Network models of phage-bacteria coevolution

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Bacteria and their bacteriophages are the most abundant, widespread, and diverse groups of biological entities on the planet. In an attempt to understand how the interactions between bacteria, virulent phages, and temperate phages might affect the diversity of these groups, we developed a stochastic network model for examining the coevolution of these ecologies. In our approach, nodes represent whole species or strains of bacteria or phages, rather than individuals, with "speciation" and extinction modeled by duplication and removal of nodes. Phage-bacteria links represent host-parasite relationships and temperate-virulent phage links denote prophage-encoded resistance. The effect of horizontal transfer of genetic information between strains was also included in the dynamical rules. The observed networks evolved in a highly dynamic fashion but the ecosystems were prone to collapse (one or more entire groups going extinct). Diversity could be stably maintained in the model only if the probability of speciation was independent of the diversity. Such an effect could be achieved in real ecosystems if the speciation rate is primarily set by the availability of ecological niches.

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INTRODUCTION

Bacteria and the bacteriophages that infect them are present in huge numbers in a wide range of natural environments, e.g., $\sim 10^6$ bacteria and 10^7 phages per ml of seawater [1]. Phages are significant factors in determining bacterial mortality [2], and thereby have a major influence on global recycling of nutrients and carbon in the biosphere [3]. The diversity of these populations is also staggering, with estimates of $\sim 10^2$ different bacterial species and $\sim 10^3$ phage genotypes per few liters of seawater [1,2] and at least 10^4 phage genotypes per kg of marine sediment [4]. Further, because most phages infect only very few host strains, the composition of phage strains is likely to be an important determinant of the composition of bacterial communities. Moreover, bacteria and phage populations are dynamic: they have been observed to fluctuate wildly on time scales ranging from weeks to months [5].

Natural phage populations comprise both virulent and temperate phages. Replication of a virulent phage kills the host bacterium (lytic life cycle), whereas a temperate phage can replicate either lytically or by temporarily combining its genome with that of the bacterium to form a lysogen (lysogenic life cycle). The phage genome in a lysogen (prophage) provides its bacterial host with immunity to lytic infection by the same strain of phage. Deterministic predator-prey modeling [6,7] of phage-bacterial ecosystems with virulent and temperate phages has shown that these may be stable (all three classes coexisting) or unstable (one or more classes collapsing), depending on predation and reproduction parameters.

In this paper we build several more coarse-grained models, consisting of a network of nodes, representing bacterial and phage strains, and links, representing interaction between strains, which evolves stochastically in discrete time steps according to a set of rules. The nodes of the network are bacterial and phage strains, i.e., subpopulations; however, we do not explicitly model the populations as dynamical variables. Instead, the rules for adding or removing nodes and links use only the structural properties of the network at that time. For instance, we take the extinction rate of a bacterial strain to be a simple function of the number (and type) of phage strains that can infect it, i.e., the number of links pointing to it from phage strains. In contrast, in a model where populations were modeled explicitly, the rule would be that an extinction occurs whenever the population of a strain falls to zero. The population, in turn, would typically be derived from a differential equation that would depend on the number of links pointing from phages to the given bacterial strain. In our modeling approach, we short-circuit this step, replacing populations and their differential equations by a simple rule based on properties like the number of links.

Our model rules incorporate various biological facts concerning phage and bacteria interactions. For instance, we take into account the ability of temperate phages to carry genes that make the lysogenic host resistant to infection by virulent phages [8], providing bacteria with weapons in the coevolutionary arms race with virulent phages. We also incorporate horizontal transfer of genes between phages sharing the same host in the rules that determine the evolution of new phage strains. Since our models coarse-grain the system at the level of strains of bacteria and phage, they are particularly suited to examine questions about the diversity of bacterial and phage populations, rather than their sizes.

MODELS AND RESULTS

The model ecosystem is built on top of a "trophic layer" of a variable number N_B of different bacterial strains. In the absence of phages, this number fluctuates because of the extinction of strains as well as the creation of new ones (which we henceforth term "speciation"). We let the speciation rate

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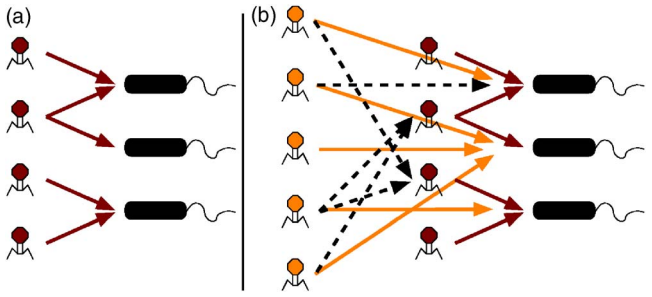

FIG. 1. (Color online) Schematic illustration of model B (a) and model C (b). Model B consists of a "trophic layer" of virulent phage strains [red (dark gray)] that can infect a layer of bacterial strains (black). The infection possibility is indicated by the directed arrows (links). Model C has, in addition, a layer of temperate phage strains [orange (light gray)] which can provide resistance to bacteria against the virulent ones. This is indicated by links between temperate and virulent phage strains. Links from either type of phage strain to bacterial strains indicate the ability of phage to infect the bacteria they point to.

define our time step. Thus at every time t two types of events occur to change the system:

Speciation. We select a random strain and duplicate it, $N_B(t)=N_B(t-1)+1$.

Extinction. We remove each strain, $i=1,2,\ldots,N_B(t)$, with a probability N_B/N_0^2.

The second rule implements random extinction associated with environmental loads common to all strains, like eukaryotic predators, scarce resources, and crowding. The parameter N_0 represents the carrying capacity of the ecosystem. In this simple noninteracting system N_B fluctuates around N_0, as illustrated in Fig. 2(a) below. Note that removing each strain with probability $1/N_0$ would produce the same behavior. Instead we choose N_B/N_0^2 to take into account the reduced extinction rate when there are fewer strains and therefore more biomass per strain. We denote this scenario of noninteracting bacterial strains, with no phages, "model A."

On top of this basic system of independent bacteria we add a self-adjusting number N_V of virulent phage strains. This extended model (B) now also contains links between phage and bacterial strains as illustrated in Fig. 1(a). Such a link represents the ability of a phage to infect and lyse the bacteria it points to. As before, the bacterial speciation rate defines the basic time step. At each time, t, the following events occur:

Bacterial speciation. We select a random bacterial strain and duplicate it, along with its original links, and then remove a random link, if possible.

Phage speciation. We select randomly a number of phage strains, the number being drawn from a Poisson distribution with mean μ. For each selected phage we create a duplicate, copying all original links, and then adding a link to a single bacterial strain. This bacterial strain is selected randomly or locally (explained below) with equal weight.

Extinction. We remove each bacterial strain i with a probability n/N_0^2 (where n is an effective total number of strains, explained below) and, in addition, with a probability β/N_0 for each link from a virulent phage to that bacterial strain. Similarly, we remove each phage strain j with a probability σ/N_0 for each link from that phage to a bacterial strain. We also remove all phages that are left without any host, i.e., with zero links.

In case the number of bacterial or phage strains falls to zero, we reintroduce a single strain with a random link to or from the other group.

The bacterial speciation rule is a simple modification from model A, the removal of one link representing the possibility of new strains improving their fitness by developing resistance to existing phages. The parameter μ specifies the rate of phage evolution, relative to the bacterial evolution, being the average number of new phage strains that arise per bacterial duplication.

The addition of a link represents the possibility of a new phage strain evolving the ability to infect a different host. A "local" choice of the new host bacterial strain models horizontal transfer, between phages, of genes for infecting bacteria. For instance, if two phages infect the same bacterium then one could gain genes from the second phage which could allow it to infect one of the latter phage's hosts. We implement this by first making a list of all other phage strains that share a common host with the phage strain just duplicated. Then we find all the bacterial strains having links from this set of phage strains, but not from the duplicated phage. Finally, we randomly choose one out of this set of bacterial strains and add a link to it from the duplicated phage. For example, if the top phage (phage 1) in Fig. 1(a) duplicates, the duplicate will add a link to the bacterium in the middle because it shares the top bacterium with phage 2 which has the middle bacterium as a host. Note that we make such a local choice half the time. The other half of the speciation events are nonlocal, i.e., the bacterial strain is chosen randomly, representing evolution of new functionality in the phage, or horizontal transfer between bacteria, which allows the phage to infect a completely new bacterial strain.

The extinction rules are also a simple extension of model A rules. Each link now results in a "load" β on the corresponding bacterial strain, which increases its extinction probability. In addition, there is an extinction rate common to all strains given by n/N_0^2, with $n=\sum_{i=1}^{N_B} e^{-\beta b_i}$ replacing N_B in the extinction rule of model A (b_i is the number of links from phages to the bacterial strain i). Instead of taking just N_B we reduce the weight of each bacterial strain to take into account the load from the phages that infect it. Then, the probability that a particular bacterial strain i survives is $(1-n/N_0^2)\times(1-\beta/N_0)^{b_i}\approx(1-n/N_0^2)e^{-\beta b_i/N_0}$ when N_0 is large. The total probability for extinction of a bacterial strain due to phage load is therefore $1-(1-n/N_0^2)e^{-\beta b_i/N_0}$.

Similarly, each link also sets a load σ on the phage because it should allocate genes to deal with the strain-specific chemistry of its potential hosts. The genes may code for proteins that change the host machinery to accommodate phage replication, or for proteins used for the attachment or injection of the phage genome into the host, or for proteins fighting the countermeasures taken by the bacteria. A phage that can enter several different strains of bacteria would need more genes, which in turn would reduce its replication rate, here represented by a phage extinction probability σ/N_0 per link. This is implemented by making the net extinction prob-

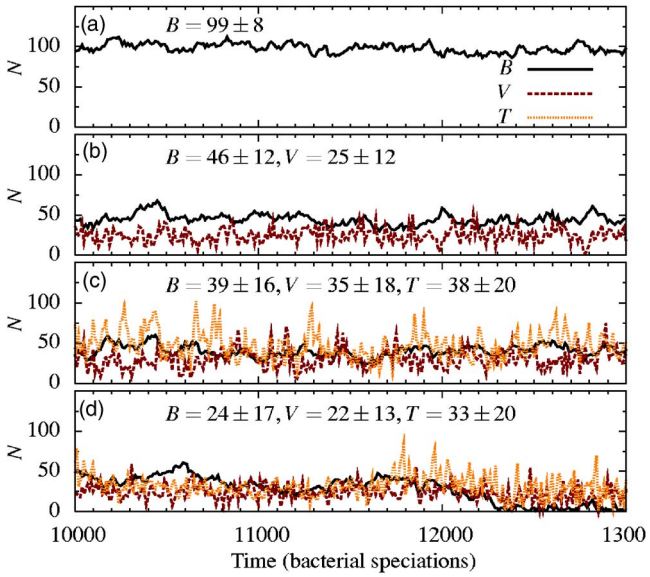

FIG. 2. (Color online) Dynamics of models A (a), B (b), C (c), and C without the resistance to virulent phages provided by temperate phages (d). Parameters are $N_0=100$ (which sets the scale for number of bacterial strains), $\mu=2.5$ (number of phage duplications per bacterial duplication), $\beta=2.0$, and $\sigma=0.2$ (the strong and weak loads; see text). All parameters are dimensionless. Plots show number of strains: bacterial (black, solid), virulent [red (dark gray) dashed], temperate [orange (light gray) dash-dotted]. Accompanying numbers show average ± standard deviation of the strain numbers for the time window shown in the figure.

ability of a phage strain $1-e^{-\sigma v_j/N_0}$, where v_j is the number of outlinks it has (this formula is similar to the bacterial extinction probability above except the extra common extinction rate n/N_0^2 which does not apply to the phage strains).

To avoid systematic errors we randomize the order in which we perform multiple duplications as well as the order in which the strains are selected in the extinction step. In general we will assume that $\beta > \sigma$, reflecting a larger load on a bacterium than on the phage infecting it. A value of the load $\beta \sim 1$ corresponds to a situation where each virulent phage imposes a load on the bacterial ecology which is similar to the background extinction rate (set by $1/N_0$).

In Fig. 2(b) we show the dynamics of model B with $\beta=2$, $\sigma=0.2$, and $\mu=2.5$. Comparing with Fig. 2(a) the first observation is the smaller number of bacteria, reflecting the load β on the bacteria imposed by the phage. Further, the number of strains in the ecosystem fluctuates relatively more than for model A. This is partly expected as adding links introduces correlations, and thus reduces the effective number of independent variables from $\sim N_B$ to a smaller number. In addition, the process of duplication in itself includes a positive feedback from the number of links to itself, a feedback that is limited only by the bound on link density set by σ.

Finally we introduce temperate phages that can insert their own genome into their hosts' genome, forming a lysogen. These phages kill a fraction of the bacteria upon infection, but also leave some of them immune to superinfection. As a consequence, they cannot drive the population of a bacterial strain to extinction. Nevertheless, they present a load on a bacterial strain (i.e., affect its extinction rate), in part because they often manipulate the bacterial metabolism and also because they increase the length of the bacterial genome and thereby its generation time (typically one finds 0–10 prophages in bacterial genomes [9]). We represent this in the network by links connecting temperate phage strains to bacterial strains [see Fig. 1(b)].

Another important characteristic of lysogenic phages is that they confer immunity not only to superinfection by their own strain, but can also provide resistance toward infection from other phages. We implement this in our model C by adding links between temperate and virulent phages, as illustrated in Fig. 1(b). Such a link implies that infection of a bacterium by that temperate phage confers on the bacterium a resistance to infection by the virulent phage the link points to. (In our model we ignore temperate phages providing resistance to other temperate phages.) Every link from a virulent phage to a bacterial strain is either "strong," if the bacteria have no link from a temperate phage that can provide resistance to the lytic phage, or "weak," if there does exist such a link (in the network picture, every link from a virulent phage to a bacterium is either part of a triangle, in which case it is weak, or not, in which case it is strong). In contrast, every link from a temperate phage to a bacterial strain is a weak link. A strong link always results in a large load β on the bacterial strain the link points to. A weak link results in a weak load σ on the bacterial strain. All links also result in a weak load, arbitrarily set to σ, on the phages from which the links originate.

The final model C incorporating bacteria, virulent phage and temperate phages is defined below. At each time step (with a time scale set by the bacterial speciation rate) the following events occur.

Bacterial speciation. We select a random bacterial strain and duplicate it by copying it with all the original links, and then remove one link if possible. In choosing which link to remove we give highest priority to strong links, then to weak links from virulent phage strains, and finally to weak links from temperate phages.

Virulent phage speciation. We choose a number of phages to duplicate, drawn from a Poisson distribution with mean μ. For each chosen phage we make a duplicate with all the original links and then make a local or random modification (as in model B) with equal weight. The modification is either the addition of a link to a bacterial strain or the removal of a link from a temperate phage.

Temperate phage speciation. We choose a number of temperate phages to duplicate, drawn from a Poisson distribution with mean μ. For each chosen phage we make a duplicate with all the original links and then either add a link to a bacterial strain or to a virulent phage (chosen locally or randomly as in model B).

Bacterial extinction. Each bacterium i is removed with probability $1-(1-n/N_0^2)e^{-\beta b_i/N_0}e^{-\sigma v_i/N_0}$, where b_i is the number of strong links pointing to it, v_i is the number of weak links pointing to it, and $n=\sum_{i=1}^{N_B} e^{-\beta b_i-\sigma v_i}$ is the effective number of bacterial strains.

Phage extinction. Each phage j is removed with probability $1-e^{-\sigma v_j/N_0}$, where v_j is the number of outlinks it has. In

addition, every phage without a bacterial host is removed.

This model is a straightforward extension of model B. Speciation is assumed to occur by duplication of an existing strain with small modifications that are likely to increase the fitness of that strain. Thus, for bacteria the modification is always the loss of a link, while for virulent phages it is the gain of a new host or the evolution of means to overcome resistance due to some temperate phage. For a temperate phage the modification is either the gain of a new bacterial host or the gain of genes that provide resistance (for the bacteria) to some virulent phage. In either case, the temperate phage receives a new link, which points to a bacterial strain or virulent phage, that is chosen locally or randomly with equal weight. As in model B, a local choice means that the temperate phage gains such a link by copying it from another phage with which it shares a common host bacterial strain.

We represent the load of temperate phages on a bacterial strain by the same weak load parameter σ as used before. Also we use σ to characterize the load that the ability to infect a bacterial strain puts on the temperate phage due to increased demand on the phage gene repertoire. In short, the overall model can be described in terms of speciation and extinction events whose rates depend on the load on bacteria and phages. Here we simplify matters by allowing only two types of load, "strong" (β) and "weak" (σ). Thus, the model has three key parameters: (1) β/σ, the ratio of strong to weak load, (2) β, which sets the scale of loads for links in the system, and (3) μ, the relative speciation rate of phages. In addition, we have a hidden parameter in the 50-50 choice of local versus random link formation in the phage speciation rules. Varying this ratio does not affect any of our conclusions (more details are below).

Figure 2(c) illustrates the dynamics of model C. Comparing with Fig. 2(b) we see that the presence of temperate phages allows the existence of more virulent strains. This is likely due to the lowering of the average load of virulent phages on bacterial strains due to the resistance provided by temperate phages. This conclusion is bolstered by Fig. 2(d) where we show the dynamics that results when the model is modified so that temperate phages provide no resistance (i.e., when all links from virulent phages to bacteria are strong). This plot also shows that the resistance conferred allows a higher number of bacterial strains to exist than when there is no resistance.

Another observation that can be made from Fig. 2(c) is that the presence of temperate phages tends to increase fluctuations. This is likely due to the intermittent increase in links from the temperate to virulent phages, which can be seen in the inset of Fig. 3. The number of links from temperate to virulent phages fluctuates especially strongly, as a result of which the network structure also varies enormously (as evident from the network snapshots in Fig. 3). Thus, one feature of our model is that the network structure is more variable and dynamic than could be guessed from observing the total numbers of bacterial and phage strains alone. This conclusion also holds if we vary the ratio between local and random choice of link formation in the phage speciation rule. Quantitatively, increasing the proportion of random link formation moderately reduces the number of links from temperate to virulent phage strains, whereas increasing the proportion of local link formation reduces the number of bacterial strains connected to phages, leaving a larger number of them isolated.

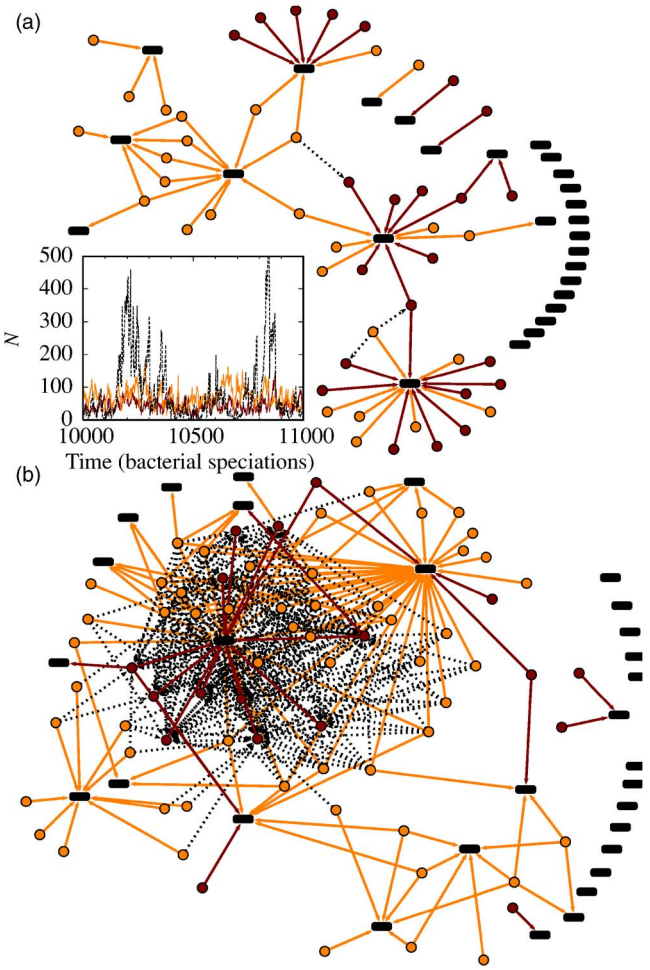

FIG. 3. (Color online) Two examples of networks generated in the same run of model C. Parameters are $N_0=100$, $\mu=2.5$, $\beta=2.0$, and $\sigma=0.2$. (a) shows a network with few links and only 2 triangles (in lower right corner). (b) shows a network with many links and more than 100 triangles. The inset shows the time development of the number of links in the system for this run (color coded as in Fig. 1).

We have examined the model against variations of the three basic parameters (β, σ, and μ). First of all, reducing β and σ while keeping β/σ fixed produces an ecology with a larger number of phages and a larger number of links per phage. One can also increase the phage to bacteria ratio without changing link density by assigning an especially weak load for temperate phages on bacteria. Thus, the overall ratio of vira to bacteria is easily rescaled. The total size of the ecosystem, bacteria plus phages, on the other hand, is primarily set by N_0.

Given fixed σ (and fixed μ) we examine, in Fig. 4, the behavior of the model ecology as a function of the strong to weak load ratio β/σ. Figure 4(a) shows that an increase in the ratio seems to reduce the overall numbers of both bacteria and phages. This is not surprising, since an increased ratio

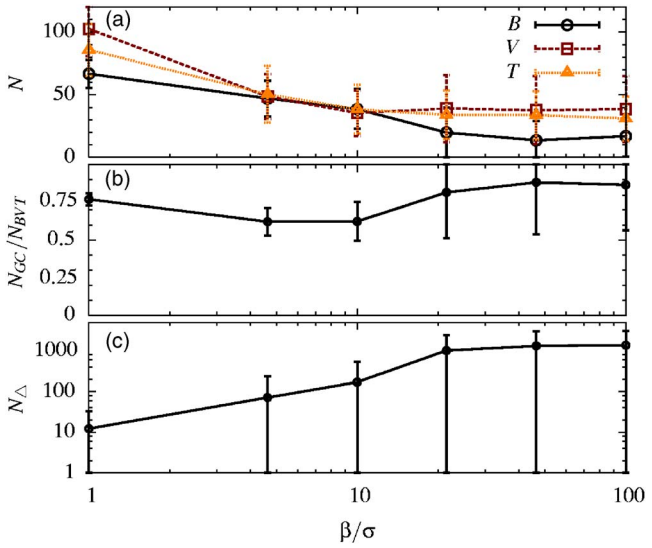

FIG. 4. (Color online) Behavior of model C as a function of the ratio of strong to weak load, β/σ. We use a fixed $\sigma=0.2$, $\mu=2.5$, and $N_0=100$ in the simulations and vary β. (a) Strain numbers for bacterial, virulent, and temperate groups (color coded as in Fig. 1), (b) fractional size of the largest connected cluster in the network (N_{GC} is the size of the largest connected cluster, N_{BVT} is the total number of strains, including both bacterial and phage strains), and (c) number of triangles in the network, denoted N_Δ. Error bars show one standard deviation.

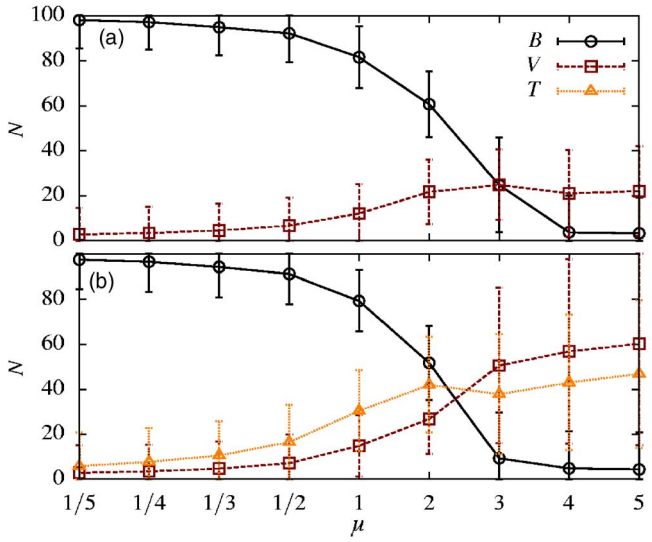

FIG. 5. (Color online) Variation in phage duplication rate μ. Other parameters are kept fixed at the same values as in Figs. 2 and 3. Plots show strain numbers for (a) model B and (b) model C.

corresponds to an increased load β. Temperate phages seem to fare marginally better than virulent ones only for intermediate values of the ratio, while bacteria do better when the ratio is smaller. Interestingly, the fractional size of the largest connected cluster in the network [shown in Fig. 4(b)] does not change much though it fluctuates more for larger β/σ ratios. This is a result of the increased interconnectedness at higher β/σ which is revealed in the number of triangles, shown in Fig. 4(c). Note that in our model a triangle necessarily has to be between one bacterial strain and one virulent and one temperate phage strain, which provides resistance to that virulent phage, i.e., the number of triangles reflects the number of weak links between virulent phages and bacteria. The figure suggests that resistance due to temperate phages plays a larger role at higher β/σ ratios but also that this resistance is intermittent, with large fluctuations from time to time. Overall, we observe a network structure that, while being usually one large, connected cluster, is nevertheless highly dynamic as indicated by the wildly fluctuating number of links and triangles.

The last important parameter of the model is μ, the speciation rate of phages relative to that of bacterial strains. Figure 5 shows that the state of the system is quite sensitive to this parameter in both model B [Fig. 5(a)] and model C [Fig. 5(b)]. Not surprisingly, when μ is increased sufficiently, the number of bacterial strains falls, while the number of phage strains increases. What is surprising is the steepness of the fall: a threefold change in μ (from 1 to 3) causes more than an eightfold change in bacterial numbers for model C.

Although the behavior is sensitive to μ, at all values the number of virulent phage strains and the number of temperate phage strains are comparable. This is in part due to a bias in the phage speciation rule. Because we always add a fixed number μ of new virulent *and* new temperate phage strains in each time step, the speciation rate per strain is not a constant. It increases as the number of strains decreases, and this negative feedback prevents strain numbers from becoming very small. A more unbiased way of implementing the speciation is to make the rate per strain constant. To investigate this scenario, we define a model D that is identical to model C in all respects except that we modify the phage speciation rule as follows. We choose (on average μ) phages to duplicate randomly from the combined set of temperate and virulent strains. This ensures that the probability for selecting a phage of a given type is proportional to the number of strains of that type. As a consequence the duplication of phages in the larger group becomes more likely and coexistence of the two groups becomes difficult. This is indeed what we see from Fig. 6(a): For standard parameters the virulent group collapses, and only temperate strategies appear viable. One remedy for this is to let virulent phages speciate faster than temperate phages. This is a realistic assumption, both because the generation time is shorter for virulent phages and because they often carry their own replication machinery.

Figure 6 shows how virulent phage strains take over when their speciation rate becomes substantially larger than that of the temperate phages. When virulent phages speciate a little over twice as fast as temperate ones, on average both types are present in equal numbers. Interestingly, however, the time plot of Fig. 6(b) shows that they do not really coexist with equal diversity. Instead the system seems to switch back and forth between one state where the temperate phages are very diverse while there are few virulent strains, and another where the virulent phages are very diverse while there are few temperate strains.

Alternatively, we also considered a variant of model D where the virulent phage population may be constantly supplemented by temperate phages that lose their immunity

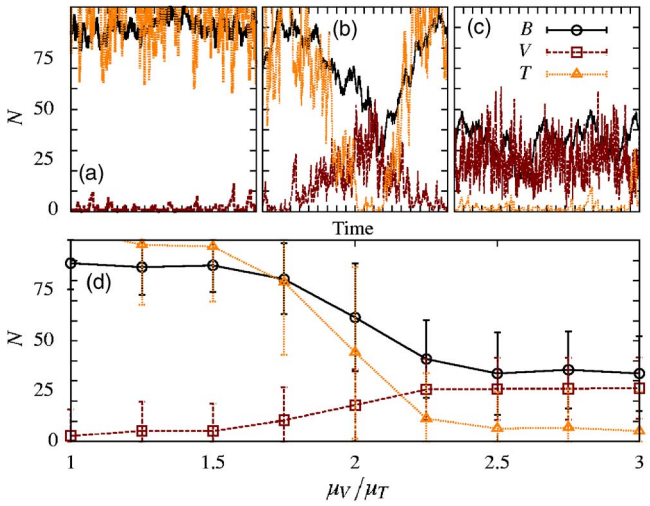

FIG. 6. (Color online) Time course of strain numbers for (a) model D, (b) a variant of model D where virulent strains speciate faster than temperate ones ($\mu_V=2\mu_T$), and (c) same variant with $\mu_V=3\mu_T$. (d) shows the average strain numbers as a function of the ratio μ_V/μ_T, with error bars showing one standard deviation. In all plots the total phage speciation rate is fixed ($\mu_V+\mu_T=3$). Other parameters as in Figs. 2 and 3.

region, e.g., [10] (the opposite is probably not possible, simply because loss of such a complicated function requires less mutations than gain). We find that the effect of this temperate-to-virulent switching is very similar to allowing virulent phages to speciate faster. That is, as the probability of mutating from being temperate to virulent increases, the number of virulent phage strains rises at the cost of the other two groups.

DISCUSSION

We have suggested a coarse-grained framework for understanding the essential ingredients in a world governed by a coevolutionary, dynamical arms race between phages and their hosts, an arms race where the elementary moves are not the fate of individual members of the community, but rather the collapse or creation of new strains by modification of old ones. The purpose in suggesting a mathematical model of this kind is to remain on a level of description that reflects our lack of knowledge of basic parameters of infection probability and replication rates in real world ecologies.

Phage-bacterial ecologies are in fact quite extensive on our planet and govern a major fraction of the known biomass: There are about 5×10^{30} prokaryotes on the planet, and viral infection is the most common way in which bacteria die, especially in the ocean. However, exceedingly little is known about this very interesting part of life on our planet. The one basic fact that a model of phage-bacterial ecologies can attempt to reproduce is the high diversity and coexistence of temperate and virulent phage strains.

In practice, ecological models, whether based on population dynamics or a more networklike approach, have great difficulty in producing viable and diverse ecosystems where many different species and strategies coexist. For instance, in population dynamics models that use Lotka-Volterra or replicator equations a handful of species can coexist for a short while but are soon destroyed by parasites [11–13]. The main reason for this is the exponential growth of self-replicating populations that results when replication rates are proportional to the population size. This typically results in a "winner-take-all" situation where the population of a slightly faster-growing species can completely repress the other populations. Only when limits are applied (sometimes artificially) on the exponential growth can species coexist [14].

One of the reasons we chose a networklike approach to modeling phage-bacterial ecologies, rather than a population dynamics approach, was to try and circumvent this problem of exponential growth and coexistence. However, our work shows that even in these kinds of models coexistence of species is not easy to achieve. We have tried several variants of the basic models, all within the same framework described above, and found that the phage speciation rule was a major determinant of the viability of coexistence of temperate and virulent phages. Model D, which makes the straightforward assumption that the speciation rate per strain is constant, does not in fact exhibit robust coexistence of virulent and temperate phage strains. There, at best, at some carefully fine-tuned ratio of virulent-to-temperate speciation rates, we find one group present at high diversity and the other at low diversity with a constant switching back and forth between these states. We also tried other variants of the rule, for instance where the speciation rate of phage strains is a fixed multiple of the bacterial speciation rate, but this scenario turned out to be even worse for coexistence. That is, even in the absence of temperate phages either all groups go extinct or only bacterial strains survive.

We succeeded in achieving coexistence only in the model C, where the speciation rate of each phage group is independent of its diversity. This "solution" parallels the solution to the problem of exponential growth in population dynamics models. In model D, the speciation rate per strain is a constant, therefore the rate of increase of strains (ignoring extinction for the moment) is proportional to the number of strains. Thus, the number of strains would grow exponentially resulting in a similar winner-take-all situation, now at the strain level. In model C, however, by making the speciation rate independent of strain number, the growth is no longer exponential and we see coexistence of a large number of strains. Thus, one "prediction" resulting from our modeling is that there may be some mechanism at work that keeps the speciation rate independent of the number of strains. We speculate that this might happen if speciation involves the discovery of new ecological niches by randomly mutated individuals. If the number of such new ecological niches is small then it could be what limits the speciation rate, rather than the population size. In that case the speciation rate would be independent of strain numbers.

Since model C was the one case where we did find robust coexistence, we mainly focus on how different phage groups influence each other in that model. The main result of this analysis was the following.

(1) Temperate phage strains in fact appear to help maintain a higher diversity of virulent strains by providing a "refuge" for a few strains of bacteria to escape to, preventing

them from being completely destroyed by virulent phages,

(2) The ecosystem is highly dynamic, especially its network structure. In particular, the numbers of links between temperate and virulent phages (and hence triangles) show large intermittent fluctuations. In other words, periods where bacterial strains are largely protected from virulent phages alternate with periods where there is little resistance and most virulent attacks present huge extinction risks for the bacterial strains. Thus, the stabilization provided by temperate phages is sometimes very important, and at other times nearly without consequence.

We emphasize that these two results hold only for model C, where each phage group produces a given fixed number of new strains at each time step of the model.

OUTLOOK

The difficulty of finding a model where phage types coexist indicates that we nevertheless miss some important insight into how such ecosystems actually work on this very basic information-exchange level. One intriguing possibility is that mutation mechanisms and speciation rates could themselves change and adjust as the network evolves. For instance, one could imagine that if viral phage strains were allowed to evolve their speciation rate, they would die out both in clusters where they had too low a rate [$\mu < 1/2$ in Fig. 5(b)] and in clusters where they had too high a rate by forcing their hosts, the bacterial strains, to collapse [$\mu > 3$ in Fig. 5(b)]. The result might be the self-organization of speciation rates to values that allow coexistence of all groups.

More realistic scenarios could also consider interactions between temperate phage species. For example prophages can confer resistance not only to virulent phages but also to temperate phages. Another feature is phage-independent genetic transfer between bacteria such as mediated by bacterial conjugation. We have loosely tried to take this into account by the random allocation of new links from time to time. However, this could be implemented more carefully in a nonrandom manner. Reference [15] is an alternate way of constructing an ecological model that coarse-grains over population variables, where the evolution rules are based on the movement of species within an underlying "genotype" space. A possible extension of our models is to implement the phage-bacteria interactions we use within ecological models that explicitly include populations [11–14,16,17].

Overall, we have presented a flexible framework for modeling phage-bacterial interactions (see [18] for a JAVA implementation). By working at strain level, ignoring detailed population dynamics, these models are particularly suited for producing questions related to the diversity of different groups in the ecosystem.

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