

ORIGINAL ARTICLE

Phage and bacteria support mutual diversity in a narrowing staircase of coexistence

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The competitive exclusion principle states that phage diversity M should not exceed bacterial diversity N . By analyzing the steady-state solutions of multistrain equations, we find a new constraint: the diversity N of bacteria living on the same resources is constrained to be M or $M + 1$ in terms of the diversity of their phage predators. We quantify how the parameter space of coexistence exponentially decreases with diversity. For diversity to grow, an open or evolving ecosystem needs to climb a narrowing ‘diversity staircase’ by alternately adding new bacteria and phages. The unfolding coevolutionary arms race will typically favor high growth rate, but a phage that infects two bacterial strains differently can occasionally eliminate the fastest growing bacteria. This context-dependent fitness allows abrupt resetting of the ‘Red-Queen’s race’ and constrains the local diversity.

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Introduction

In biology, the ‘competitive exclusion principle’ states that complete competitors cannot coexist when preying on the same resource (Gause, 1936; Hardin, 1960). More generally, coexistence of a number of consumers requires at least the same number of distinct resources (Levin, 1970). In this light, observations showing large diversity, even for consumers with shared resources have been paradoxical (Hutchinson, 1961) and alternative mathematical formulations have been brought forward (Haigh and Smith, 1972). Apart from the upper limit imposed by resource richness (Levin, 1970), the exclusion principle makes no statement on the relative abundance of predators. However, in experimental studies strong correlation between the strain richness of the consumer and the resource were found (Armbrecht *et al.*, 2004; Sandom *et al.*, 2013). These observations suggest that the predator also has a critical role in shaping the network of strains, even when direct interaction between predators is absent (Holt, 1977). A mechanism, known as niche differentiation, has been proposed as a way to produce a varied fitness of the consumers as

measured by their relative competition for the resources. These findings have triggered a range of hypotheses on mechanisms causing the observed coexistence and coevolution (Wilson, 1990).

With about 5×10^{30} prokaryotes on earth (Whitman *et al.*, 1998), these small single celled organisms dominate the global biomass. The bacterial biomass is not only limited by available resources, but also by their predators, the bacteriophages (d’Herelles, 1917; Ellis and Delbruck, 1939; Breitbart and Rohwer, 2005; Suttle, 2005, 2007). Current estimates rank the total population of all existing phages to be approximately an order of magnitude larger than that of bacteria (Breitbart and Rohwer, 2005) and they accordingly should have an important role in shaping the microbial ecology. Further, the global diversity of phages and bacteria was also reported to be tremendous (Rohwer, 2003). In the same paper it has been suggested that the diversity of phages could outnumber that of bacteria by an order of magnitude and that single bacteria could be infected by a large array of distinct phages. Applied locally, this finding would be difficult to reconcile with the ‘competitive exclusion principle’ or ‘Gause’s principle’, by which precisely this cannot be the case.

The present work explores sustainable microbial ecosystems, using classical predator–prey relations as introduced to the phage–bacteria ecology by Campbell (1960) and others (Levin *et al.*, 1977; Weitz *et al.*, 2005). These equations parameterize the dynamics of a well-mixed system of virulent phages

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and their hosts, while ignoring the duration of the latency period and its dependence on bacterial growth conditions (Weitz and Dushoff, 2008; Wang and Goldenfeld, 2010). Because of the extreme maximal growth rate of phages, the modeled ecology can exhibit a dramatic dynamic response to addition of strains, and transient oscillations can drive some strains to population levels equivalent to extinction. However, these extinctions may not represent real ecosystem behavior, as they would be softened by both the heterogeneity between individuals and spatial heterogeneity (Kerr *et al.*, 2002; Haerter and Sneppen, 2012; Heilman *et al.*, 2012). We therefore simplified our analysis to focus on the composition and evolution of sustainable ecosystems, defined as systems with coexistence steady states. Our approach allows us to clearly derive the coexistence conditions.

Previous analysis of the ecological network structure has focused on specific geometries of the sub-network formed by the interaction between phages and their hosts (Thingstad, 2000; Flores *et al.*, 2012; Jover *et al.*, 2013). Here we take the entire interaction matrix of all bacterial and phage strains as a starting point of the analysis. We investigate the limits that phage predation sets on the topology of phage–bacteria networks when considering purely virulent phages. These phages interact directly with the bacteria but phage–phage interaction occurs only indirectly (Holt, 1984, 1977).

We characterize the networks formed by well-mixed ecologies of resources and consumers. We then use the Campbell-type relations to demonstrate the formation of a staircase of coexistence, where steps along the staircase become successively more difficult. We support our theory by numerical simulation of the evolutionary dynamics. Evolutionarily, addition of a new strain demands niche differentiation in terms of network links and compression of production rate parameters. We finally explore the possible application of our theory to observational ecological data (Moebus and Nattkemper, 1981; Flores *et al.*, 2012).

Materials and methods

Computer simulations

We perform numerical simulations of the equations for bacterial (B_i), and phage (P_k) population densities, $\left(\frac{1}{B_i}\right)(dB_i/dt) = k_i(1 - \sum_{j=1}^N p_{ji}B_j) - \alpha_i - \sum_{k=1}^M \eta_{ki}P_k$ respectively $\left(\frac{1}{P_k}\right)(dP_k/dt) = \sum_{m=1}^N \beta_{km}\eta_{km}B_m - \delta_k$ with the constant parameters k_i , p_{ji} , α_i , η_{ki} , β_{km} and δ_k (compare Equation 1). To obtain Figure 3b, the number of bacterial strains N and phage strains M are sampled uniformly between 0 and 10, and the coefficients of \mathbf{R} and \mathbf{k} are sampled in the following way: for each sample ecosystem, we draw a random number $\gamma \in (0, 1)$ from a uniform distribution, which determines the network connectivity. We then let $\eta_{i,i} = 1$ with probability γ and otherwise set it to

zero for all $j = 1, \dots, M$ and $i = 1, \dots, N$. Further, β is assumed to be dependent on the phage only, that is, $\beta_{ji} = \beta_j$, and $\delta_j / \beta_j \eta_j$ is sampled uniformly in $(0, 1)$. α_i is fixed to 0.001 and $k_{i \in (0, 1)}$ is sampled randomly. Then matrix inversion is applied to obtain strain densities. If all population densities are positive, the parameter combination is accepted and added to the histogram. To simulate coevolution of ecologies in Figure 5, we start from a system with only one bacterial strain with randomly assigned parameters in steady-state, and add either a new phage or bacterial strain at equal probability. The bacteria are sharing the resources fully ($p_{ji} = 1$). Parameter assignments are summarized below for each plot. Using matrix inversion of \mathbf{R} , we determine whether steady-state coexistence is possible. If not, we continue with the dynamics to determine which strains are eliminated. We apply the Runge–Kutta method to integrate the time-evolution equations (Equation 1), starting at the steady-state population for previously existing strains and assigning the population of 10^{-3} for the newly added strain. To determine extinction of any given strain we set a small threshold of 10^{-30} for population density, below which a strain is considered extinct. Once the system reaches steady-state, we confirm the coexistence solution by matrix inversion, and proceed by adding the next strain. For Figures 5a and b, newly assigned bacteria B_N are resistant to all existing phages (that is, $\eta_{jN} = 0$ for $j = 1, \dots, M$), while a newly added phage M will be able to infect only the newest bacteria B_N (that is, $\eta_{MN} = 1$ and $\eta_{Mi} = 0$ for $i < N$), to achieve the diagonal interaction network, the values of α_N/k_N , k_N and $\delta_M / (\beta_{M,N} \eta_{M,N})$ are assigned randomly in $(0,1)$. For Figures 5c and d, newly added phages P_M will be able to infect all the bacteria (that is, $\eta_{Mi} = 1$ for $i = 1, \dots, N$), to assure the nested interaction network. α_N is fixed to 0.001, and $\beta_{M,i} = \beta_M$ is assumed to depend on the phage only. α_N / k_N and $\delta_M / (\beta_M \eta_{M,i})$ are assigned randomly in $(0,1)$. The simulation for Figures 5e and f is the same as that for figures 5c and d) except that $\eta_{M,i}$ and $\beta_{M,i}$ can depend on the bacteria and the values are assigned randomly in $(0,1)$. In figure 5f the average per phage $\sum_{i \in j's \text{ host}} \delta_j / (\beta_{ji} \eta_{ji}) / \sum_{i \in j's \text{ host}} 1$ is plotted.

Units

In the equations of the main text, all parameters and observables have been made dimensionless by normalizing population densities to the bacterial carrying capacity B_{\max} , growth and decay rates to the maximal bacterial growth rate k_{\max} , and adsorption rates to the ratio of k_{\max} and B_{\max} . A detailed derivation is given in the Supplementary Material, Section 1.

Experimental data

The dataset was acquired from the literature (Flores *et al.*, 2012) and analyzed using the R-programming language.

Results

The phage–bacteria ecology

The phage–bacteria ecology serves as a comparably simple model system, where a predatory link between a given phage and bacterial strain is justified by the strong specificity of phage predation. To describe the interacting bacteria–phage ecology, we assume a well-mixed system, that is, a system where spatial fluctuations are negligible. Fluctuations in environmental conditions have been shown to permit the coexistence of a greater number of strains when compared to a homogeneous environment, for example, by the ‘storage effect’ in Chesson (1986). In reality, a homogeneous system may be most closely observed in populations immersed in water, for example, marine bacteria–phage ecologies. We further focus on ecological dynamics without rapid evolution, that is, systems where diversity is not a consequence of transient rise and decay of strains. In the systems we discuss, the community is allowed to reach equilibrium population densities before new or mutant strains are introduced. In the following we define a distinct phage or bacterial strain as an organism of distinguishable phenotype, noticeably including the possibility of a resistant mutant bacterial strain (Bohannan and Lenski, 2000). The general form of the time-evolution equations for a system of N bacterial strains with populations B_i , where $i=1, 2, \dots, N$, interacting with M phage strains with populations P_j , where $j=1, 2, \dots, M$, is (Lotka, 1925; Volterra, 1926; Campbell, 1960; Jover *et al.*, 2013)

$$\begin{aligned} \frac{dB_i}{dt} &= k_i B_i \left(1 - \sum_{j=1}^N p_{ji} B_j \right) - \alpha_i B_i - B_i \sum_{k=1}^M \eta_{ki} P_k \\ \frac{dP_k}{dt} &= P_k \sum_{m=1}^N \beta_{km} \eta_{km} B_m - \delta_k P_k \end{aligned} \quad (1)$$

In Equation 1, $B_i(P_k)$ is the density of the i 'th (k 'th) bacterial (phage) strain in units of the bacterial carrying capacity. For a strain's phenotype to be distinguishable, it must be characterized by a unique combination of parameters: k_i and α_i are the bacterial growth and decay rates corresponding to B_i , respectively p_{ji} , is the interaction between bacterial strain j and bacterial strain i , η_{ki} is the adsorption of phage strain k by bacterial strain i , β_{km} are the respective phage burst sizes and δ_k is the decay rate of phage strain k . All rates have been made dimensionless by normalizing to the maximal bacterial growth rate (details: Supplementary Material). In Equation 1, bacteria compete for resources (Campbell, 1960; Levin *et al.*, 1977; Jover *et al.*, 2013) when $p_{ji} > 0$ for $i \neq j$, but the equation can also allow for discussion of the case of direct competition, cooperation, as well as growth on non-overlapping resources ($p_{ji} = \delta_{ji}$) (Supplementary Material).

The staircase of phage–bacteria coexistence

In the steady-state, the time-derivatives in Equation 1 are set equal to zero and non-zero solution for all densities require

$$\begin{aligned} \tilde{k}_i &= \frac{k_i - \alpha_i}{k_i} = \sum_{j=1}^N p_{ji} B_j + \sum_{k=1}^M \frac{\eta_{ki}}{k_i} P_k \\ \delta_k &= \sum_{m=1}^N \beta_{km} \eta_{km} B_m \end{aligned} \quad (2)$$

The LHS of these equations is now a constant $N + M$ —element vector of rescaled bacterial growth rates and phage decay rates while the RHS defines a matrix multiplication

$$\begin{aligned} \mathbf{R} \cdot \mathbf{S} &= \begin{pmatrix} p_{11} & \cdots & p_{1N} & \frac{\eta_{11}}{k_1} & \cdots & \frac{\eta_{M1}}{k_1} \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ p_{N1} & \cdots & p_{NN} & \frac{\eta_{1N}}{k_N} & \cdots & \frac{\eta_{MN}}{k_N} \\ \beta_{11}\eta_{11} & \cdots & \beta_{1N}\eta_{1N} & 0 & \cdots & 0 \\ \vdots & \ddots & \vdots & \vdots & \ddots & \vdots \\ \beta_{M1}\eta_{M1} & \cdots & \beta_{MN}\eta_{MN} & 0 & \cdots & 0 \end{pmatrix} \begin{pmatrix} B_1 \\ \vdots \\ B_N \\ P_1 \\ \vdots \\ P_M \end{pmatrix} \\ &= \begin{pmatrix} \tilde{k}_1 \\ \vdots \\ \tilde{k}_N \\ \delta_1 \\ \vdots \\ \delta_M \end{pmatrix} = \mathbf{k} \end{aligned} \quad (3)$$

where \mathbf{R} is now the matrix containing all interactions between strains with a structure that is highlighted in Figure 1a. \mathbf{S} is the vector of strain densities, and \mathbf{k} is the vector of rescaled growth and decay constants. Importantly, \mathbf{R} is equal to zero for any interaction between the M last columns and M last rows.

To obtain a unique solution for \mathbf{S} in Equation 3 the matrix \mathbf{R} should be invertible, thus a unique solution requires that $\det(\mathbf{R}) \neq 0$. If $\det(\mathbf{R}) = 0$, at least two lines in Equation 3 become linearly dependent, thus include a value of either B or P which is zero. As a result, $\det(\mathbf{R}) = 0$ implies that there are no stable coexistence solutions.

When $M > N$, the determinant $\det(\mathbf{R}) = 0$, and coexistence solutions therefore require that

$$M \leq N, \quad (4)$$

a statement consistent with the competitive exclusion principle (Levin, 1970). Accordingly, a well-mixed ecology can only be in a steady-state with a number of phage strains that does not exceed the number of bacterial strains.

We have so far shown that coexistence solutions do not exist when $M > N$. To demonstrate that solutions can be found for lower phage diversities, we consider the case where $M = N$ with interaction coefficients between phages and bacteria $\beta_{km} \eta_{km} \geq 0$. The lower left quadrant of \mathbf{R} (which we define

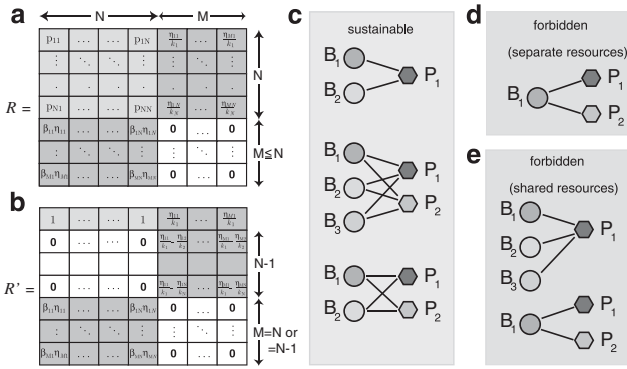


Figure 1 Coupling of phage–bacteria strain richness. **(a)** General interaction matrix for bacteria and phages. Colored blocks highlights bacteria–bacteria interaction (blue shades), bacteria–phage interaction (red shades) and absence of interaction (white). This block of white spaces leads to the condition $N \geq M$ for sustainable ecosystems. For convenience we define the lower left quadrant, that is, the block defining interactions between phage and bacteria, as R_r . **(b)** Shared resource interaction matrix when subtracting rows 2 to N from row 1. Note that an additional $(N-1) \times M$ dimensional block of white spaces is introduced, leading to the additional conditions $N = M$ or $N = M + 1$. **(c)** Examples of sustainable phage–bacteria networks. **(d)** Examples of forbidden networks when bacteria grow on separate resources. **(e)** Examples of forbidden networks when bacteria share resources. The full colour version of this figure is available at *ISME Journal* online.

as R_r , Figure 1a) then generates the system of N equations $\sum_{m=1}^N \beta_{km} \eta_{km} B_m = \delta_k$ or in matrix form $R_r \cdot \mathbf{B} = \mathbf{d}$. This equation will have a solution for linearly independent rows of R_r , an independence that will occur for most interaction parameters $\beta_{km} \eta_{km}$. When all B_i are positive, the product $R_r \cdot \mathbf{B}$ will be a vector with all positive entries, and when choosing the decay rates \mathbf{d} accordingly, this set of positive B will constitute a unique solution. Since realistic phage decay rates δ_k are much smaller than the growth rate parameter $\beta_{km} \eta_{km}$ (De Paepe and Taddei, 2006), the relevant solution space will have all $B_i \ll 1$ and easily fulfill the constraints necessary for all phage populations to be positive ($\sum p_{ji} B_i \leq \bar{k}_j$). Requiring all P_k to be positive further constrains the parameters k_i , α_i and η_{ki} .

Equation 1 was very general in the sense that it allowed for both negative and positive interactions between the bacteria and in particular included the case where the different bacterial strains were limited by different food sources. However, bacterial strains in a given environment may be quite similar leading to strong competition for similar resources. In the extreme limit where all resources are shared, all interactions between bacterial strains become unity, $p_{ji} = 1$, and the upper left quadrant of R becomes a block of ones. The shared bacterial resources now introduce a second level of competitive exclusion (Gause, 1936; Hardin, 1960), that is, bacteria act now both as consumers and resources, sandwiched between two trophic levels. Subtracting rows 2 to N from row 1, we obtain the matrix shown in Figure 1b. Checking again for $\det(\mathbf{R}) = 0$, we now obtain an additional necessary condition, namely

$M \geq N - 1$. Together, with Equation 4 a number of N bacterial strains can only coexist when supported by a number of phage strains

$$M = N - 1 \text{ or } M = N, \quad (5)$$

a result that generalizes the fact that one susceptible and one resistant strain of bacteria can coexist with the support of one virulent phage (Campbell, 1960; Levin *et al.*, 1977). Examples of interaction networks are displayed in Figures 1c–e.

Our theoretical results suggest that in systems where prey share resources, predator and prey have balanced diversity and coexistence is thus only possible along a line in the space spanned by the diversities of prey and predator. Thus not only do phages allow bacteria to speciate as suggested by Weitz *et al.* (2005), they do so in a precise counting sequence where each new phage strain allows for exactly one new bacterial strain and vice versa. We further explore this mutual support of diversity in the following.

Evolutionary climbing of a narrowing staircase

We have seen that high diversity steady-state solutions can be constructed, provided that phage and bacterial parameters are carefully chosen. In reality, phages and bacteria expose themselves to ecosystems with a variety of parameters (Supplementary Figure S1 and (De Paepe and Taddei, 2006)), and it is in general unlikely that a given set of phage parameters allows for sustainable high diversity (May, 1972). In fact, as we shall see now, the possible constraints on parameters of interactions that support coexistence are becoming much stricter as overall diversity increases. To make contact with Equation 5, the community must start to grow by the presence of a single bacterial strain B_1 (hence $N = 1$). Expansion of diversity is only then possible by adding a phage P_1 that preys on the existing bacterial strain ($\eta_{11} > 0$ and $M = 1$). Subsequently, again a new bacterial strain B_2 must enter, that is, $N = 2$, $M = 1$. Note that the second bacterial strain must have distinct immunity to the existing phage ($\beta_{12} \eta_{12} \neq \beta_{11} \eta_{11}$), otherwise the first two rows of R_r would become linearly dependent and one of the two bacterial strains would collapse. At this stage we have the classical resistant versus susceptible phage–bacteria system considered by Campbell (1960) and Levin *et al.* (1977) (compare also Supplementary Information).

For additional growth of the $N = 2$, $M = 1$ ecosystem, the only possible addition now is another phage strain P_2 . To ensure linear independence of the final two rows in R_r , the replication rates of P_2 , $\beta_{2m} \eta_{2m}$, must not be a linear combination of $\beta_{1m} \eta_{1m}$. Analogously, when considering the entry of a new phage into the existing community, the probability of the new phage to reach a finite population increases with the number of bacterial strains it can infect. Re-writing Equation 1 for a new phage

strain P_N , the condition for positive growth of small P_N is: $\delta_N < \sum_{m=1}^N \beta_{Nm} \eta_{Nm} B_m$, a condition that becomes easier to meet the more effectively bacterial strains are infected.

For bacteria, the condition for positive growth is

$$k_i \left(1 - \sum_{j \neq i} B_j \right) - \alpha_i - \sum_k \eta_{ki} P_k > 0, \quad (6)$$

implying that a reduction of the number of interactions with phages would lead to more parameters compatible with the increase in diversity.

Continuing further, these findings suggest that an interaction matrix results with phages interacting with many earlier bacteria, whereas new bacteria tend to be resistant. This type of interaction has features of the previously proposed nestedness (Bastolla *et al.*, 2005, 2009; Flores *et al.*, 2012). Alternatively, new phage strains may choose to interact preferentially with the most abundant bacterial strain, which is commonly the bacterial strain immune to all existing phages. This ‘killing the winner’ situation (Thingstad, 2000) would result in a diagonal interaction matrix. It has to be kept in mind that evolution of large ecologies demands both successful entry of new strains and the system’s ability to sustain all previous ecologies with large diversity. Using the nested and diagonal network geometries, we examine this further in the following.

Quantifying the narrowing staircase

We analyze and contrast ecosystem growth as a diagonal interaction matrix (Figure 2a, compare Winter *et al.* (2010); Thingstad (2000)) respectively a nested matrix (Figure 2b, compare Jover *et al.*, 2013)). Thingstad (2000) investigated the case of bacterial strains sharing a common resource and phages that were always host specific. Starting with bacteria of equal clearance rates (that is, decay rates) but distinct growth rates the authors present the relations between bacterial and phage parameters leading to coexistence of an equal number of bacterial and phage strains. Jover *et al.* (2013) discuss the case of a perfectly nested infection network, implying equal numbers of bacterial and phage strains. In their case, bacterial decay occurs only through phage infection, no natural decay is present. We point out the implications of the lack of bacterial decay below.

The following discussion highlights the relation between the two extreme cases of the diagonal and nested network when the system evolves from a single bacterial strain along the coexistence staircase. We initially specialize to the case where $\beta_{km} \equiv \beta_k$ and $\eta_{km} \equiv \eta_k$, that is, burst sizes and adsorption rates are only phage-specific, but relax this assumption later in the discussion. In both the diagonal and nested cases, each added bacterial strain is resistant to all previously added phage strains. In the former, each added phage strain

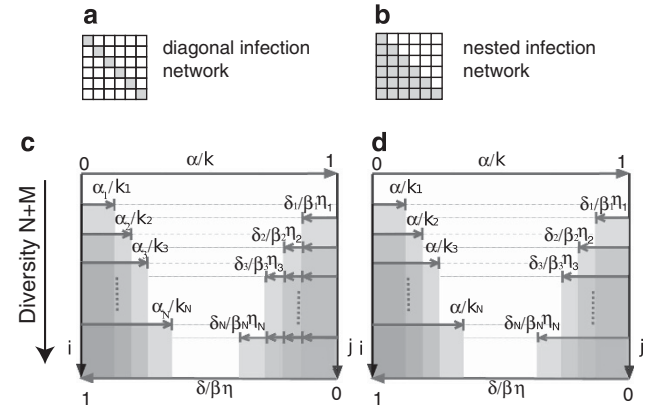


Figure 2 Congestion of parameter choices for diagonal and nested cases. (a) Diagonal interaction network. (b) Nested interaction network. (c) Schematic illustrating the congestion of parameters with increasing diversity (increasing from top to bottom) for diagonal infection network. Solid blue (red) arrows denote addition of bacterial (phage) strains with corresponding parameters marked near the arrows. Black vertical arrows denote increase in diversity. (d) same as c but for the nested infection network. The full colour version of this figure is available at *ISME Journal* online.

infects only the latest added bacterial strain while in the latter, each added phage infects all previously existing bacterial strains. In the diagonal case, upon addition of a new bacterial strain, a necessary condition for sustainability of the resulting ecosystem is

$$\frac{\alpha_n}{k_n} > \frac{\alpha_{n-1}}{k_{n-1}}, \quad (7)$$

that is, newly added bacteria must always have larger normalized decay rates. This condition generalizes the form presented previously (Thingstad, 2000). The condition equally applies to the nested case, when all $\alpha_i \equiv \alpha$ are taken to be equal (general case: Supplementary Material). Note that the condition can never be fulfilled in the special case of vanishing decay rates α_i (Jover *et al.*, 2013). There, addition of a predator-less bacterial strain would always lead to eventual removal of the existing strains. The subsequent relations Equations 9 and 11 should be seen as enabling evolutionary climbing of the narrowing staircase, a feature not compatible with vanishing α_i . A further condition applies for successful growth of the added bacteria. For diagonal interactions

$$\frac{\alpha_n}{k_n} < 1 - \sum_{j=1}^{n-1} \frac{\delta_j}{\beta_j \eta_j} - \frac{\alpha_{n-1}}{k_{n-1}} \quad (8)$$

and similarly for the nested case with equal α_i

$$\frac{\alpha}{k_n} < 1 - \frac{\delta_{n-1}}{\beta_{n-1} \eta_{n-1}}. \quad (9)$$

Similarly, subsequently added phage strains are subjected to an upper bound on $\delta_n / (\beta_n \eta_n)$,

$$\frac{\delta_n}{\beta_n \eta_n} < 1 - \sum_{j=1}^{n-1} \frac{\delta_j}{\beta_j \eta_j} - \frac{\alpha_n}{k_n}, \quad (10)$$

to be viable in the diagonal case. In the nested case, both an upper and a lower bound are induced by the existing strains.

$$\frac{\delta_{n-1}}{\beta_{n-1}\eta_{n-1}} < \frac{\delta_n}{\beta_n\eta_n} < 1 - \frac{\alpha}{k_n}. \quad (11)$$

The first inequality in Equation 11 results from the fact that phages are coupled to one another by the overlapping infection of bacteria, distinguishing the nested from the diagonal network.

These relations emphasize that the sustainability of any newly added strain is constrained in its parameter values by all previously added strains (Figure 2a). Upon addition of bacterial strains where Equation 7 is violated, all previous bacterial strains with larger ratios are removed from the ecosystem along with their respective phages. When strains are added randomly, the presence of bacterial strains with large decay α_i/k_i should often be eliminated by subsequent addition of bacteria with lower decay rates. As a result, much of the diversity frequently collapses and high diversity is only sustained when all bacteria have low relative decay rates. Note that for additions of new phage strains to the diagonal network, entry as determined by Equation 10 always guarantees sustainability of the resulting ecology while this is not guaranteed in the nested network (Equation 11). There, a newly added phage strain can also cause collapses of those sections of the ecosystem that are determined by less-productive phages and their corresponding bacterial strains.

Both the diagonal and the nested system have in common that completely balanced ecologies ($N=M$) are phage limited, that is, all bacterial densities are constrained by phage predation (Figure 3a and Supplementary Information). Systems with a surplus of bacterial strains ($N=M+1$) are resource limited, that is, total bacterial biomass is constrained by the food resources (details: Supplementary Information). Finally, we note that the rapid reduction of probabilities with diversity also carries over to the case of randomly shared resources and a random interaction network (Figure 3b).

Discussion

Experimental network characterization

Exact ecological characterization of phage–bacteria networks remains elusive, with the largest characterized network dating back from an expedition across the Atlantic in 1981 (Moebus and Nattkemper, 1981). This network was recently re-visited and analyzed extensively by Flores *et al.* (2012). The network included 215 phage strains and 286 bacterial host strains with a total of 1332 interactions. An important aspect of this network is that to a large extent it was formed by predatory links between a phage and a bacterial strain that were not in the same location. In fact, when examined in detail at the scale of

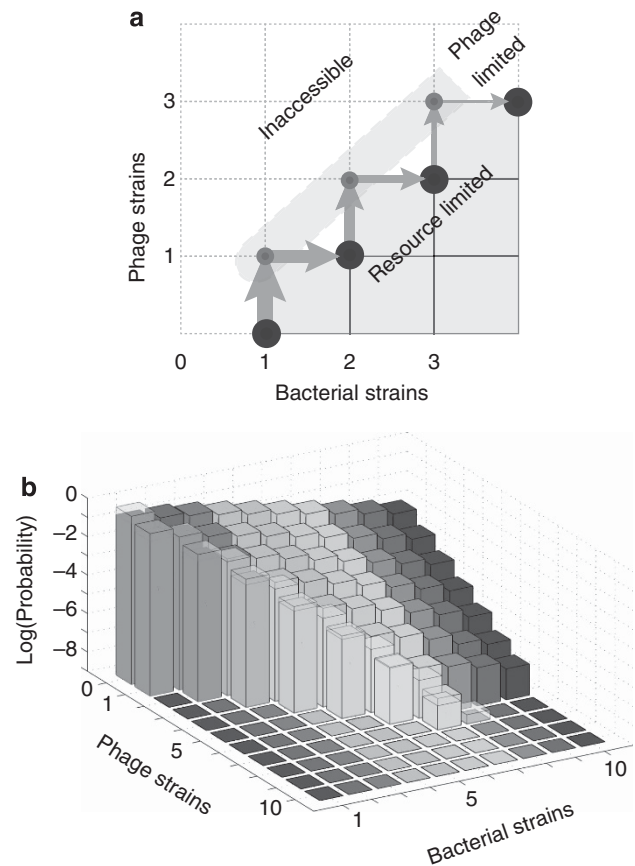


Figure 3 The narrowing staircase of coexistence. **(a)** Schematic of the narrowing staircase formed by the coexistence of phage and bacteria in the space of strain numbers. Blue shaded region indicate possible configurations when bacteria consume separate resources. Blue and red circles show accessible configurations when bacteria consume shared resources. Red (blue) circles are generally phage (resource) limited (see Supplementary Information for details). Thickness of orange arrows indicates transition probability between configurations for shared resources. Upper triangle is inaccessible to the phage–bacteria ecology. **(b)** Two-dimensional histogram of the probability for coexistence at given configurations of phage (M) and bacterial (N) strains for random resource sharing of bacteria, that is, random $p_{ij} \in (0, 1)$, for $i \neq j$ but $p_{ij} = 1$ for $i = j$ in Equation 1. Shaded columns mark the case of completely shared resources ($p_{ij} = 1$ for all i and j). Phage–bacteria interaction network η_{ki} and other parameters are sampled randomly, details given in Materials and methods. The full colour version of this figure is available at *ISME Journal* online.

individual locations, for each of these local phage–bacteria networks the interaction matrix between phage and bacterial strains is predominantly diagonal as indicated by the proximity of the average interaction number to unity (Figure 4a). It is well-known that—owing to spatial effects—strain diversity strongly increases when considering an area of increasing size (Harte, 2011). In stark contrast to the suggested large global phage diversity (Rohwer, 2003), the local samples obtained at the stations show only modest diversity (Figure 4a, inset). Diversity is generally low and the number of stations reaching high diversity declines approximately

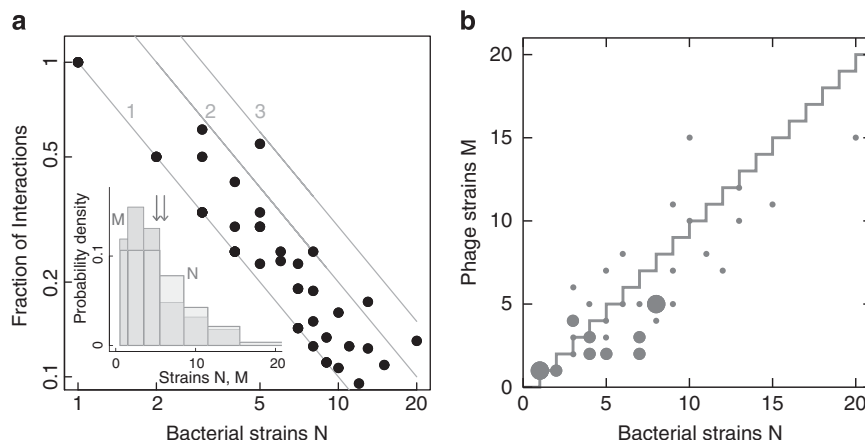


Figure 4 Experimental phage versus bacterial diversity. Local diversity measured at stations in the Atlantic Ocean (Moebus and Nattkemper, 1981). **(a)** Fraction of interactions versus total number of coefficients in the interaction matrices. The fraction was computed by counting the number of interactions in each block formed by the N bacterial and M phage strains at a given station and dividing by the total number of possible interactions $N \times M$. Each black circle corresponds to one station, orange lines correspond to number of bacterial strains infected by each phage strain as labeled. One interaction per bacterial strain (as labeled) is equivalent to a diagonal matrix. The average of interactions per phage strain is 1.4. Note the double-logarithmic axis scaling. Inset: histograms of number of phage (M , red) and bacterial (N , blue) strains, red (blue) arrows indicate the mean of $M(N)$. **(b)** Blue circles are pairs of phage strain richness versus bacterial strain richness. Size of circles corresponds to the number of distinct observations. Dark grey step function (that is, 'staircase') is a function obeying $M=N$ or $M=N-1$. The full colour version of this figure is available at *ISME Journal* online.

exponentially. When comparing the number of phage strains M and bacterial strains N at each station, one finds them to be nearly balanced (Figure 4b) with an overall slight dominance of N (Figure 4a, inset). These observational features are consistent with our theoretical ecological analysis (Equation 5). Returning to the numerical simulations on diversity (Figures 3 and 5), we find that also in the experimental data the number of ecologies reaching a given diversity drops rapidly, both for bacterial and phage strains (Figure 4a, inset).

Representative sampling of actual phage–bacterial diversity in the wild constitutes a cumbersome task owing to the difficulty of allowing accurate assessment of possible under-sampling. Nonetheless, the data gathered by Moebus and Nattkemper (1981), constitute an enormous achievement and a possible reflection of the theoretical balance of predator and prey strain richness of Equation 5. Additional support for the balance of diversity may be established from the data for other ecosystems, such as between plants and animals (Murdoch *et al.*, 1972), for example, twigs and ants (Armbrecht *et al.*, 2004).

Dynamics of coevolution and niche differentiation

Realistic phage–bacteria networks are generally neither fully diagonal nor fully nested. However, the marine phage–bacteria ecosystem we study is in fact quite close to a completely diagonal system (Figure 4a), that is, the interaction matrix is sparse, that is, it has few non-zero off-diagonal elements. Notably, this is true also for the larger ecologies where the number of off-diagonal elements is similarly low. In Figures 5a and b we present the coevolution of the phage and bacteria parameters for the diagonal case. As expected from Equation 7, the

value of the lowest bacterial effective decay rate α_1/k_1 is a monotonously decreasing function of time. Within periods where α_1/k_1 is constant (such as the one emphasized by arrows in Figure 5a) a similar behavior for the second fastest grower (α_2/k_2) holds there. All bacterial strains with larger decay rates can be seen as living in a temporary ecology, contingent on the respective fastest grower. When inspecting the figure more closely, one finds that cascades of such temporary ecologies exist, sustainable on ever shorter timescales. Any replacement of the correspondingly fastest grower leads to its destruction. The result is a compression of effective decay rate parameters, allowing the conditions shown in Figure 2 to be subsequently met at greater ease. The rules of successful additions dictate that removal of existing strains becomes more and more unlikely as growth rates are further and further optimized.

When removal of fast growers does occur, avalanches of strain collapse can result. The result is a narrowing of parameter differences as the staircase is climbed toward higher diversity (Figure 3a). Every subsequent step along the staircase will take longer, and be interrupted by a sequence of drops. For the phage strains, no such obvious structure emerges as there is no condition on hierarchy for $\delta/\beta\eta$ for the diagonal network. For the nested network (Figures 5c and d) both the bacterial and phage parameters have to obey a hierarchy (Equation 8 and 11). The pattern of lowering minimal decay rates now also applies to the phage parameters and a mutually perturbed structure emerges where collapses of the ecology can both result from the entry of bacterial and phage strains. We estimate the ratio $\delta/\beta\eta \sim 10^{-3} - 10^{-4}$ (Supplementary Figure S1 (De Paepe and Taddei, 2006)) hence additions of phages will be

possible until high diversity is reached for the diagonal case, while in the nested case the hierarchy imposed on phage parameters can lead to an additional restriction on diversity. Some reduction in η can be caused by modification of bacterial receptors in realistic environments and thereby lead to an increased ratio $\delta/\beta\eta$.

Finally, in our numerical simulations we now also relax the assumption of bacterial-independent adsorption rate (η_{ki} in Equation 1) and allow burst size β_{ki} to be both dependent on the phage and bacterial strains (Figures 5e and f). Again, patterns with steadily decreasing α_1/k_1 emerge. However, these are now interrupted by jumps in α_1/k_1 where the ratio increases, that is, evolution does not produce ever improving absolute fitness.

Fitness is context dependent

Further investigation is demanded by the observed recycling of parameter values in Figures 5e and f. It exemplifies that continuously increasing growth rate of the fastest bacterial strain may be a rule of thumb, but situations can arise where the fastest grower is replaced by a slower grower. To explain the observation, we maintain variable η_{ki} and β_{ki} , but limit to a system of only a single phage P_1 preying on a single bacterial strain B_1 . A new strain B_2 with $k_2 < k_1$ can replace B_1 if

$$\frac{\eta_{12}}{\eta_{11}} < \frac{k_2^*}{k_1^*}$$

with $k_i^* \equiv k_i - \alpha^*$ (where $\alpha^* \equiv \alpha / (1 - \max(\frac{\delta_1}{\beta_{11}\eta_{11}}, \frac{\delta_1}{\beta_{12}\eta_{12}}))$) which in turn constrains $\eta_{12} > 0$, a reshuffling condition that in fact is similar for both the case of shared and separate food resources, that is, it can be

done with identical β 's, purely using a bacterial strain with few receptors and thus low η (Details: Supplementary Information, section 3). These situations reflect a phage strain that weakly adheres to the slow grower B_2 , but causes relatively more damage to B_1 . From this result we predict a possible virulent-phage variant of the experimentally observed 'suicide bombing' recently found for temperate prophages (Duerkop *et al.*, 2012). In other words, although bacterial strains are under evolutionary pressure to increase their net growth rate $\sim k - \alpha$, a fast grower occasionally can be attacked by a combination of a bacterial strain with slow growth rate and a phage strain that preferentially eliminates the fast grower. Replacement of a fast by a slow grower is thus possible, reflecting the fact that existence is context-dependent.

When viewing evolution as hill-climbing where reaching the top means higher fitness, this situation exemplifies, that the hill is not invariant, but is exposed to changes of context—reshaping the landscape dynamically (details: Supplementary Information). To build up diversity, strains compete in a 'Red-Queen's race' on a fixed fitness slope (van Valen, 1973), but phages transiently disrupts the landscape and reset the race. The overall dynamics resembles the behavior explored in the Bak-Sneppen model of coevolution (Bak and Sneppen, 1993), where the stability of strains was inherently assumed to be context-dependent.

The lack of on-going absolute improvement and the steady-state reshuffling of growth properties also implies that ecosystems cannot build up an infinitely long and fine-grained staircase of coexistence. Our simulations of growing ecologies were persistently constrained to relatively small diversities owing to collapses (Figures 5e and f).

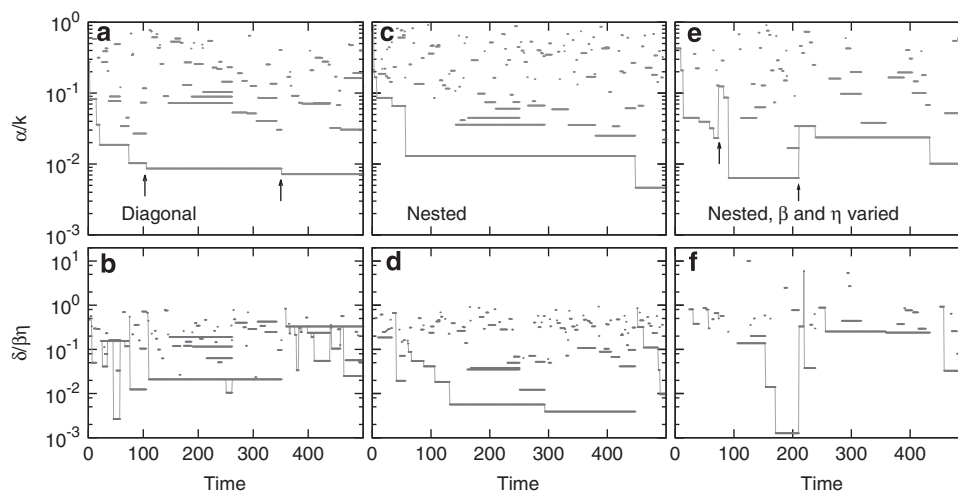


Figure 5 Coevolution of diversity. (a) Evolutionary simulation of bacterial effective decay rate α/k for a diagonal infection network. Note the build-up of parameter cascades, for example, in the range between the two arrows. (b) Similar to a but for phage effective decay rate $\delta/\beta\eta$. Note the lack of cascade for the parameters $\delta/\beta\eta$. (c, d), Similar to a and b, respectively but for the nested infection network. (e, f), Similar to c and d, respectively but for variable β_{km} and η_{km} . Note that the lowest decay rate α/k can now be replaced by larger decay rates (indicated by the vertical arrows). Time is given in ecology updates by additions or removals of strains. Note the logarithmic vertical scale in all panels. The full colour version of this figure is available at *ISME Journal* online.

Concerning the experimental data (Figure 4), we speculate that it may be precisely the sparseness of the network and its nearly diagonal structure that support the relatively large diversity observed at some of the stations. In a diagonal phage–bacteria interaction network, even variable η_{ki} cannot give rise to Bak-Sneppen-type replacements, and one could therefore generate large diversity by evolutionary compression of the parameter space (see Figures 5a and b).

Conclusion

The main lesson of our study is that the possible compositions of sustainable microbial ecosystems are highly constrained. Addition of a phage and bacterial strain to an existing ecology demands its infection strategy to be independent of all combinations of the previous strategies, a process reminiscent of niche differentiation (Hutchinson, 1959). Phage diversity cannot exceed the diversity of bacterial strains, and if the bacteria compete for the same limiting resource, bacteria–phage coevolution is bound to a staircase structure (Figure 3a). When moving along this staircase, diversity of phage and bacteria must be balanced. Reaching a further step is exponentially less likely than the previous (a narrowing). In other words, while infection strategies must diversify from one addition to the next, the opposite is true for production rates: growing diversity of ecologies demands production rate ranges to become increasingly narrow.

Importantly, our analysis only considers virulent phages. Temperate phages could in contrast be plentiful, as they may hide inside bacteria indefinitely (Little and Michalowski, 2010). Such phages were in fact included in the data sampling used for Figure 4, and presumably contribute to total diversity. Furthermore, a bacterial strain, where parts of the population contain a temperate prophage, may appear as two strains with different immunity to some virulent phages as immunity can be provided by some prophages (Bachi *et al.*, 1979; Parma *et al.*, 1992). Hence, presence of temperate phages may supplement the natural immunities of bacteria (Makarova *et al.*, 2013) and thereby further increase the sustainable diversity of virulent phages. Further, transiently present phages could temporarily increase M and thus explain the data points above the diagonal in Figure 4b.

Fitness of a strain is usually defined in terms of its growth rate (Fisher, 1930), and the evolutionary climbing of a fitness gradient is interpreted as an improvement of the net growth $k - \alpha$ for bacterial strains. Noticeably, however, the selection for steady-state survival in phage–bacteria ecologies is more complex than optimization of the maximal bacterial net growth rate $k - \alpha$ (Williams, 2013). In fact, with phage predation we find that it is the ratio k/α that is under evolutionary pressure. In a

chemostat, α is the on-going dilution rate, whereas α in the ocean would have the role of bacterial decay owing to non-phage predators. As a consequence a slow grower with very low death rate α , may still out-compete a bacterial strain with high growth rate $k - \alpha$. Further, if one includes the fact that phages may infect different bacterial strains with widely different adhesion coefficients η_{ki} , one finds that even extremely high k/α does not secure long term survival: Such highly ‘fit’ bacteria may be eliminated by a combination of a hostile phage strain and a less fit bacterial strain with partial immunity to this phage. As a consequence the ‘survival of the fittest’ concept of Darwin and Spencer becomes context-dependent, and a globally superior bacterial strain does not exist.

On a more general note, our focus on steady-state sustainability also speaks to a fitness measure for phages that goes beyond their growth rates. As each phage strain suppresses its bacterial prey to a marginally sustainable steady-state population, $\sim \delta/(\beta\eta)$, one phage may eliminate another phage by exposing their common host to more fierce predation—a predation ‘fierceness’ that is quantified in terms of big bursts multiplied by adhesion rates, in units of the phage death rate. Importantly, the sustainability of a phage relies on support from interaction with at least one of its hosts, reinforcing a coevolution that acts on the links—not nodes—of the ecological network.

Conflict of Interest

The authors declare no conflict of interest.

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