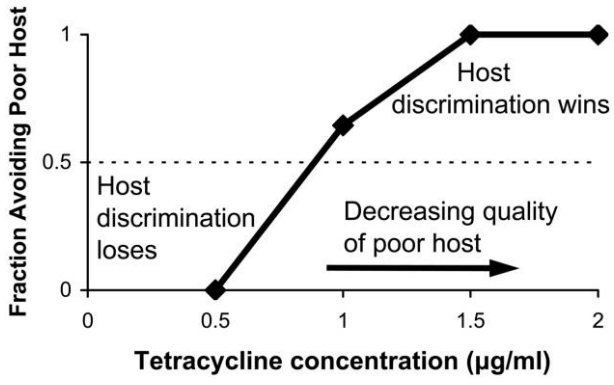

Figure 2: Advantage of host avoidance depends on relative host quality. Phages were grown in a mixture of a good host ($C_{tet\text{-}R}$) and a poor host (K12), with 5×10^6 and 10^8 cells/mL of each host, respectively. The quality of tetracycline-sensitive K12 hosts was progressively reduced by increasing the concentration of tetracycline, as indicated on the horizontal axis. A 50 : 50 mixture of choosy phages that avoided K12 ($T7_{choosy}$) and indiscriminate phages that infected both strains ($T7^+$) was used to initiate the competition; at 2 h, the final proportion of each phage type was measured. At least two assays were performed for each point. Confidence bars of 1 SE, smaller than the point, are provided.

were maintained at intermediate frequencies throughout the 2-h competition, although it appeared that discrimination had a slight advantage. Phage $T7^+$ did grow on K12 impaired by tetracycline at 1.5 μg/mL (data not shown), so these results were not due to the trivial case that K12 simply prevented phage reproduction whenever discrimination was favored.

Prediction 2, that increasing the density of good hosts favors discrimination, was tested as well. Using tetracycline at 1.5 μg/mL and a constant high density of K12 hosts (10^8 cells/mL), we found that avoidance spread when the density of $C_{tet\text{-}R}$ was high but not when it was low (fig. 3). This result also supports the model. Again, selection was weak enough at one intermediate density of $C_{tet\text{-}R}$ that peaks from both phage types were evident in the sequence profiles from the end of the competition.

From prediction 3, once good host density is high enough to favor discrimination, avoidance should evolve regardless of the density of the poor host. In contrast to the other successes of the optimal foraging model, this prediction was not supported: although discrimination was favored when $C_{tet\text{-}R}$ and K12 densities were 5×10^6 and 1×10^8, respectively, discrimination was selected against when K12 density was dropped by two orders of magnitude to 1×10^6 (tetracycline at 1.5 μg/mL in all cases; fig. 3). The basis for this model failure is suggested by the next result.

An implicit assumption of the optimal foraging model is that the only fitness cost of discrimination is realized from the missed opportunities of infecting the poor host (which may be advantageous or not, depending on the environment). Thus, the two phages should have equal fitness when exposed to just the good host. In contrast to this assumption, a competition of both phages on 5×10^6 $C_{tet\text{-}R}$ (with 1.5 μg/mL tetracycline) revealed that $T7^+$ grew faster than $T7_{choosy}$. Phage $T7_{choosy}$ was only 38% of the population after 2 h (differing from a 50 : 50 ratio, with $P < .003$, two tailed). Thus, there is a modest cost to discrimination between C and K12, independent of differential host effects. This cost is likely to be caused by the lower adsorption rate of $T7_{choosy}$ to C ($P < .001$; fig. 1) and is specific to the gene *17* substitution of $T7\_K^-$ (which can be inferred from the fact that $T7_{choosy}$ was engineered to differ from $T7^+$ by only the single substitution).

The choosy phage fared best (in competition with $T7^+$) at high densities of poor hosts (10^8), next best in the absence of poor hosts, and, paradoxically, slightly worse at intermediate densities of poor hosts (10^6). However, the difference between $T7_{choosy}$ performance with few poor hosts and with no poor hosts is not significant, and it seems likely that there is merely little difference between these two conditions.

Discussion

Phage T7 evolved to discriminate among host strains of *Escherichia coli* with single amino acid substitutions in the

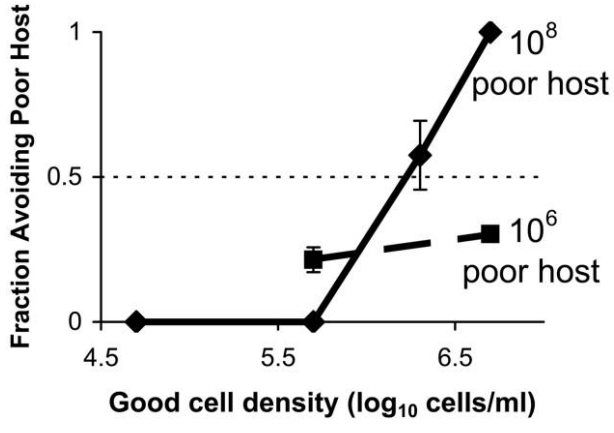

Figure 3: Evolution of avoidance depends on the density of both good and poor hosts. When the relative productivity of good and poor hosts is held constant (1.5 μg/mL tetracycline) and the density of poor hosts is high (10^8 K12/mL; *solid line*), increasing the density of good-host cells ($C_{tet\text{-}R}$) ultimately favors avoidance of poor hosts. When the poor host is at low density (10^6 K12/mL; *dashed line*), the same pattern is expected as for the solid line, yet the discriminating phage is at a disadvantage, even when the good host is common. At least two assays were performed for each point, except the lowest-density $C_{tet\text{-}R}$ 10^8 K12 point. Confidence bars of 1 SE, sometimes smaller than the point, are provided.

tail fiber gene. In contrast to the commonly applied selection for expanding host range, our selection was for host range contraction, which we could achieve because infection of one strain was lethal to the virus. Although few host strains were tested, our results may be cautiously extended to suggest that tail fiber genes can readily evolve to discriminate among many bacterial strains. Furthermore, given the many ways bacteria can be nonproductive hosts for a phage—for example, superinfection exclusion (McAllister and Barrett 1977), restriction modification systems (Twomey et al. 2000), and abortive infection systems proper (Molineux et al. 1989; Chopin et al. 2005)—we may expect that phages are often selected to shrink as well as shift host range. The three strains used here differ in their surface molecules, which provide a likely basis for discrimination (Wright et al. 1980).

A tantalizing possibility concerns cells in stationary phase, a physiological state in which bacteria largely cease to reproduce. While T7 is reported to grow somewhat on stationary phase cells, those cells are unproductive for many other phages due to altered physiological states that are in some cases related to mechanisms of abortive infection (Slavcev and Hayes 2003). Adsorption is generally lower on cells in stationary phase, a point argued to stem from physical constraints such as reduced cell size or lower density of receptors to which the phage can adsorb (Adams 1959). Our study raises the possibility that reduced adsorption to stationary phase cells might also be an adaptive response of lytic phages to avoid nonproductive physiological host states. Cells in nature may spend much of their time in stationary phase, possibly providing lytic phages with a consistent selective pressure to avoid them, especially if other, better, hosts are relatively common. Our results also suggest a way that viruses could avoid ecological traps that have been proposed as a way of limiting the growth of viral populations (Dennehy et al. 2007).

Our results mostly supported optimal foraging theory in an evolutionary context that is nonbehavioral. Phages have no behavioral plasticity in the usual sense, yet they evolved to make host range choices that qualitatively match optimality predictions. Although most animal foraging may include at least some plasticity, our study shows that plasticity is not vital to the success of optimal foraging predictions when the environment is constant. Selection was measured directly in evolutionary competition between a broad and a narrow host range genotype, avoiding the usual difficulties of inferring fitness from a proxy such as energy intake. Few studies have actually measured evolution in response to optimal foraging predictions, although there are some precedents (Abedon et al. 2003; Heineman and Bull 2007; K. L. Hillesland, personal communication).

Our tests were qualitative because they did not predict the thresholds at which selection should shift from favoring avoidance to favoring host range breadth. It should be possible in principle to estimate the threshold and thus conduct quantitative tests, but this requires estimates of burst size, latent period, and adsorption rates of the different phages (Bull 2006), and the combined uncertainty in these estimates would likely thwart any overall accuracy.

Our results deviated in one way from the simple optimal foraging model: avoidance of the poor host compromised performance/growth rate on the good host. This cost seems to be caused by decreased adsorption on the good host, a pleiotropic effect of the same mutation that far more greatly reduces adsorption to the poor host. When avoidance of the poor host was otherwise favored but the poor host was uncommon, the cost to discrimination outweighed the advantage of discrimination, and the nondiscriminating phage was favored, contrary to the model. This intrinsic cost to choosiness is analogous to a predator needing metabolically expensive sensory organs in order to reject unsuitable prey, with the cost remaining even when unsuitable prey are absent.

In one respect, this cost overlaps the second body of theory developed to deal with optimal diets—the specialist-generalist trade-off paradigm. Within this paradigm, the drawback to diet breadth is generally thought to stem from a cost to utilizing many food items efficiently. In $T7_{choosy}$, we observed the opposite—a cost to specialization. The generality of this cost to discrimination by phage is not apparent, however. There may be other T7 mutations that would have caused avoidance of K12 without affecting fitness on C, though one would expect that if such changes existed, they would have been favored during the initial adaptation over a change that has a cost on C. In any case, $T7\_B^-$ does not appear to have decreased adsorption to C cells, suggesting that a cost to discrimination is by no means a universal pattern in phages (also see Duffy et al. 2006).

The advantage of discrimination depends not only on the relative quality of hosts but also on the growth rate of the phage population (Bull 2006). Here, we adapted phages in conditions that allowed high growth rates and maintained cells in excess. The benefits of discrimination decrease as phage growth rate slows or hosts become limiting. Under some conditions, any infection that results in at least one phage progeny will be favored, while others will not (Bull 2006). Thus, the potential for avoidance revealed here should be general, even though the experimental details may pertain to a narrow spectrum of nature.

The principles demonstrated here for phages may operate in other viral systems. The main requirement is that a virus that avoids infecting one host (or cell type) will have opportunities to infect a different type. This property may apply to many viruses infecting multicellular hosts

with respect to tissue tropisms—differences in the ability to infect various tissues within the body. Viruses within a host are likely to be selected to use some tissues and not others, and the nature of selection on tissue tropism may parallel those found here for phages (Kelly et al. 2003; Orive et al. 2005). For many viruses, such as HIV, extensive evolution occurs on a semireproducible basis within the body (Guindon et al. 2004) and is important to both transmission and virulence (Zhang et al. 1993). The importance of spatial structure in these systems may complicate the simple predictions of the basic model somewhat, but this framework may help explain patterns of adaptation seen in some human diseases.

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