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LETTER

Virus population extinction via ecological traps

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Abstract

Populations are at risk of extinction when unsuitable or when sink habitat exceeds a threshold frequency in the environment. Sinks that present cues associated with high-quality habitats, termed ecological traps, have especially detrimental effects on net population growth at metapopulation scales. Ecological traps for viruses arise naturally, or can be engineered, via the expression of viral-binding sites on cells that preclude viral reproduction. We present a model for virus population growth in a heterogeneous host community, parameterized with data from populations of the RNA bacteriophage Φ6 presented with mixtures of suitable host bacteria and either neutral or trap cells. We demonstrate that viruses can sustain high rates of population growth in the presence of neutral non-hosts as long as some host cells are present, whereas trap cells dramatically reduce viral fitness. In addition, we demonstrate that the efficacy of traps for viral elimination is frequency dependent in spatially structured environments such that population viability is a nonlinear function of habitat loss in dispersal-limited virus populations. We conclude that the ecological concepts applied to species conservation in altered landscapes can also contribute to the development of trap cell therapies for infectious human viruses.

Keywords

Bacteria, ecological trap, habitat spatial structure, phage, *Pseudomonas phaseolicola*, source–sink.

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INTRODUCTION

One of ecology's most powerful assertions is that habitats either support or do not support local population growth. Such habitats are termed sources and sinks respectively (Holt 1985; Pulliam 1988; Dias 1996). While obvious, this observation is general enough to bear upon any biological system at any scale and has spawned research on a wide range of subjects including population viability (Morris & Doak 2002) and host shifts by pathogens (Dennehy *et al.* 2006; Sukorenko *et al.* 2006). In this study, we adopt population viability concepts stemming from the source–sink paradigm to develop and parameterize a model for the forced extinction of virus populations.

Sink populations are maintained by migration from source populations, usually driven either by migrants that randomly disperse (Holt 1985) or by those that are competitively excluded from higher quality habitats (Pulliam 1988). Although habitat selection is expected to help organisms avoid sinks (Holt 1985, 1997; Kokko & Lundberg 2001), optimal behaviour can be foiled by misleading cues about habitat quality. For example, mayflies lay eggs on asphalt in response to polarized light cues that mimic those produced by water, the insect's natural oviposition site (Kriska *et al.* 1998; Robertson & Hutto 2006). Sink habitats that present signals normally associated with sources are termed ecological traps (Dwernychuk & Boag 1972; Schlaepfer *et al.* 2002; Battin 2004) and constitute the inevitable risk associated with behavioural choices based on incomplete information (Kokko & Sutherland 2001). Ecological traps are often considered in light of anthropogenic changes to the environment (Schlaepfer *et al.* 2002).

Sinks, whether traps or not, can significantly affect the ability of a landscape to support net population growth. When the fraction of unsuitable habitat in a landscape exceeds a threshold frequency, a population will decline towards extinction (Lande 1987; Nee 1994; Kareiva & Wennergren 1995; Hanski *et al.* 1996). This extinction threshold is contingent upon habitat selection, and the distribution of source and sink habitats. Without dispersal limitation, a population avoiding sink habitat can persist as long as some source habitat remains in the landscape (Delibes *et al.* 2001). Incorporation of spatial structure and dispersal limitation dramatically alters this result.

Populations can withstand large amounts of habitat loss only if source habitat remains connected by short-distance dispersal. Hence, the extinction threshold in a structured environment increases with increasingly aggregated patterns of habitat loss (Urban & Keitt 2001; Fahrig 2002). Ecological traps are expected to have a more severe effect on population growth than are unattractive sinks (Delibes *et al.* 2001; Kokko & Sutherland 2001), but extinction thresholds are still substantially higher if sources are spatially aggregated (Hill & Caswell 1999).

The above hypotheses have primarily been applied to the conservation of populations in changing landscapes (e.g. Doak 1995; Donovan & Thompson 2001; Keagy *et al.* 2005). However, the problem can be inverted to ask how pest and disease organisms might be controlled via the introduction of sinks (*sensu* Nee 1994). Such a perspective has been applied to the control of agricultural pest insects. Trap crops divert pests from plants of economic importance and act to aggregate pest populations, thereby reducing total insecticide use. Trap crops may also foster larger populations of the pests' natural predators (Hokkanen 1991; Badenes-Perez *et al.* 2005).

As with all organisms, viruses choose their habitats (host cells) based on limited information (viral receptors on the host cell membrane). Viruses cannot assess habitat suitability beyond the presence of the proper receptor and will infect cells possessing the correct receptor even if these cells are unsuitable for viral replication (Repik *et al.* 2005). This aspect of virus biology makes them vulnerable to subversion by ecological traps. Ewald (1994) proposed a 'fake doorknob trick' to treat human immunodeficiency virus (HIV) infection; here an infected individual would be administered CD4 molecules (HIV's receptor on T cells of the human immune system) that would bind to attachment proteins on the virus exterior. Ewald speculated that by 'grasping knobs that do not open any doors' HIV would become more vulnerable to the antibody neutralization process of the immune system. Beyond mere neutralization, a trap cell could bind one or more virus particles to its surface and perhaps allow viral entry, thus creating a dead-end infection. Asher *et al.* (2005) engineered mouse erythrocytes to express a viral receptor; by thus reducing systemic levels of Coxsackievirus, mice experienced prolonged survival and reduced mortality. The trap cell concept is an extreme, cellular analogue of the 'dilution effect' (Schmidt & Ostfeld 2001; Keesing *et al.* 2006) wherein variance in competency among host species reduces a pathogen's force of infection. That is, the rate at which susceptible individuals (or cells) are infected with a pathogen will decrease when less productive hosts are added to a community.

The link between source–sink theory and the trap cell concept is novel and may prove useful for testing ideas of both ecological and medical importance. The therapeutic application of trap cells would probably involve repeated introductions of engineered cells following measurements of viral titre and target cell abundance. Thus, the system roughly approximates creating a sink habitat by exploiting the dilution effect to diminish the force of infection of a pathogen. Our current goal was to present a first-order approximation of the effect of trap cells on virus fitness, taking virus population growth rate to be a net measurement of all effects, both direct (e.g. trap interference with host cell infection) and indirect (e.g. differential production of cell types). We acknowledge the importance of the underlying dynamics; more sophisticated models will be developed in future investigations.

We fit our model to experimental infections involving the RNA bacteriophage Φ6 and three naturally occurring variants of its host bacteria, *Pseudomonas syringae* pathovar *phaseolicola* (Lythgoe & Chao 2003) acting as source, neutral and trap habitats. This system is especially useful for evaluating generalities of trap cell efficacy because it allows study of virus dynamics in both unstructured (liquid) and structured (semisolid agar) environments. Thus, we could compare the effect of trap cells on viral growth rate with qualitative predictions regarding population viability in mean-field and spatially structured models.

Overall, the effect of replacing hosts with non-host cells resembled patterns predicted for a species experiencing habitat loss. Trap cells were more effective than neutral non-hosts at reducing virus growth rate, and spatial structure reduced the ability of traps to prevent the infection of rare hosts. Our results suggest that factors identified as crucial to conservation biology – habitat aggregation and connectivity – may also affect the development of trap cell therapy for infectious human viruses.

MODEL

Our model describes virus growth rate as a function of the frequency of source habitat (host cells) and either neutral or trap habitats (collectively, non-host cells). In allegiance with the source–sink habitat paradigm, we assume that the total abundance of cells is not appreciably altered by the virus, allowing us to treat virus growth rate as a function of the relative abundance of cell types. This assumption will hold for cases where target cells remain abundant enough to saturate viral kinetics (Hethcote *et al.* 2005), but is otherwise an overt but practical simplification. Rather than model host dynamics, which greatly increases model complexity (e.g. Begon & Bowers 1994), we focus on the net effect of non-host frequency on virus growth rate. This approach will allow us to predict viral fitness in the short term from the initial frequency of cell types.

If hosts make up a proportion p of target cells and non-hosts the proportion $1 - p = q$, the virus achieves a fraction, a, of its maximum birth rate,

$$a = \frac{H_1 k_1}{H_1 k_1 + H_2 k_2} = \frac{p}{p + qc}, \quad (1)$$

where H_1 is the abundance of hosts, H_2 is the abundance of non-hosts, and the k_i are the probabilities that an encounter with cell type i results in attachment to that cell (*sensu* Emlen 1973), and $c = k_2/k_1$ measures the efficacy of the non-host cell for viral subversion. For $c > 1$, non-hosts are more attractive to the virus than are hosts and, therefore, are very effective traps.

Efficacy itself may change with the frequency of cell types, increasing or decreasing with host community composition. As an analogy, the effect of progressive habitat loss on net population growth rate can be highly nonlinear if the loss is spatially aggregated (Hill & Caswell 1999; Urban & Keitt 2001; Fahrig 2002). Changes in interaction strength with abundance or frequency are also common in predator–prey models, reflecting processes that affect resource capture rates including handling time, predator learning and prey aggregation (Real 1977). Manly *et al.* (1972) incorporated frequency-dependent prey preference into a foraging model by making the coefficients k_i in eqn 1 linear functions of prey frequencies. We take a similar approach by making the efficacy of non-hosts a linear function of their frequency, such that

$$a = \frac{p}{p + q(c - mq)}, \quad (2)$$

where c is now defined as the intrinsic efficacy of the trap and m describes the change in efficacy with increasing trap frequency. The proportion by which efficacy differs between a community dominated by hosts and one dominated by non-hosts is the ratio m/c, which we will refer to as the frequency dependence of efficacy. For $m > 0$, the trap cell is the least effective when hosts are rare.

Consider a virus population with maximum birth rate b (attained when all cells are hosts). Trapped and free viruses are removed or degraded at a constant loss rate, u, analogous to death and emigration. We assume that u is independent of trap cell frequency. Incorporating eqn 2, virus per capita growth rate as a function of host frequency is,

$$\frac{\mathrm{d}V}{V\,\mathrm{d}t} = \frac{bp}{p + q(c - mq)} - u. \quad (3)$$

Note that by pooling all virus particles into a single population, rather than tracking free and trap-bound particles separately, eqn 3 assumes that bound viruses can dissociate from traps and may then infect hosts. The reversible attachment of phages has been widely demonstrated (see Goldberg *et al.* 1994 and references therein). Dissociation decreases intrinsic efficacy, c, by allowing trapped viruses the chance to subsequently infect hosts.

Setting eqn 3 to zero and solving for q gives the threshold frequency of non-host cells, q^*, necessary to arrest virus population growth. The biologically relevant solution in the range $0 \le q^* \le 1$ is

$$\begin{cases} m = 0 & q* = \frac{r}{r+cu} \\ m \neq 0 & q* = \frac{r+cu-\sqrt{(r+cu)^2-4mur}}{2mu}, \end{cases} \quad (4)$$

where $r = b - u$. The conditions in eqn 4 correspond to frequency-independent ($m = 0$) and -dependent ($m \neq 0$) efficacy.

A frequency of trap cells higher than q^* leads to extinction of the virus. As demonstrated in Fig. 1, the extinction threshold is generally high when loss (u) is low. Higher loss rates, for instance, due to emigration (wash-out) or predation (immune response), decrease q^* (Fig. 1). With strong negative frequency-dependent efficacy, q^* remains high at low loss rates but decreases substantially if loss increases (Fig. 1). Hence, frequency-dependent effects of ecological traps can produce abrupt changes in population viability with decreasing local recruitment.

EXPERIMENTAL SYSTEM AND METHODS

Bacteriophage Φ6 is a lipid-coated, lytic virus with a double-stranded RNA genome (Vidaver *et al.* 1973; Mindich *et al.*

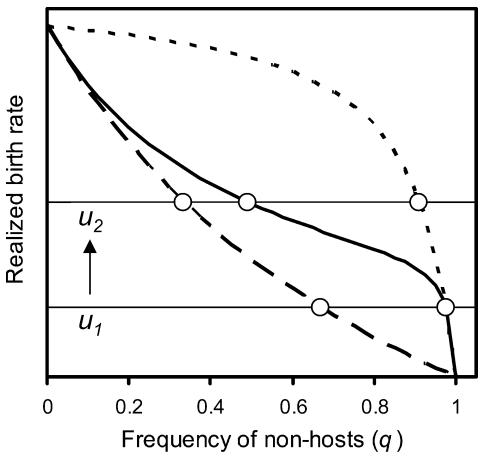

Figure 1 The effect of intrinsic efficacy and the frequency dependence of efficacy on the threshold frequency of traps for viral extinction (q^*, eqn 4). Curves show birth rates as a function of the frequency of a non-host. Dotted curve: low, constant efficacy: $c = 0.05$, $m = 0$. Dashed curve: high, constant efficacy: $c = 2$, $m = 0$. Solid curve: high intrinsic efficacy and strong negative frequency dependence: $c = 2$, $m = 1.95$. The horizontal lines labeled u_1 and u_2 are two loss rates. Open circles give the q^* for each curve at each loss rate. With an increase in loss from u_1 to u_2, q^* drops most with frequency-dependent efficacy.

1976a,b). The strain used in this study was derived from a single clone of wild-type Φ6 obtained from L. Chao (University of California, San Diego, CA, USA). The wild-type *Pseudomonas phaseolicola* host is a plant pathogenic bacterium purchased from American Type Culture Collection (ATCC #21781). Phage Φ6 infects wild-type *P. phaseolicola* by binding to type-IV pili produced by the bacteria for host–plant attachment (Romantschuk & Bamford 1985; Romantschuk *et al.* 1988). When *P. phaseolicola* retracts a pilus, data suggest that some of the attached phage then encounter and enter the cell (Bamford *et al.* 1987; Daugelavicius *et al.* 2005).

Two naturally occurring *P. phaseolicola* mutants, non-piliated and super-piliated, were used as neutral and trap cells respectively. The non-piliated mutant (strain #LM2509) was obtained from L. Mindich (Public Health Research Institute, Newark, NJ, USA), and was used as a control to gauge the effect on phage growth rate of replacing hosts with unusable habitat. This neutral non-host presents no binding sites (pili) for phage attachment and thereby resists infection. The super-piliated mutant (strain #KL6) was obtained from L. Chao (University of California), and used to test whether a trap cell can drive the phage towards extinction. The super-piliated cell presents many sites for phage attachment but precludes infection by failing to retract its pili.

Methods overview

We quantified the rate at which Φ6 binds to these three variants of *P. phaseolicola* to judge whether our classifications of neutral, trap and host were justified. We also compared the growth rate, stationary phase density and relative fitness of the three host cell types to ensure that direct competition did not distort cell frequencies. Subsequently, we evaluated trap cell efficacy in both structured and unstructured habitats by enumerating virus growth rate over a range of frequencies of non-host *P. phaseolicola*, obtaining model parameters by non-linear regression. Finally, we used our parameterized model to forecast phage dynamics during transmission between mixed-host communities, and used serial passage experiments to test these predictions.

Culture conditions

All viruses and bacteria were grown, plated, incubated and diluted at 25 °C in LC medium, Luria broth (10 g NaCl, 10 g Bacto® tryptone [Becton, Dickinson and Co., Sparks, MD], and 5 g Bacto® yeast extract per litre) at pH 7.5, using published methods (Dennehy *et al.* 2006). Briefly, bacterial cultures were grown by placing a single colony in a sterile flask with 10 mL of LC medium. The flask was then placed on a shaker (120 rpm) inside an incubator. After 24 h, cultures attained stationary-phase densities.

Virus stocks were cultured by placing Φ6 and 200 μL of stationary-phase wild-type *P. phaseolicola* into 3 mL of 0.7% top agar (liquid at 45 °C), overlaid onto a 1.5% agar plate (solid). After 24-h incubation, viruses formed visible plaques (holes) in the bacterial lawn growing in the agar overlay. Cell-free lysates of virus were prepared by collecting the plaques in the top agar, re-suspending them in 4 mL of LC broth, and centrifuging at 3000 rpm (1620 **g**) for 10 min. The supernatant was then filtered (0.22 μm, Durapore®; Millipore, Bedford, MA, USA) to remove bacteria. Lysates were stored at −20 °C in a 4 : 6 glycerol/LC (v/v) solution. Lysates were titered (i.e. number of phage per ml was quantified) by serially diluting lysate and plating dilutions on wild-type *P. phaseolicola* lawns. At dilutions where 30–500 plaques were visible and non-overlapping, plaques were counted to estimate the titre of the lysate.

Bacterial growths

We measured both the log-phase growth rate and stationary-phase density (i.e. carrying capacity) of the three bacterial strains by monitoring culture optical density using a spectrophotometer. To measure each strain's growth rate, 1 mL of overnight stationary phase culture was added to 9 mL of LC broth, with threefold replication. Log_{10} bacterial abundances were calculated from hourly samples taken during log-phase growth of cultures, and regressed against time to estimate growth rate. Stationary-phase $\log_{10}$ densities of the three bacterial strains were estimated in threefold replicated experiments. Growth rates and carrying capacities were compared among strains by analysis of variance (ANOVA).

Competition assays

We assayed bacterial relative fitness by competing each strain against a common competitor: *P. phaseolicola* strain no. LM3313 (provided by L. Mindich) containing a plasmid that allows ampicillin resistance (Amp^r) at 100 μg mL^{-1} of the antibiotic. Competition occurred using the culture conditions described above. Strains were preconditioned through separate growth for 1 day in the experimental medium, ensuring their comparable physiological states. They were then mixed 1 : 1, diluted 1 : 100 into fresh medium, and allowed to grow and compete for 24 h. Initial and final densities of competitors were estimated by comparing colony growth on LC and LC + ampicillin plates. The number of antibiotic-sensitive test bacteria was determined by subtraction.

The initial and final (24 h) densities of competitor i can be symbolized as N_{i0} and N_{i1} respectively. The

time-averaged rate of increase, m_i, for the competitor was then calculated as $m_i = \ln [N_{i1}/N_{i0}]/(24\text{ h})$. The fitness ($W$) of strain i relative to the Amp^r reference strain j was expressed as the ratio of their rates of increase, m_i/m_j (Lenski *et al.* 1991). Relative fitness values were compared among strains by ANOVA, using $\log_{10}(W)$ values – equivalent to the selection coefficient – as $\log_{10}(W)$ scales linearly (Burch & Chao 1999).

Attachment rates

To measure virus attachment rates (Stent 1963; Dennehy & Turner 2004; Pepin *et al.* 2006), 4×10^3 virus particles were mixed with 4×10^8 exponential-phase bacteria in 10 mL of LC medium, with twofold replication. The mixture was incubated with shaking for 40 min. Immediately after mixing and every 10 min thereafter, a volume of 500-μL sample from the mixture was centrifuged at 4000 rpm (2880 **g**) for 1 min to pellet the cells, and the supernatant was titered on wild-type *P. phaseolicola* lawns. As bound phages are pelleted with the bacterial cells, only unbound phage remained in the supernatant. We then calculated the log-linear decay rate $-kCt$, where k is the per capita attachment rate, C is the concentration of host cells in the mixture, and t is time in minutes. The units of k are per phage per cell per millilitre per minute. Estimates of k were compared among strains by ANOVA.

Effect of non-hosts on virus growth rate

To observe phage performance in an unstructured habitat, a lysate of Φ6 was obtained from wild-type *P. phaseolicola*. Using this lysate, *c.* 2000 virus particles were added to sterile vials containing 6 mL of LC broth and a total of 6.64×10^8 host cells. Vials contained 11 different ratios of trap (super-piliated) hosts to wild-type hosts ranging from 0 : 100 to 100 : 0, with twofold replication ($n = 22$). As a control, identical experiments were performed using mixtures of ordinary and neutral (non-piliated) cells. Volumes were twice those used in structured habitats (see below) to ensure efficient mixing of shaking cultures.

Structured habitat experiments were performed similarly: from a lysate, *c.* 1000 virus particles were added to 3 mL of 0.7% top agar containing 3.32×10^8 cells, varying the percentages of trap cells from 0% to 100%, and overlaid on a 1.5% agar plate. Each trap cell percentage was replicated fourfold ($n = 44$). Increased replication was used because preliminary trials showed that structured environment results were more variable.

In both experimental habitats, cell-free lysates were prepared after 24 h (see above), and titered on wild-type *P. phaseolicola* lawns. From these data, the per capita population growth rate in each treatment was estimated using the equation, $r = \ln [n_t/n_0]/t$, where t is time (24 h), n_0 is the number of individuals in the initial inoculum, and n_t is the number of individuals in the recovered lysate. For one replicate in which we recovered no phage, we estimated phage abundance to be the minimum detectable by our dilution series, 100. This estimate yielded the same results as discarding the replicate.

Model parameters and confidence intervals were estimated from data using the nonlinear platform in JMP 5.01® (SAS Institute, Cary, NC), which employs the common Gauss–Newton iterative method for minimizing least squares and generates likelihood confidence intervals. Parameter estimates in different treatments were deemed qualitatively distinct in pairwise comparisons if neither estimate was included in the confidence interval of the other. R^2 values were approximated as 1 − SSE/SST, where SSE is the sum of the model's squared residual errors and SST is the sum of the total squared error about the mean. The benefit and cost of model complexity was judged using Akaike information criteria (AIC) adjusted for high model complexity (Burnham & Anderson 2002).

Serial passage experiments

Serial passage experiments are highly useful for examining the dynamics of model host–parasite systems under controlled laboratory conditions (Ebert 1998). We used serial passage experiments in a structured environment to examine the effects of the trap cells on virus sustainability. Populations were bottlenecked during transmission to a new habitat after having achieved maximal growth in a previous habitat as in Dennehy *et al.* (2006). A stock lysate containing 6.0×10^{11} phages was serially diluted to 10^{-6} and 10^{-7} and 100 μL was plated on bacterial lawns, producing inocula of 60 000 and 6000 Φ6 phages respectively. Three separate bacterial lawn treatments were used with threefold replication: 100% wild-type host, 50% host plus 50% neutral non-host and 50% host plus 50% trap. The first two treatments control for the ability of phage to persist in the absence of traps and the third was the experimental treatment. During 24-h incubation, the bacteria lawns seeded at low density grew exponentially until reaching a very high density (confluent lawn) that signified stationary phase, whereby Φ6 plaques ceased to expand in size. After 24 h, virus progeny (plaques) were harvested to obtain a lysate for each replicate. Lysates were titred on the ordinary host and the resulting number was adjusted for dilution to track inoculum sizes over time. Diluted inocula were then passaged to new plates for a total of four consecutive passages in each treatment.

For model predictions of virus population dynamics in the presence of trap cells, we estimated loss rate, u, as $\ln(\text{dilution})/24 + u_0$, where u_0 is the loss rate found in the structured environment. All other parameter values were

Table 1 Estimates of parameters for virus growth rates and effects of non-host cells (and 95% likelihood confidence interval)

Environment	Control (neutral)	Unstructured (trap)	Structured (traps)
Birth (b)	0.86 (0.78 to 0.93)[1]	0.86 (0.81 to 0.92)[1]	0.82 (0.77 to 0.86)[1]
Loss (u)	0.10 (0.04 to 0.17)[2]	0.12 (0.08 to 0.17)[2]	**0.03** (−0.01 to 0.07)[1]
Intrinsic efficacy (c)	**0.09** (−0.05 to 0.24)[1]	0.68 (0.41 to 0.96)[2]*	1.09 (0.87 to 1.33)[3]*
Decrease (m) in efficacy with non-host frequency	**0.04** (−0.11 to 0.19)[1]	**0.25** (−0.15 to 0.61)[2]	0.92 (0.66 to 1.18)[3]
ΔAIC vs. $m = 0$	+2	+2	−10
Constant efficacy (c with $m = 0$)	0.06 (0.04 to 0.07)[1]*	0.52 (0.42 to 0.65)[2]*	0.48 (0.35 to 0.66)[2]*

*Efficacy estimate is lower than expected from attachment rates. Bold values are not significantly different from zero. Across each row, parameter estimates with the same superscript share overlapping confidence intervals. ΔAIC < 0 suggests that the model improves with the addition of frequency-dependent efficacy.

taken from the structured environment, unmodified (Table 1). Equation 3 was then iterated discretely as $V_t = V_0 e^{\dot{}^{eqn3'\times 24\times t}}$ for $t = 0$ to four passages.

RESULTS

Measurements of the growth rate, stationary-phase density and relative fitness of the three bacterial strains suggest that we can safely apply eqn 3 under the assumption that total cell density did not vary with bacteria community composition. The three strains showed similar growth rates: wild-type *P. phaseolicola* (0.183 ± 0.004 SEM per hour); super-piliated trap strain (0.187 ± 0.008 SEM per hour); neutral non-piliated strain (0.166 ± 0.005 SEM per hour). Growth rate did not differ among the strains ($F_{2,6} = 3.79$, $P = 0.09$). The three strains had very similar stationary-phase densities: wild-type *P. phaseolicola* ($1.66 \times 10^9 \pm 3.22 \times 10^7$ SEM cells mL^{-1}); super-piliated trap strain ($1.61 \times 10^9 \pm 3.71 \times 10^7$ SEM cells mL^{-1}); neutral non-piliated strain ($1.71 \times 10^9 \pm 2.96 \times 10^7$ SEM cells mL^{-1}). Stationary-phase densities did not differ according to the genotype ($F_{2,6} = 1.99$, $P = 0.22$). Competition assays were used to estimate the mean $\log_{10}(W)$ of each bacterial strain: wild-type *P. phaseolicola* (0.010 ± 0.006 SEM); super-piliated trap strain (0.029 ± 0.009 SEM); neutral non-piliated strain (−0.010 ± 0.012 SEM). Although $\log_{10}(W)$ of the three strains differed in competition ($F_{2,12} = 4.68$, $P = 0.031$), wild-type fitness did not differ significantly from that of the neutral non-piliated strain, or from that of the super-piliated trap strain (Tukey's HSD, $\alpha = 0.05$). These results indicated that the strain combinations used in our virus growth experiments were competitively equivalent (i.e. wild-type bacteria paired with either the neutral strain or the trap strain).

Significant differences were found in the ability of the three *P. phaseolicola* strains to bind phage Φ6 ($F_{2,3} = 477.11$, $P = 0.0002$). Figure 2 illustrates that attachment rate was high to trap cells, intermediate to wild-type hosts, and low to neutral non-hosts. Under our experimental conditions, these

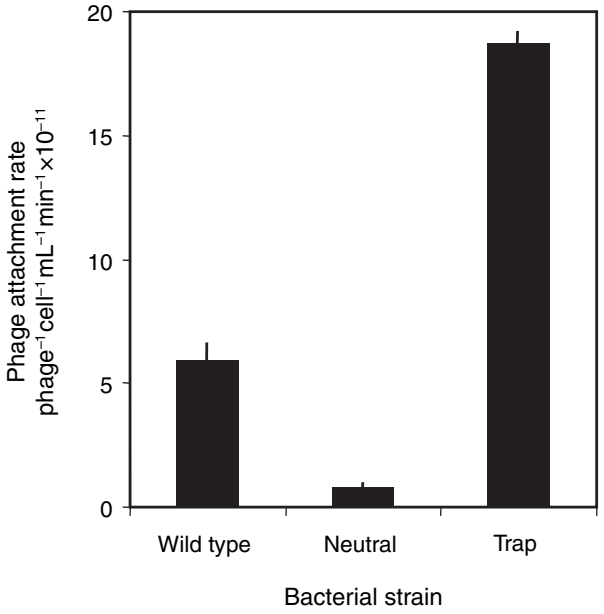

Figure 2 Attachment rates estimated from replicated 40-min assays ($n = 2$). Error bars are 1 SD.

attachment rates translate to the binding of 99%, 75% and 18% of free particles per hour for the trap, host and neutral cells respectively. The high rate of phage attachment to trap cells in pure culture indicates that they should be effective at reducing the population growth rate of the virus. Intrinsic efficacy, c, ought to reflect differential attachment to each cell type in pure culture. Our null expectation of the efficacy of trap cells was therefore $c = k_{trap}/k_{host} = 3.2$, where k_i is the attachment rate. We expected a neutral cell efficacy of $c = k_{neutral}/k_{host} = 0.14$.

Neutral non-host cells caused little interference with host infection other than taking up physical space. Figure 3a shows the response of virus population growth rate to the frequency of neutral non-hosts in an unstructured environment. Here, growth was characterized by low values of c and

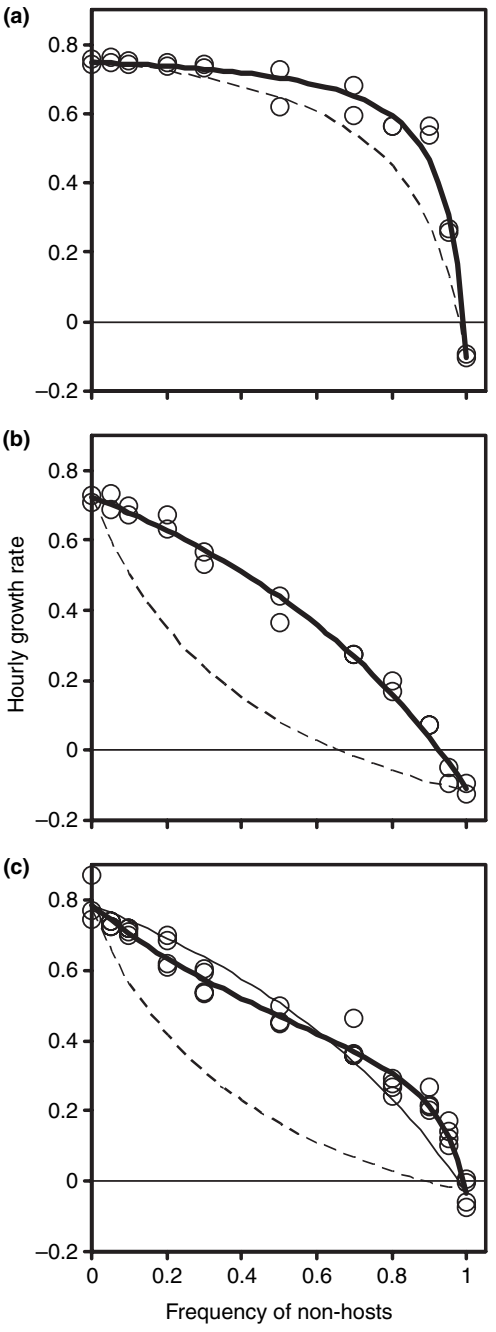

Figure 3 The effect of host type and environment on phage growth rate. Solid curves are the best fit of eqn 3 (parameters in Table 1). Dashed curves are null model predictions estimating efficacy as the ratio of attachment rates measured on each cell type in isolation (given in text), assuming constant efficacy ($m = 0$). (a) Neutral non-hosts in the unstructured environment. (b) Trap cells in the unstructured environment. (c) Trap cells in the structured environment (heavy curve) superimposed on efficacy of traps in the unstructured environment (fine curve) for comparison. Best fit curves assume frequency-dependent efficacy only in the structured environment.

m that were not statistically different from zero ($P > 0.05$, Table 1). Such low values of c and m imply, respectively, that neutral non-hosts are unattractive to phage, and that this relationship does not change as the ratio of hosts is manipulated. The virus population maintained a high growth rate until neutral cells were the vast majority of the target cell population. The AIC indicated that m could be removed from the model and c estimated as constant efficacy (Table 1). Figure 3 shows the effect of including and excluding m on our inference of the realized efficacy of neutral cells (control). The ability of neutral cells to reduce virus growth was similar to our expectation based on attachment rates ($c \approx 0.14$, Table 1, dashed curve in Fig. 3a).

Trap cells displayed significant efficacy, decreasing the growth rate of the virus even at low frequency (Fig. 3b,c). This impact was manifest in significant values of c ($P < 0.05$, Table 1) in both structured and unstructured environments. In the unstructured environment, m was not significantly different from zero and the AIC indicated that it could be removed from the model (Table 1). In the structured environment, trap cell efficacy was negatively frequency dependent (decreased with the prevalence of traps) reflected by a significant positive m ($P < 0.05$, Table 1). Trap cell efficacy in both environments was lower than expected from attachment rates ($c < 3.2$, Table 1, dashed curves in Fig. 3b,c). Loss rates differed between environments (Table 1).

We concluded that the ability of a trap to reduce virus population growth is frequency independent in well-mixed environments and negatively frequency dependent in spatially structured environments. With this information, the correct form of eqn 4 can be selected to estimate the extinction threshold, q^*.

To validate the concept of trap cell therapy, we observed virus population dynamics in serially passaged populations. Virus passaged at 10^{-7} dilution between plates of pure host populations persisted at high numbers over all four passages, as did virus passaged between plates containing 50% neutral non-hosts (Fig. 4). In contrast, virus passaged at 10^{-7} dilution between plates containing 50% trap cells declined in abundance towards extinction (see Dennehy *et al.* 2006) after three passages (Fig. 4). Reducing virus mortality by decreasing dilution to 10^{-6} was not sufficient to ensure virus population survival in the presence of trap cells (Fig. 4). These results support the hypothesis that trap cells can be used to reduce or eliminate viral loads in diseased organisms.

For comparison of model predictions with the population dynamics of the serially passaged populations, we parameterized eqn 3 using values from the structured environment (Table 1). With the additional loss due to dilution, the total loss rates (u) at 10^{-6} and 10^{-7} dilution were 0.61 and 0.70 respectively. The initial rates of virus population decline in

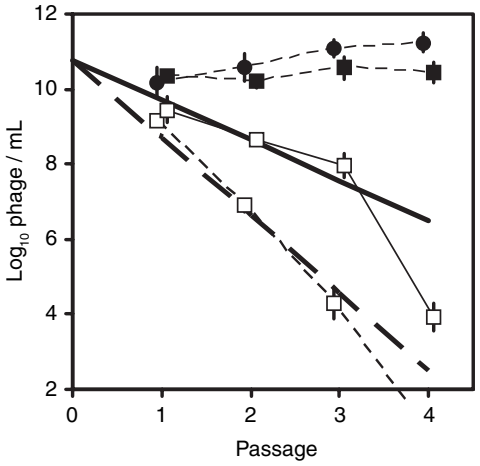

Figure 4 Phage abundance over the course of four serial passages in the structured environment with 10^7-fold dilution (dashed lines) or 10^6-fold dilution (solid lines) between passages. Filled circles: pure host community; filled squares: 50% neutral non-host community; open squares: 50% trap cell community; heavy lines without symbols are phage dynamics predicted by eqn 3. Parameters are provided in text and Table 1. Points are offset for clarity. Error bars are ± 1 SD.

the presence of trap cells predicted by the model qualitatively matched those observed in the serial passage experiment (Fig. 4). We conclude that parameterization of eqn 3, although simplistic, is sufficient to predict short-term virus population dynamics in response to trap cell treatment.

DISCUSSION

The concept of population viability is often applied to landscapes, real or theoretical, in which source habitats are gradually replaced with sinks (Morris & Doak 2002). In such situations, effective habitat choice by resident species minimizes the impact of sinks on population persistence (Delibes *et al.* 2001; Kokko & Sutherland 2001). However, sink habitats can present cues associated with sources, thereby foiling optimal habitat selection (Schlaepfer *et al.* 2002) and reducing population viability via an increased use of sinks (Doak 1995; Delibes *et al.* 2001; Kokko & Sutherland 2001). Our observations of phage population growth in cell communities of varying composition support the basic predictions of population viability theory. Replacement of host cells with neutral non-hosts did not have a large impact on phage fitness unless the vast majority (90%) of hosts were replaced (Fig. 3a). In contrast, a similar decrease in phage growth rate was obtained by replacing only 30% of hosts with traps in both structured and unstructured environments, demonstrating significant efficacy in trapping virus particles (Fig. 3b,c). In serial passage, replacement of 50% of the host population with traps led to phage extinction, while 50% neutral non-host communities allowed phage persistence (Fig. 4).

The efficacy of trap cells was frequency dependent in the structured environment. In spatially structured landscapes, the dispersion of sources and sinks is expected to affect population viability greatly (Fahrig 2002). An aggregated (as opposed to random) pattern of habitat loss maximizes the connectivity between source habitat patches (Urban & Keitt 2001) and decreases the net rate at which sinks are sampled by local dispersal (Hill & Caswell 1999), thereby increasing a population's robustness to increasing sink frequency. The spatial structure of bacterial lawns may explain why virus populations were able to grow on plates even when 95% of cells were traps (Fig. 3c). Bacterial lawns are seeded with mixtures of two host types at low density. Over time, spatially autocorrelated reproduction produces a confluent patchwork of locally homogenous micro-colonies (S. Abedon, personal communication). A phage that locates a micro-colony of suitable hosts is colonizing a resource patch that will support limited local population growth even if most other patches consist of trap cells. Our findings suggest that the utility of virus traps for therapy will depend on the spatial structure of the target tissues.

Trap cells will be most effective in open systems, such as bodies of water or tissues subject to high turnover rates. In such systems, wash-out will elevate the loss rate of trapped and free viral particles. Figure 1 illustrates the consequences of two loss rates in terms of the threshold for virus population growth, q^* (eqn 4). With little or no loss, as in our experimental system, the threshold is high unless traps have acute efficacy independent of frequency (u_1, Fig. 1). The threshold decreases with increased loss (u_2, Fig. 1), especially in the case of frequency-dependent efficacy. In serial passage with periodically inefficient transmission, and therefore high loss, trap cells drove the phage towards extinction under conditions that would otherwise allow persistence (Fig. 4). Initial rates of population decline in the presence of traps agreed qualitatively with model predictions. The sudden drop in phage population size in the fourth passage (Fig. 4) resembles dynamics observed in a previous study (Dennehy *et al.* 2006) and may be attributable to reduced population growth at low phage density.

Trap efficacy was not as high as expected from the difference in phage attachment rates to host and trap cells. Traps bound phage over three times faster than did wild-type hosts in attachment assays (Fig. 2), producing the expectation of a rapid decrease in virus growth with increasing trap frequency (dashed curve, Fig. 3b,c). Lower efficacy may result from dissociation rates that were not captured in our attachment assays. Alternatively, our measure of efficacy may be integrating the direct and

indirect effects of phage on host dynamics. More detailed measurements of host competition in the presence of phage are required before we can increase the complexity of our model. However, it would be surprising if host dynamics were entirely responsible for the patterns in Fig. 3b,c, given the similarity in virus growth between the unstructured and structured environments. Whereas the virus affects all hosts with equal probability in a shaken liquid culture, it is only acting in the vicinity of plaque growth on the surface of a plate, where each generation of phage encounters an essentially naive bacterial community. Strong effects of phage on bacterial competition would therefore be expected to cause dramatically different responses of phage growth in structured and unstructured environments, but our estimates of constant trap efficacy (ignoring frequency dependence) did not differ between environments (Table 1).

Reduction in virus population growth rate should benefit the host population in the long term. Not only is this prediction relevant to the application of trap cell therapy, it also raises an interesting scenario regarding the evolution of phage resistance. Both of the non-host cell types used in our experiments are naturally occurring mutants resistant to phage infection. If selection for resistance results in a neutral non-host, virus growth rate remains high until susceptible hosts are eradicated. [We note that naturally occurring non-piliated mutants may seem implausible given they cannot attach to leaf surfaces. However, research shows that *P. phaseolicola* may be facultatively pathogenic; it can survive long periods in soil (Wimalajeewa & Nancarrow 1980).] Conversely, resistance obtained by becoming a trap may result in virus extinction before susceptible hosts are lost from the system. Therefore, some mechanisms of resistance can confer indirect positive effects on susceptible hosts.

Recent theoretical and empirical investigations suggest that biodiversity is a driver of disease ecology and evolution. The particular effects of each species in a community on the fitness of a disease organism can act to either increase or decrease the sustainability of an infection of the target host population (Holt *et al.* 2003). While the net effect of diversity on disease prevalence varies among systems, it appears that disease is suppressed by increased diversity in many cases (Keesing *et al.* 2006). In particular, variance in competence among diverse hosts can reduce a disease's force of infection (Schmidt & Ostfeld 2001). As we have shown, strong negative interactions with some species in a community can lead to pathogen extinction even when productive hosts are abundant (Fig. 4).

It is possible that natural trap cells exist in humans, whether or not they directly evolved to combat viral infection. While no definite trap cells have been defined, some evidence suggests that red blood cells (erythrocytes) may be natural virus traps (Gagneux & Varki 1999; Baum *et al.* 2002). Erythrocytes are ideal trap cells because they lack nuclei (where many viruses replicate) and circulate systemically in large quantities throughout the body. In fact, putative HIV co-receptors have already been defined on erythrocytes (Hammache *et al.* 1999). Erythrocytes have also been characterized with multiple antigens (i.e. blood types) (Daniels 1995), which may actually be a diverse set of germline-encoded viral receptors.

Indeed, erythrocytes are an obvious choice for engineering trap cells to reduce viraemia. As can be seen in Fig. 1, the frequency of trap cells may have to be very high to achieve viral extinction. In patients with fully developed acquired immunodeficiency syndrome (AIDS), the frequency of red vs. T4 white blood cells per volume can easily exceed the threshold levels that might be required to engineer HIV population inviability. HIV attacks T4 white blood cells, which are typically 5000-fold fewer per volume than red blood cells in healthy humans (Hoffman *et al.* 2004). A transfusion of engineered red blood cells as virus traps could easily achieve trap cell frequencies well in excess of 90%, especially in AIDS patients suffering T-cell destruction. While some viruses can persist for long periods in refuge tissues, trap cell therapy may aid in the recovery of immune function and reduce the need for antiviral medications, just as trap crops foster natural enemy populations and reduce the need for insecticides (Hokkanen 1991; Badenes-Perez *et al.* 2005). Our tests of phage trap efficacy, while limited to contrived environments, suggest that a consideration of the ecological source–sink concept will be useful to the process of engineering and applying trap cell therapies.

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