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abstract: Viruses can occasionally emerge by infecting new host species. However, the early phases of emergence can hinge upon ecological sustainability of the virus population, which is a product of both within-host population growth and between-host transmission. Insufficient growth or transmission can force virus extinction before the latter phases of emergence, where genetic adaptations that improve host use may occur. We examined the early phase of emergence by studying the population dynamics of RNA phages in replicated laboratory environments containing native and novel host bacteria. To predict the breadth of transmission rates allowing viral persistence on each species, we developed a simple model based on in vitro data for phage growth rate over a range of initial population densities on both hosts. Validation of these predictions using serial passage experiments revealed a range of transmission rates for which the native host was a source and the novel host was a sink. In this critical range of transmission rates, periodic exposure to the native host was sufficient for the maintenance of the viral population on the novel host. We argue that this effect should facilitate adaptation by the virus to utilize the novel host—often crucial in subsequent phases of emergence.

Keywords: bacteriophage, density-dependence, emergence, host shift, source-sink dynamics, virus.

Viruses occasionally "emerge" by expanding the range of host types they infect (Morse and Schluederberg 1990; Morse 1993; Moya et al. 2004). Emerging viruses initially tend to be less productive on the new host relative to the original host (Crill et al. 2000; Turner and Elena 2000; Cuevas et al. 2003). This trade-off is consistent with the general expectation that organisms evolved on one resource are inefficient at using alternative resources (Futuyma and Moreno 1988; Holt 1996; Whitlock 1996). Although an emerging virus or other pathogen may initially suffer low fitness, it may persist on the new host solely because of chance effects (Antia et al. 2003; Andre and Hochberg 2005) or via subsidy from the original host population (i.e., through source-sink dynamics; Holt 1985; Pulliam 1988; Pulliam and Danielson 1991; van Baalen and Sabelis 1995). Through time, the pathogen may be selected to better exploit the new host, achieving some optimal level of virulence (Lenski and May 1994). When there is only a single bout of cross-species infection, there is a race between extinction and potential adaptive evolution on the novel host, and extinction is likely to win if the novel host provides a low-quality environment for pathogen growth and genetic variation is constrained (Gomulkiewicz and Holt 1995; Antia et al. 2003). Therefore, emergence may be fostered by ecological factors that permit establishment of a persistent population on a novel host, which can then be followed by genetic adaptation to improve host use (Morse and Schluederberg 1990; for instance, key mutations in the SARS coronavirus may have facilitated epidemiological spread in humans; see Li et al. 2005 and Qu et al. 2005).

The ecological obstacles encountered by emerging viruses can be formidable. With low initial abundance and low within-host fitness, persistence will depend on the mode and frequency of virus transmission (Morse 1993, 1995; Rogers and Packer 1993), which can be influenced by the spatial or temporal proximity of alternative host populations. Insufficient transmission can prevent sustained growth of emerging virus populations (Rand et al. 1995), causing extinction before genetic adaptation to the novel host can take place (Gomulkiewicz and Holt 1995; Antia et al. 2003). Thus, transmission rate constitutes a component of fitness at the between-host scale.

It follows that the initial persistence of an emerging viral population is literally the product of fitness at two scales. If we define transmission as the proportion of a viral pop-

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ulation that locates a fresh host, a simple model of viral dynamics assumes hosts are ephemeral resources, and only the transmitted proportion of the viral population survives. A novel host is a "source" if within-host growth compensates for the bottleneck in population size incurred during transmission; the host is a "sink" if the growth rate or transmission is so low that total population growth falls below the replacement rate (for analyses of source-sink dynamics in spatially heterogeneous landscapes, see Holt 1985, Pulliam 1988, and the review in Kawecki and Ebert 2004; our use of these terms broadens their reference to include alternative hosts, as in Holt and Hochberg 2002).

In this study, we experimentally explored the interaction of within-host ecology and between-host transmission rate. In particular, we investigated the ability to predict the fitness of an emerging virus from its performances on a native and novel host separately. The process of emergence necessarily involves cross-host transmission, the pattern of which will determine whether viral persistence is driven by fitness on the novel host. We developed and tested predictions of viral population dynamics in the early phase of viral emergence using the RNA bacteriophage (phage) Φ6. Phage Φ6 has been successfully used as a laboratory model to address questions in virus ecology and evolution (Turner and Chao 1998; Lythgoe and Chao 2003; Dennehy and Turner 2004; Froissart et al. 2004). We used Φ6*h*, a host-range mutant of phage Φ6 that is able to infect novel host species in addition to the typical laboratory bacterial host. In pilot studies, we observed two key features of the ecology of this two-host system. First, phage Φ6*h* had a reduced growth rate on the novel host, making it a good candidate for emergence studies. Second, the yield (titer) of phage Φ6*h* grown on a single plate (containing either host) appeared to peak at intermediate inoculum densities, suggesting that growth rate is density dependent and possibly even positively density dependent at low numbers.

Material and Methods

Experimental Overview

We developed a simple model for the population dynamics of serially passaged Φ6*h*, using in vitro data on phage growth rate over a range of inoculum densities on both hosts. The model predicts the lower limit of transmission necessary for viral persistence on each host. Where growth on the native host exceeds that on the novel host, the model also predicts a range of transmission rates between host types over which the novel host is a sink. We used the serial passage experiments to validate the model and to examine in vitro the dynamics of phage persistence when transmission rate is externally manipulated. To do so, we serially transferred fixed fractions of phage to new media (fig. 1) and assessed long-term fitness. (Laboratory serial passage experiments are a powerful approach for examining the sustainability of microbial populations in the face of fixed mortality rates [Lenski et al. 1991; Ebert 1998].) We manipulated transmission rate by diluting the purified lysate obtained from each 24-h period of growth, the equivalent of about four viral generations. Experiments were limited to four passages to minimize the opportunity for viral evolution while permitting enough time to observe population dynamics. This experimental system is analogous to a rudimentary natural history in which a virus grows within a host (a single plate of host bacteria) and then infects another host via the transmission of a proportion of its population. The infective stage of the host is therefore instantaneous, following a discrete, experimentally imposed latent phase.

Study Organisms

Pseudomonas syringae pathovar *phaseolicola* (hereafter PP) used in our experiments was purchased from American Type Culture Collection (#21781). *Pseudomonas pseudoalcaligenes* East River isolate A (hereafter ERA) was obtained from the laboratory of L. Mindich (Public Health Research Institute, Newark, NJ). In our study, PP was the "source" host, and ERA was the "sink" host.

Phage Φ6 (family *Cystoviridae*) is a lytic (lethal) virus featuring a ~13,379 bp double-stranded RNA genome (Mindich 1988). Wild-type Φ6 can infect several plant pathogenic Pseudomonads (Cuppels et al. 1979), but PP is the typical host bacterium used in the laboratory (Vidaver et al. 1973; Turner and Chao 1998). One or more spontaneous mutations for host-range ability allow Φ6 mutants to shift onto ordinarily resistant hosts, including ERA (Mindich et al. 1976; S. Duffy, P. Turner, and C. Burch, unpublished data). In this study, we used strain

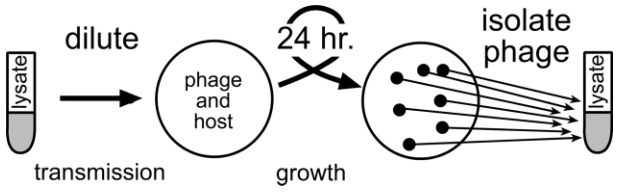

Figure 1: Schematic diagram of the steps involved in a single passage of the serial passage experiment. A lysate solution is diluted according to the treatment's transmission rate and plated with a constant density of host bacteria. After 24 h of growth (about four phage generations), all phage progeny are isolated from the host plate. A fraction of the resulting lysate is used to census the population, while another fraction is diluted as before to be used as an inoculum in the next passage.

PT590 (hereafter Φ6*h*), a spontaneous host-range mutant of Φ6; Φ6*h* has been minimally grown on PP and ERA in the laboratory (approximately four generations per host to create frozen stock), and therefore, as with its immediate predecessor Φ6, the mutant cannot be considered well adapted (Turner and Chao 1998).

Culture Conditions

All phages and bacteria were grown, plated, incubated, and diluted at 25°C in LC medium, Luria broth (10 g NaCl, 10 g Bacto tryptone, and 5 g Bacto yeast extract per liter), at pH 7.5 (Turner and Chao 1998). Bacterial cultures were grown by inoculating a single colony into 10 mL LC medium in a sterile flask with shaking (120 rpm) incubation. By 24 h, bacterial cultures attained stationary-phase densities. Stationary-phase density of PP in liquid LC medium is $\sim 4 \times 10^9$ cells/mL; for ERA, it is $\sim 5 \times 10^{10}$ cells/mL. To produce a bacterial lawn in top agar, we used 200 μL of stationary-phase (overnight) PP or 10 μL of overnight ERA culture; thus, initial host density in lawns was held relatively constant in our experiments at between $\sim 5 \times 10^8$ and 8×10^8 cells/mL. All bacterial stocks were stored at −80°C in a 4 : 6 glycerol/LC medium (v/v) solution.

Viruses were cultured by placing phage and stationary-phase bacteria into 3 mL of 0.7% agar (stored liquid at 45°C; gels to solid at 25°C) overlaid onto a 100 × 15-mm Petri dish containing 1.5% agar. By 24 h, viruses underwent four generations of growth to form 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, resuspending them in 4 mL of LC broth, and centrifuging at 3,000 rpm for 10 min. The supernatant was then filtered (0.22 μm Durapore; Millipore, Bedford, MA) to remove bacteria. Lysates were stored at −20°C in a 4 : 6 glycerol/LC medium (v/v) solution.

Plaque-Yield Assays

To examine differences in plaque yield across hosts, a lysate of Φ6*h* was prepared on PP, and dilutions were plated onto both PP and ERA lawns to isolate single plaques on each host. Five plaques were chosen at random from each host lawn, and each plaque was resuspended in 3 mL LC broth through gentle vortexing. Serial dilutions of the plaque suspension were plated on a PP lawn to estimate the mean titer of plaques grown on each host after 24 h. In microbiology, titer is standard nomenclature for virus population size and is defined as the number of viable virus particles per volume of solution, presented in our study as plaque-forming units (PFU) per milliliter on PP. Otherwise identical assays were performed where the Φ6*h* lysate was prepared on ERA.

Density-Dependence Assays and Model Predictions

We inoculated 3 mL of 0.7% agar with 10 μL ERA or 200 μL PP and a lysate volume containing between five and 10^8 phage particles (i.e., 5, 10, 50, 100, 500, 10^3, 5×10^3, 10^4, 5×10^4, 10^5, 5×10^5, 10^6, 5×10^6, 10^7, 5×10^7, or 10^8 individual viruses). This mixture was overlaid onto 1.5% agar plates. After 24 h, cell-free lysates of virus were prepared as described above. Lysates were titered by dilution series on PP lawns.

Our response variable was the ratio of final to initial titer (a measurement of growth rate), which we log transformed for presentation and analysis. We fitted log ratio of increase versus log inoculum density with second-degree polynomial regressions and used the resulting parameter estimates to generate predictions of population dynamics both analytically and numerically. We chose the quadratic model as a parsimonious and general method for accommodating curvilinearity. Our purposes did not require the derivation of a biologically mechanistic model.

Serial Passage Experiments

Serial passage experiments were used to examine the effects of mortality rate on virus sustainability (fig. 1). Serial passage experiments in bacteria have traditionally involved unstructured habitats, such as populations grown in shaking flasks containing liquid nutrient media. However, it is increasingly recognized that natural populations of bacteria and other microbes thrive in structured habitats where resources are not uniformly distributed, such as solid surfaces that may allow formation of bacterial biofilms. Agar in Petri dishes is a laboratory environment that allows microbes to be grown in a structured habitat.

A high-titer ($\sim 6 \times 10^{11}$ PFU/mL) master lysate of Φ6*h* was obtained on PP and used to initiate a serial passage experiment. On day 0, the master lysate was serially diluted up to seven times (i.e., to a strength of 10^{-2}, 10^{-3}, …, 10^{-8} of the original lysate), creating seven virus populations differing in initial population size (N_0). A 100-μL sample from each tube was then plated on a PP lawn. After 24 h of incubation, virus progeny were harvested to obtain a day 1 (t_1) lysate for each of seven treatment populations. The t_1 lysates were titered on PP, and the resulting number was multiplied by the transmission rate to estimate N_1 (i.e., we tracked inoculum sizes over time such that N_1 was the estimated number of phage particles that would be delivered to a second plate). The t_1 lysates were then diluted to the same extent as on day 0. A 100-μL sample of each dilution was then plated on a PP lawn. This process

was repeated for a total of four consecutive passages. Each dilution treatment in a serial passage experiment was replicated threefold. Identical experiments were performed on ERA and on alternating hosts (PP → ERA → PP → ERA). In all experiments, the treatment populations were titered on PP, where plaque yield is very high (see "Results").

We measured absolute fitness as the geometric mean population growth over the period of persistence, obtained as the *n*th root of the ratio of the *n*th inoculum to the initial inoculum. We defined each replicate's period of persistence as the number of passages over which estimated inoculum size exceeded 0.1 phage particles. While growth rate is expected to be a number much greater than 1.0, absolute fitness can be greater or less than 1.0. Populations with absolute fitness <1.0 are defined as living in a "sink" habitat, whereas populations with absolute fitness >1.0 live in a "source" habitat (Holt 1985). Populations with a geometric mean growth rate <1 are expected to approach extinction asymptotically. Absolute fitness was log transformed and analyzed in a two-way ANOVA.

Results

Comparison of Plaque Yield across Hosts

Preliminary assays compared the plaque yield (titer in PFU/mL) of Φ6*h* on the native host, *Pseudomonas phaseolicola* (PP), and on the novel host, *Pseudomonas pseudoalcaligenes* (ERA). When a "parent" lysate of Φ6*h* was created on PP and viruses were then reared for 24 h under low-density conditions (formation of nonoverlapping plaques), the mean titer ($n = 5$) of a resulting plaque on PP was $(4.35 \pm 2.60) \times 10^8$ PFU/mL, but on ERA it was only $(5.68 \pm 1.79) \times 10^7$ PFU/mL. This roughly tenfold increase in plaque yield on PP relative to ERA was highly significant ($t = 3.364$, df = 8, $P = .0099$).

We also conducted the complementary experiment, where a parent lysate of Φ6*h* was prepared on ERA and individual plaques were then reared on both hosts. Average ($n = 5$) plaque titer was $(4.26 \pm 2.44) \times 10^7$ PFU/mL for plaques reared on PP but only $(4.40 \pm 2.51) \times 10^6$ PFU/mL for those grown on ERA ($t = 6.499$, df = 8, $P = .0002$). Thus, regardless of the prior host, Φ6*h* produced roughly 10 times as many particles per plaque on the native host, PP, as on the novel host, ERA. In addition, the fact that mean plaque yield on both hosts was roughly one-tenth that when the Φ6*h* parent lysate was prepared on ERA relative to PP strongly suggests the importance of a "maternal effect" in our system (sensu Mousseau and Fox 1998; PP yield comparison: $t = 3.249$, df = 8, $P = .0117$; ERA yield comparison: $t = 3.488$, df = 8, $P = .0082$; see "Discussion").

Density Dependence of Phage Growth Rates

For each host, PP and ERA, we inoculated host lawns with a range of initial virus population sizes. By keeping the ratio of initial virus population size to host population size less than 1.0, we avoided high multiplicity of infection, and any confounding effects of virus coinfection could be largely ignored (Turner and Chao 1998; Dennehy and Turner 2004). For treatments with discernible plaques (inocula of 5, 10, 50, 100, and 500 individuals), plaque numbers were counted to determine whether expected and observed inocula differed; inoculum size was overestimated by 4.8%, but this difference was not significant ($t = 0.69$, df = 4, $P = .52$) and may be due in part to rare instances of viruses coinfecting the same cell or to plaque overlap. We then measured the resulting population size (titer on PP) of the virus progeny after 24 h of growth. As shown in figure 2*a*, titer (N') peaked at intermediate inoculum size (N) and was fitted with the estimated function

$$\log(N') = -0.14 \log(N)^2 + 1.27 \log(N) + 7.57 \quad (1a)$$

($r^2 = 0.81$, $F = 28$, df = 2, 13, $P < .001$). On ERA, titer was highest near the maximum inoculum size used in the experiment (fig. 2*a*). Regression on the ERA titer data produced the function

$$\log(N') = -0.20 \log(N)^2 + 2.81 \log(N) + 1.51 \quad (1b)$$

($r^2 = 0.99$, $F = 499$, df = 2, 13, $P < .0001$). The equations in (1) can be rearranged in the form $N' = N \times 10^B$, where B represents the logarithm of the ratio of increase. The growth rate functions for Φ6*h* on PP and ERA, respectively, were

$$B_{\text{PP}} = -0.14 \log(N)^2 + 0.27 \log(N) + 7.57 \quad (2a)$$

and

$$B_{\text{ERA}} = -0.20 \log(N) + 1.81 \log(N) + 1.51. \quad (2b)$$

Equations (2*a*) and (2*b*) are the same as those given by quadratic regression of log growth rate on log inoculum size (fig. 2*b*) and are the same as equations (1*a*) and (1*b*) divided by initial population size. Growth rate on PP decreased with inoculum size ($r^2 = 0.98$, $F = 301$, df = 2, 13, $P < .0001$), indicating negative density dependence. Growth rate on ERA peaked at intermediate inoculum size ($r^2 = 0.91$, $F = 66$, df = 2, 13, $P < .001$), indicating regions of positive and negative density dependence, suggesting an Allee effect (see "Discussion"). All parameter estimates in equations (1) and (2) were highly significant

except the first-order coefficient for growth on ERA (1.81; eq. [2*b*]; $F = 2.1$, df $= 1, 13$, $P = .17$).

The growth curves in figure 2*b* and equation (2) allowed us to predict the effect of transmission rate on phage population dynamics. For a transmission rate with magnitude 10^{τ}, where $\tau \leq 0$, the phage population will be at equilibrium if $B = -\tau$. For instance, a population that grows by four orders of magnitude ($B = 4$) is in equilibrium if one in 10^4 resulting viral particles is transmitted to a fresh host ($\tau = -4$).

Sustainability of Emerging Viruses on the Native and Novel Hosts

We used the simple model depicted in figure 2*b* to predict the sustainability of the phage growing on either its native or a novel host. The model predicted that Φ6*h* could be sustained on PP if transmission was of an order $\tau \geq -7.7$. On the novel host, ERA, where the maximum growth rate was two orders of magnitude lower than on the native host, the model predicted sustainability for $\tau \geq -5.56$. Lower transmission rates (lower τ) would lead to population decline and eventual extinction.

Serial passage experiments (fig. 1; see "Material and Methods") tested our predictions of phage sustainability on the native host, PP, or the novel host, ERA. Experimental results corroborated the model predictions for both hosts. On PP (fig. 3*a*), $\tau = -8$ led to phage extinction in 4 d, while $\tau \geq -7$ resulted in phage persistence. Phage populations on ERA (fig. 3*b*) showed sustainability at transmission rates of $\tau = -5$ and above and extinction by the fourth passage at $\tau = -7$. A transmission rate of 10^{-6} on ERA yielded a decline in phage population size over time, but the population was still extant in the fourth passage. There is a clear downward trend, suggesting that extinction would occur with longer sequences of passages at this transmission rate.

The sustainability of Φ6*h* in a homogeneous host regime is summarized by its absolute fitness on each host. We used serial passage data from treatments with transmission rates from 10^{-5} to 10^{-7} on PP and ERA to calculate log absolute fitness (fig. 4*a*, 4*b*). At high transmission rates, both hosts produced phage fitness statistically indistinct from unity. However, fitness on ERA declined below replacement (i.e., population growth rate did not compensate for the bottleneck of transmission) at lower transmission rates (fig. 4*b*). Mean log absolute fitness on PP at 10^{-7} transmission was -0.16 ± 0.32, not different from replacement ($t = 0.9$, df $= 2$, $P = .46$) and not different from fitness at 10^{-6} or 10^{-5} transmission (Tukey's HSD; $\alpha = 0.05$). Mean log absolute fitness of Φ6*h* on the novel host at 10^{-5} transmission was 0.016 ± 0.017 ($t = 1.6$, df $= 2$, $P = .26$). Transmission of 10^{-6} yielded log absolute fitness of -1.4 ± 0.29, significantly less than replacement ($t = 8.6$, df $= 2$, $P = .013$). We concluded that there is a range of transmission rates over which the novel host is a sink while the native host is a source for Φ6*h*. These results agreed closely with our model's predictions of long-term dynamics (fig. 4*a*, 4*b*).

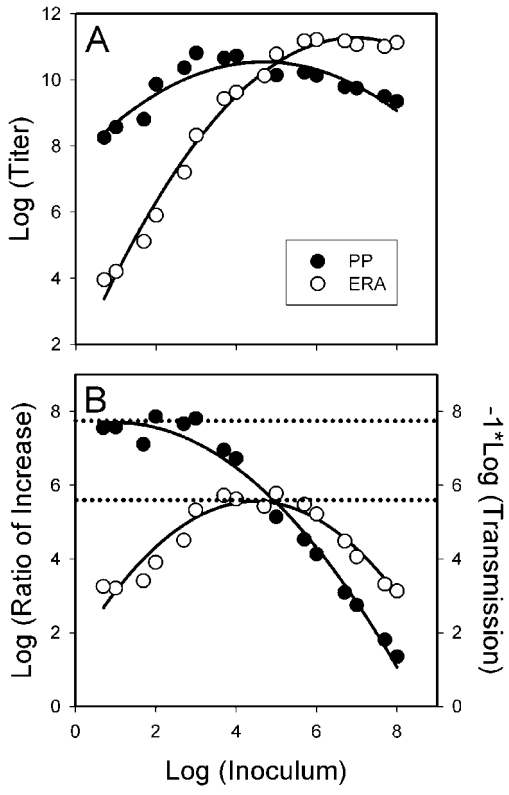

Figure 2: Log-log plots of population size and growth rate as functions of the estimated number of particles placed on a lawn of either the native (PP) or novel (ERA) host bacteria. *A*, Titer, an estimate of population size after 24 h of growth from an initial population size, is indicated on the *X*-axis. *B*, Growth rate of the populations plotted in *A* is indicated on the left axis, and the inverse log transmission rate is indicated on the right axis. As transmission rate decreases, inverse transmission rate increases. Horizontal lines indicate the minimum transmission rate for deterministic persistence of the phage growing on PP or ERA, where growth is equal to the inverse of transmission. For calculation of growth rate, see "Material and Methods." Curves are second-degree polynomial regressions through data from each host species. See "Results" for regression statistics.

Sustainability of Emerging Viruses in Heterogeneous Environments

It is widely recognized that environmental heterogeneity, whether spatial or temporal, has implications for population size and sustainability. We ran serial passage experiments using alternating hosts to mimic a coarsely het-

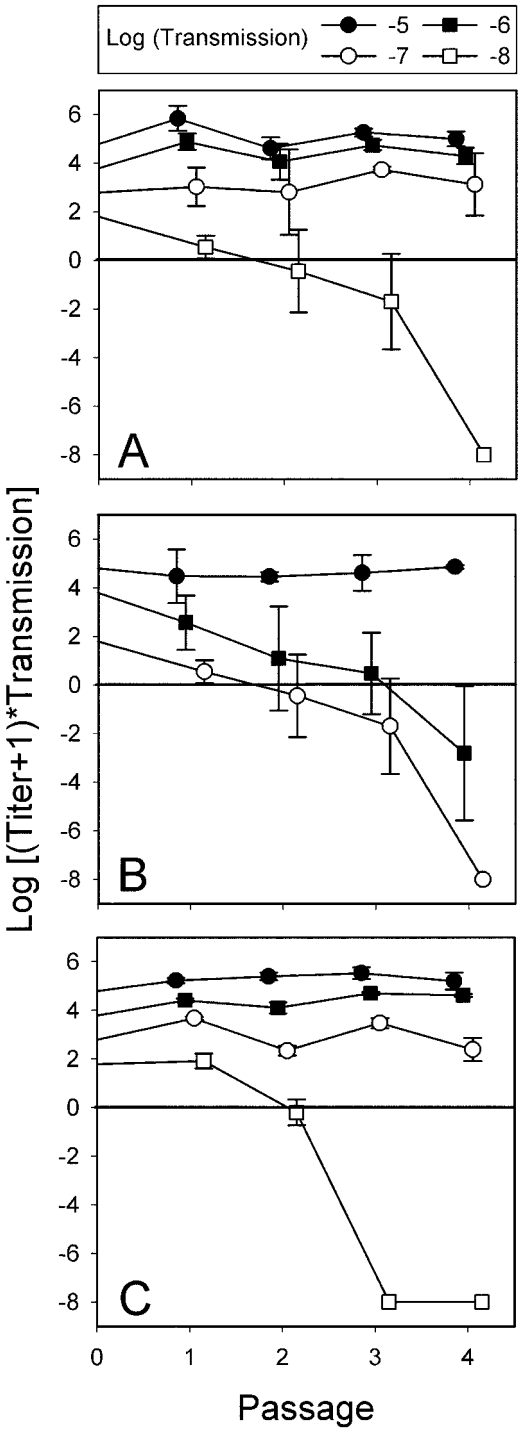

Figure 3: Population sizes after each cycle of growth and transmission in serial passage experiments in three host regimes. *A*, Populations passaged on PP at transmission rates from 10^{-8} to 10^{-5}; *B*, populations passaged on ERA at transmission rates from 10^{-7} to 10^{-5}; *C*, populations passaged on alternating hosts, beginning with PP, at transmission rates from 10^{-8} to 10^{-5}. Points are offset for clarity. Error bars are ± 1 SD.

erogeneous environment; all replicates began with growth on PP, followed by alternating host passages. We imposed transmission rate treatments from 10^{-8} to 10^{-5}. Population dynamics on alternating hosts were qualitatively similar to those on PP alone. Figure 4*c* shows log absolute fitness predicted by iterating equations (2*a*) and (2*b*) through four simulated passages of alternating host types. Absolute fitness measured experimentally for transmission rates from 10^{-7} to 10^{-5} on alternating hosts is also summarized in figure 4*c*. While the model predicted net population decline with 10^{-7} transmission, the observed log absolute fitness at 10^{-7} transmission was -0.10 ± 0.12, a value indistinct from replacement ($t = 1.4$, df $= 2$, $P = .29$). Two-way analysis found a significant interaction of host regime and transmission rate ($F = 4.7$, df $= 4, 18$, $P = .009$), attributable entirely to the phage's performance on ERA at low transmission. Log fitness did not differ between the PP and alternating-host treatments at any transmission rate (Tukey's HSD; $\alpha = 0.05$). We concluded that the phage can persist in an alternating environment as long as PP is consistently present as a source host.

Discussion

A clear analysis of the conditions for disease emergence into novel hosts has profound implications for the ecology, evolution, and population biology of viruses, their hosts, and the communities in which these organisms reside (Morse 1995; Daszak et al. 2000; Moya et al. 2004). For example, emerging RNA viruses of humans, such as the influenza A virus, human immunodeficiency virus 1, and the SARS coronavirus, have caused major and minor epidemics during the past century. The increasing importance of emerging viruses for human, agricultural, and wild populations of hosts has stimulated a pressing need to better understand the ecological dynamics underlying emergence processes (Daszak et al. 2000).

Viral emergence may sometimes occur through multiple phases. Typically, virus fitness is lower on a novel host than on a native host, a consequence of a lack of viral adaptation to the novel host. The pathogen must experience a new host and maintain a population in it before any subsequent adaptation may occur (Morse and Schluederberg 1990).

Using the bacteriophage Φ6*h* as a biological model, we examined the early phase of viral emergence, the establishment of a persistent population on a novel host. Phage Φ6*h* is a mutant that infects *Pseudomonas phaseolicola* (i.e., PP, the native host) but has expanded its host range to include a novel host, *Pseudomonas pseudoalcaligenes* (i.e., ERA). Although phage Φ6*h* is not ideally adapted to the typical host PP, it is expected to be more productive on PP than on the novel host ERA (Turner and Chao 1998).

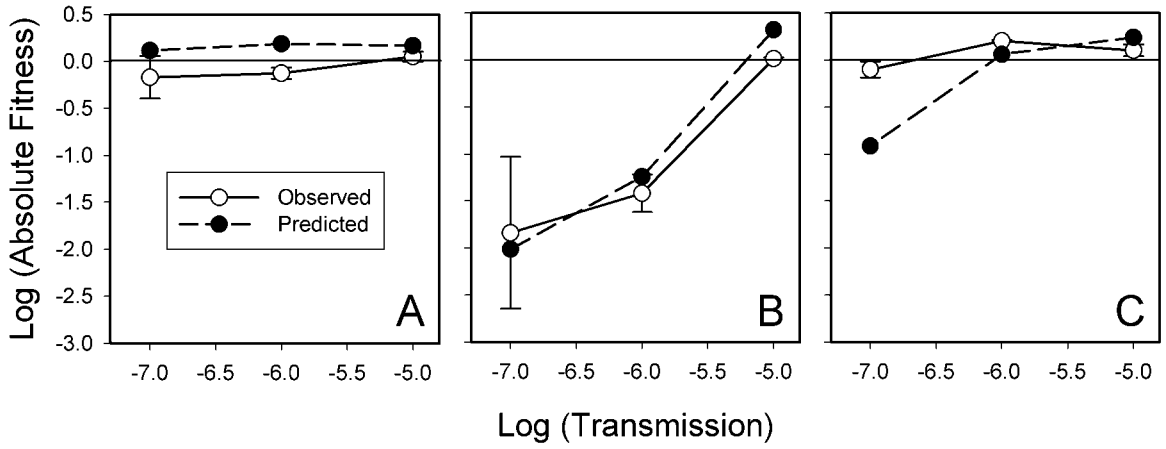

Figure 4: Observed and predicted absolute fitness as a function of transmission rate in three host regimes: PP (*A*), ERA (*B*), and alternating hosts, beginning with PP (*C*). Fitness was calculated from serial passage data presented in figure 2 (see "Material and Methods"). Predictions were generated by iterating equation (2) through four simulated passages. Points are offset for clarity. Error bars are ± 1 SE.

Our experiments confirmed this expectation; 24-h plaques on PP contained an order of magnitude more viral particles than plaques on ERA. This may be due to a difference in cellular attachment rate and/or burst size (progeny produced per infected cell) for the virus between the two hosts (Dennehy and Turner 2004).

Regardless of the underlying mechanism, this discrepancy in productivity was amplified in our growth-rate assays. Growth rate was density dependent, and peak productivity occurred at different phage densities on each host (fig. 2). While phage populations were able to increase quickly from low initial density on the native host, low initial density on the novel host yielded low growth rates. Low maximum growth rate, combined with a region of positive density dependence ranging over initial inoculum sizes from five individuals to 10^5 individuals, makes a host shift by Φ6*h* unlikely at low transmission rates.

Several mechanisms may explain the region of positive density dependence on the novel host (fig. 2*b*). Localized dispersal on a plate may limit the virus's access to suitable hosts (Holt 2000) if host cells differ in suitability. This spatial contribution to demographic stochasticity, as well as chance events after infection, can closely resemble the results of Allee effects (Lande 1998). Alternatively, there could be a real biological Allee effect (Allee et al. 1949) at work at low densities, where viruses benefit each other in some way. There are many idiosyncratic mechanisms that can lead to Allee effects (Courchamp et al. 1999; Stephens et al. 1999; Holt et al. 2004). For instance, if host cells can mount defenses against viral infection and these defenses can be saturated with sufficiently high viral attack rates, then there will be positive density dependence in viral population growth.

Positive density dependence may also result from the influence of population size on the appearance of rare mutants capable of improved growth on the novel host. Rather than being monomorphic, as RNA phage populations grow large, they may contain a considerable variety of genotypes as a consequence of the high error rate associated with RNA replication (Domingo et al. 1998). Thus, assuming that phages with improved growth characteristics can arise spontaneously in the population, the positive density dependence may result from the increased likelihood that these rare mutants will appear in large populations. However, we note that only four virus generations occurred during the experiments that showed positive density dependence, suggesting that these mutational effects would need to be of large magnitude to affect the average behavior of the phage population. Further work will be required to distinguish among the effects of demographic stochasticity, genetic heterogeneity, and an intrinsic Allee effect in generating the growth pattern we observed.

The increased productivity (plaque yield) offered by the native host comes at a population-wide cost. A bacterial lawn is essentially a renewable resource for the phage, and figure 2 clearly illustrates the effect of rapid host exploitation when Φ6*h* infects a lawn of PP. Maximum titer is achieved at a fairly low inoculum size. We speculate that PP host cells are lysed so quickly that large inocula of phage deplete the host population early in its course of geometric growth. The 24-h incubation period of a daily passage allows for roughly four virus generations to occur

when Φ6*h* forms distinct plaques (i.e., initial inoculum less than 1,000 viruses) on a bacterial lawn (Turner and Chao 1998), but fewer generations are expected when the phage is grown under crowded conditions (i.e., more than 1,000 viruses per plate) because phage can exhaust the available hosts in the lawn before peak growth can be realized. By this logic, the lower growth rate afforded by ERA explains its relatively higher titer and growth rate at large inoculum sizes (fig. 2b). The observed trade-off between growth rate when rare and apparent carrying capacity is consistent with general predator-prey theory. The resource for the virus "predator" in this system is a living population and therefore can be overexploited.

The growth profiles of Φ6*h* on the two hosts have profound implications for Φ6*h* as it negotiates the early and late phases of emergence. The maintenance of Φ6*h* on native and novel hosts depends on the interaction of within-host growth rates and between-host transmission (Rand et al. 1995). Our experimentally derived estimates of density-dependent growth on PP and ERA (fig. 2*b*) predicted the lower limit of transmission for the persistence of Φ6*h* when it experienced its two hosts homogeneously. The virus was able to persist at lower transmission rates on PP than on ERA (fig. 3*a*, 3*b*; fig. 4*a*, 4*b*). On PP, phage growth was able to compensate for transmission rates as low as 10^{-7}. In contrast, on ERA, phage growth could only compensate for a transmission rate of 10^{-5}. The difference in the extinction threshold on the two hosts means that there is a range of transmission rates over which PP is a source and ERA is a sink (Holt 1985; Pulliam 1988). Once created, these experimental source-sink systems can be used to address a wide range of ecological and evolutionary questions.

The implications of source-sink dynamics for viral emergence will depend on a pathogen's pattern of exposure to a given host. Broadly speaking, a pathogen may experience a novel host in one of two ways: homogeneously or heterogeneously. In the homogeneous case, a population of the pathogen initially comes into contact with the novel host and is henceforth isolated from the native host; sink dynamics on the novel host, particularly when the host is of low quality, so that the initial absolute fitness of the pathogen is well below 1, are likely to cause pathogen extinction before adaptation can occur (Morse and Schluederberg 1990; Holt and Gaines 1992; Gomulkiewicz and Holt 1995; Antia et al. 2003).

In the heterogeneous case, the pathogen experiences both hosts from time to time, or from place to place. The heterogeneity of host experience can be coarse grained or fine grained in space, time, or both, and such heterogeneity may facilitate initial persistence on the novel host. Our study tested an extreme case of coarse-grained temporal heterogeneity in which the phage experienced alternating host environments from one passage to the next, a case of 100% dispersal (migration) between source and sink. This situation is analogous to a pathogen population challenged with shifts in the seasonal or annual availability of hosts, such as an agricultural pest that infects rotated crops. We found that intermittent exposure to the native host could "rescue" (Brown and Kodric-Brown 1977) emerging phage populations in the novel host in a critical range of transmission rates. In a future study, we will address the effects of other classes of heterogeneous host use, such as the coarse-grained pattern of spatiotemporal variation expected in a metacommunity of spatially and temporally segregated hosts, or the fine-grained variation expected in well-mixed host communities.

The persistence of the virus with alternating host exposure suggests that a temporally heterogeneous host regime may provide emerging viruses time to adapt to novel hosts and facilitate such a shift via effects on viral population size, but is host expansion or a host shift likely to result? The literature on evolution in heterogeneous environments is large, and we will not address it in depth here because our focus is on the initial ecological challenges preceding adaptation to a novel host. However, it is interesting to contemplate that pathogens may be sustained on novel hosts despite low fitness, permitting new genes and gene combinations adapted to the new host to arise by mutation and recombination. (Because Φ6 is a segmented RNA virus, genetic exchange is achieved by segment reassortment [Turner and Chao 1998].) The fate of new genes or gene combinations may depend on the magnitude of their effect (Kawecki 2000). Holt and Gomulkiewicz (1997; see also Gomulkiewicz et al. 1999) suggested that adaptation cannot occur in a sink until a new mutant (or linked combination of mutants) arises that is fully capable of persisting on the novel host without immigration from the native host. This stringent "absolute fitness criterion" is unlikely to be met when the transmission rate is low and the novel host is a strong sink. However, the stringency of conditions permitting adaptation assumes a negative correlation of mutant fitness on its two hosts and also assumes that there is a largely unidirectional flow of immigrants from source to sink, with little or no backflow. Our research (S. Duffy and P. E. Turner, unpublished data) indicates that alternating host exposure can select for mutations that improve growth rate of Φ6 viruses on both the native and novel hosts. The possibility of positively correlated responses to selection makes emergence much more likely (Gandon 2004). Likewise, bidirectional transmission between hosts may in some situations increase the probability of adaptation to the novel host (Kawecki and Holt 2002).

Perhaps the least surprising result of our study is also the most fundamental to the process of emergence. As

shown in figure 4, transmission rate is such a significant component of viral fitness that a virus can emerge on novel hosts even when poorly adapted, if the transmission rate is high enough. The logical consequence is that emergence of a directly transmissible virus will occur more frequently at increasing host-population densities, including those containing admixtures of native and novel hosts. With a sufficient transmission rate, emergence requires only cross-host transmission; further adaptation is not necessary. It is thus important to consider the evolution of the transmission rates themselves, which might arise from the evolution of a novel vector.

The frequency and mode of transmission might also influence the evolution of host use by a pathogen. For instance, if the interval between successful transmission events increases, a lower growth rate of the pathogen (analogous to lower virulence; Turner and Chao 1998; Dennehy and Turner 2004) might afford a higher harmonic mean population size, reducing the risk of pathogen extinction (sensu Andre and Hochberg 2005). Therefore, the spatial or temporal availability of hosts may exert selection for reduced pathogen virulence, thus prolonging the window of opportunity for a host range expansion (sensu Claessen and de Roos 1995; Rand et al. 1995; Pugliese 2002; Gandon 2004). However, the paradox of within-host competition that produces the familiar "tragedy of the commons" (Hardin 1968) by favoring short-term gains may forestall a reduction in virulence on a productive native host, resulting in pathogen extinction before a host shift can take place.

We end by noting that our experimental observations and our model predictions differed in only one respect. The model underestimates the fitness (and therefore sustainability) of phage in alternating environments at low transmission rates (10^{-7}; fig. 4*c*). This underestimate may simply be a result of the simple and rigid form of the model, but it is more likely that it has a biological cause. When Φ6 progeny exit the cell, they incorporate certain host-membrane lipids into the viral capsid (Mindich 1988). Thus, one possibility is that PP-derived host lipids enhance the ability of virus particles to infect ERA (e.g., by elevating virus attachment rate), relative to that of phages that transmit directly between ERA cells. (Bacterial restriction-modification systems are classically shown to similarly affect growth of DNA phages [Luria and Human 1952; Bertani and Weigle 1953]; however, these systems are not shown to operate on RNA phages.) In preliminary follow-up experiments, we observed an increase of an order of magnitude in the phage's growth rate on ERA if it was derived from PP host cells rather than ERA, an increase sufficient to explain the disparity between our model predictions and our experimental results. This "legacy" effect occurred in every replicate of a 24-h assay, implying that it is indeed analogous to a maternal effect and not evidence of adaptation (Mousseau and Fox 1998). More research is necessary to adequately describe the mechanism.

This experimental demonstration of a host legacy effect between hosts has important implications. The capacity of a virus to propagate on a novel host is apparently conditional on the recent experience of preceding generations. This is intrinsically interesting, suggesting a kind of complexity in viral population dynamics that has not been widely regarded. Additionally, given this host legacy effect, the total viral population size experiencing the novel environment is greater than would otherwise be expected. Because the amount of genetic variation that can be exposed to selection via novel mutations should scale with population size, this provides a more fertile ground for adaptive evolution to the new host.

The overarching goal of emerging-disease research is to resolve which ecological factors are most important in fostering emergence. The hope is that such data would aid our power to predict which host populations are most at risk for infection by emerging viruses in the future (Moya et al. 2004). We have shown that the persistence of a bacteriophage in a homogenous environment can be predicted from measurements of its growth and transmission rates. However, these measurements do not prove as useful for cases of more complex patterns of host exposure, which are likely to occur during emergence. Our unexpected demonstration of a putative "maternal" effect in this RNA phage implies that ecological and evolutionary dynamics can depend on emergent properties of the community, even in the very simple community provided by our one-predator, two-prey laboratory experimental system.

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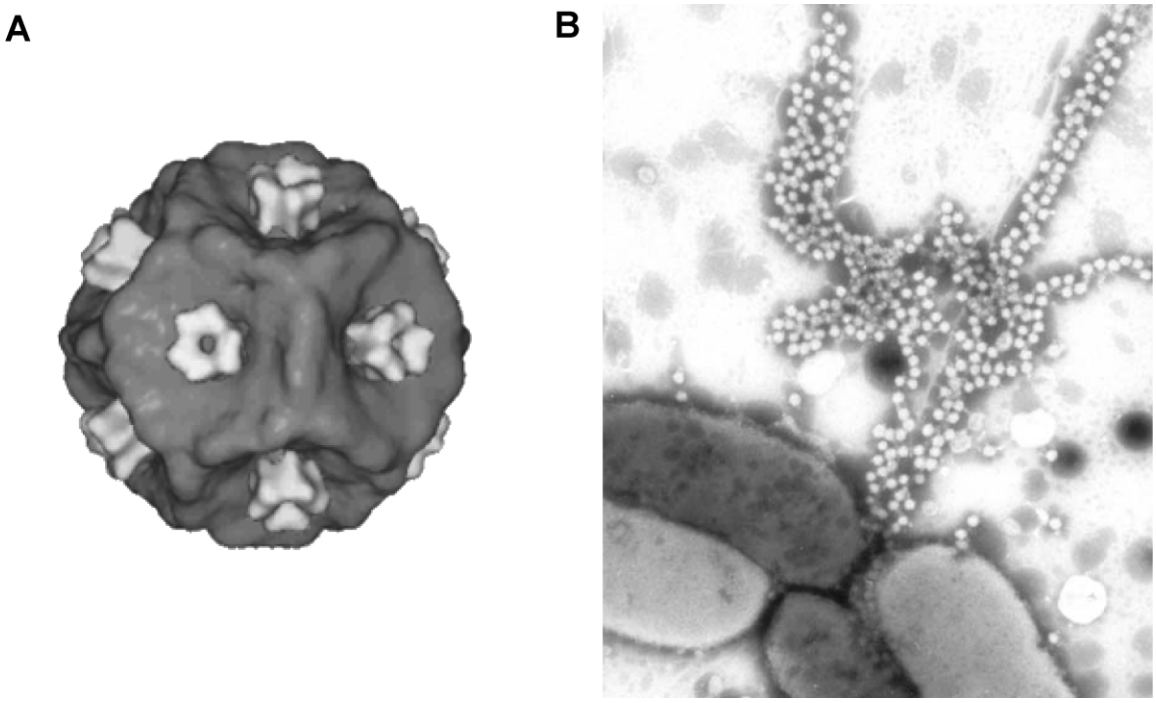

A, Cartoon image of the phage Φ6 procapsid; once filled with the viral RNA genome and coated with protein P8, it becomes the infectious nucleocapsid. *B*, Phage Φ6 particles attached to the pilus of their host bacteria. Photos courtesy of Dr. Dennis Bamford, University of Helsinki.