

MIGRATION ENHANCES ADAPTATION IN BACTERIOPHAGE POPULATIONS EVOLVING IN ECOLOGICAL SINKS

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Migration between populations can be a major evolutionary force. However, some disagreement exists as to precisely how migration affects population adaptation. Some theories emphasize the inhibitory effects of gene flow between locally adapted populations, whereas others propose that migration can enhance adaptation. Migration has also been theorized to rescue sink populations from extinction. In our experiments, we serially passaged bacteriophage $\Phi 6$ host range mutants under sink conditions on a novel host while manipulating the source and number of migrants into these experimental populations. Migrants from two sources were used: mutant $\Phi 6$ phage able to infect a novel host (treatment) and wild-type $\Phi 6$ phage unable to infect a novel host (control). We used quadratic regressions to determine the relationship between the number of migrants per passage and the absolute fitnesses of experimental populations following 30 passages. Our results showed that migration from a control population had no effect on absolute fitnesses of our serially passaged populations following 30 passages. By contrast, the relationship between migrants per passage and absolute fitnesses for populations receiving migrants able to infect the novel host was best described by an upwardly concave curve. These results suggest that intermediate levels of migration can have favorable impacts on evolutionary adaptation.

KEY WORDS: Emergence, experimental evolution, gene flow, immigration, local adaptation, source-sink.

The movement of individuals from one population to another has long been cited as an important evolutionary force (Slatkin 1985, 1987), but the consequences for the receiving population can be difficult to ascertain. Some treatments emphasize that gene flow can be inhibitory factor in organismal adaptation (Mayr 1963; Ehrlich and Raven 1969; Antonovics 1976; Lenormand 2002; Kawecki and Ebert 2004; Bolnick and Nosil 2007; Bridle and Vines 2007; Yeaman and Guillaume 2009). Environmental variation creates ecologically heterogeneous landscapes across species' ranges, and alleles favored in one habitat may not be

favored in others. The movement of individuals between populations tends to introduce less-fit alleles to local populations, thus swamping adaptation. Experimental evidence for the inhibitory effect of migration (sometimes termed migration load) comes from diverse species, including fish (Bolnick et al. 2008; Huff et al. 2011), plants (Etterson et al. 2007), insects (Peer and Taborsky 2005), and nematodes (Dolgin et al. 2007).

By contrast, some theories maintain that migration can enhance adaptation. For example, Wright's "shifting balance theory" implies that migration can liberate populations trapped on



relatively low adaptive peaks, allowing populations to access higher peaks on an adaptive landscape (Wright 1932). Other theory asserts that migration can enhance fitness, particularly in situations where per capita growth rates show positive density-dependence (Gomulkiewicz et al. 1999; Holt et al. 2004). Here, migration affects local adaptation in at least two ways. First, migration increases local population density, which can lead to greater population growth rates because of the fitness-enhancing effects of increased density. For example, population growth rates in some sexually breeding species are improved in denser populations due to greater access to mates (Courchamp et al. 1999). A greater local population growth rate means more individuals are recruited to the population, which implies that the probability of novel beneficial mutations arising within the population is higher. This effect may be especially important in sink populations (i.e., populations with $R_0 < 1$) because the indigenous supply of beneficial mutations may be small and declining (Holt and Gomulkiewicz 1997; Gomulkiewicz et al. 1999; Holt et al. 2003, 2004). Second, migration can directly contribute genetic diversity to a population. More individuals dispersing into a population means increased probabilities of beneficial mutations entering the population, thus providing more genetic variation for natural selection to act upon.

In fact, migration itself may introduce beneficial genetic variation to evolving populations despite disruptive selection (Holt et al. 2004; Dennehy et al. 2010). That is, although a source population may occupy a habitat experiencing a widely different selection regime, it can still contribute alleles advantageous to populations persisting in another habitat. In fact, even alleles that are neutral or deleterious in the source population can be favorable in the receiving population. Migration can also assist adaptation in changing environments, such as when hosts and parasites are engaged in coevolutionary arms races (Gandon 2002). Ultimately, migration may permit sufficient adaptation that the receiving populations are able to escape sink conditions.

Migration and gene flow, therefore, may have both negative and positive effects on local adaptation (Kawecki 2008). The movement of individuals into a population may increase genetic diversity and introduce beneficial mutations, but too much migration swamps the receiving population with genotypes reflective of the source population, thus limiting the response to selection in the receiving population. This hypothesis suggests that there exists an optimal intermediate migration rate (Lythgoe 1997; Gomulkiewicz et al. 1999; Tufto 2000; Lopez et al. 2009).

Several studies have experimentally explored the role of migration on adaptation in controlled laboratory settings. The results of such experimental evolution studies support the idea that migration can enhance adaptation (Miralles et al. 1999; Morgan et al. 2007; Perron et al. 2007; Venail et al. 2008; Bell and

Gonzalez 2011). Here, dispersal among laboratory cultures of microbes were shown to significantly affect evolutionary adaptation relative to controls. Often the greatest positive impact on adaptation occurs at intermediate rates of migration. These laboratory studies are supported by field experimental studies using, for example, plants (Leinonen et al. 2011), amphibians (Fitzpatrick and Shaffer 2007), and rotifers (Tortajada et al. 2010).

We used laboratory populations of mutant $\Phi 6$ bacteriophage ($\Phi 6h$ phage) able to infect a novel host, *Pseudomonas pseudoalcaligenes* East River isolate strain A (hereafter ERA), to test the hypothesis that migration can enhance adaptive evolution among phage populations in sink habitats. As a corollary to this hypothesis, we inquired whether intermediate levels of migration lead to greater evolutionary adaptation than lower or higher levels of migration. In our experiments, we serially passaged a mutant $\Phi 6$ phage able to infect the ordinarily nonpermissive host *P. pseudoalcaligenes* ERA. In these experiments, the bottleneck between passages was greater than the basic reproductive rate (i.e., sink conditions, $R_0 < 1$) (Dennehy et al. 2006). To prevent population extinction, migrants from frozen, ancestral stocks were added at each passage. These migrants were derived from two sources: $\Phi 6$ phages able to infect the novel host (our experimental treatment, $\Phi 6h$ phages) and $\Phi 6$ phages unable to infect the novel host (control, wild-type $\Phi 6$ phages). A third treatment, where no migrant phages were added to sink populations, was used as an additional control. We expected that the populations in the latter two treatments would go extinct because of a lack of recruitment. Although we did not explicitly measure migration rate, we used simulations to produce crude theoretical estimates of the ratio of locals/migrants at each passage. These simulations are included as Online Supporting Information.

Material and Methods

STUDY ORGANISMS AND CULTURE CONDITIONS

Bacteriophage $\Phi 6$ (family Cystoviridae) is a dsRNA virus whose genome is divided into Small (2948 bp), Medium (4061 bp), and Large (6374 bp) segments (Mindich 2006). Like most RNA viruses, these phages experience exceptionally high mutation rates (Drake and Holland 1999). Coupled with extremely large population sizes and rapid generation times, adaptation can occur rapidly (Dennehy et al. 2010). However, unlike most organisms, these phages are not believed to undergo homologous recombination (Mindich 1996, 2004). Rather coinfection of the same host by two or more phages can lead the shuffling of parental genomic segments among progeny phages, a phenomenon termed reassortment (O'Keefe et al. 2010). Coinfection depends on the ratio of phage to host (termed multiplicity of infection or MOI). When phage significantly outnumbers hosts (i.e., $MOI > 2$), host

cells are commonly infected by multiple phage. These features may make host and phage population density important for the evolution of these phages.

Phage $\Phi 6$'s standard laboratory host is the plant pathogen, *P. syringae* pathovar *phaseolicola* (ATCC # 21781) (Vidaver et al. 1973). Phage $\Phi 6h$ (strain PT590, kindly provided by Paul Turner, Yale University, New Haven, CT) is a previously described spontaneous mutant of wild-type $\Phi 6$ (Turner and Chao 1998). This strain differs from the wild type due to a nonsynonymous substitution (E8K) in the gene for host attachment protein P3 (Duffy et al. 2006). This mutation allows the virus to infect the novel host species used in our experiments: *P. pseudoalcaligenes* ERA, kindly provided by Leonard Mindich, Public Health Research Institute, Newark, NJ). $\Phi 6h$'s productivity on ERA is approximately an order of magnitude less than its productivity on the standard host, *P. phaseolicola* (Dennehy et al. 2006).

All phages and bacteria were propagated using LB medium (10 g NaCl, 10 g Bacto™ tryptone, and 5 g Bacto™ yeast extract per liter of water) at pH 7. ERA cultures were initiated by transferring a single colony from a streak plate into 10 mL LB medium in a sterile 50-mL flask capped with a 20-mL beaker. Culture flasks were incubated with shaking (220 rpm) at 25°C for 18 h, allowing bacteria to attain stationary-phase density ($\sim 6 \times 10^9$ cells/mL).

High-titer lysates of phage $\Phi 6h$ were prepared by adding 1 μ l frozen stock and 20 μ l of stationary-phase ERA to 3 mL top agar (LB medium with 0.7% Bacto™ agar; stored as liquid at 45°C, solidifies at 25°C), and pouring onto 35 mL bottom agar (1.5% Bacto™ agar) in a sterile Petri dish. After 24 h, the resulting plaques were harvested and resuspended in 4 mL of LB medium, followed by 10-min centrifugation at 3000 rpm (RCF = 1.4) to pellet agar and bacterial debris. Bacteria-free lysates were obtained by filtering the supernatant through a 0.22- μ m filter (Durapore®; Millipore, Bedford, MA). Phage particles per milliliter in the lysates were quantified via serial dilution and titering. Plaques were counted on plates where 30–500 plaques were visible. The number of plaque-forming units per mL (pfu/mL) in the original lysate was obtained by multiplying the number of plaques times the dilution factor. Lysates were stored at –20°C in a 1:1 glycerol/LB (v/v) solution. Similar procedures were used to obtain stock isolates of $\Phi 6$, except that 200 μ l *P. phaseolicola* was substituted for ERA.

EXPERIMENTAL PROCEDURE

A total of 10^4 $\Phi 6h$ phages, 1.2×10^8 ERA cells, and 10 mL LB medium were added to each of 33 sterile 50-mL flasks capped with a 20-mL beaker. After approximately 18-h incubation at 25°C on a rotary shaker (220 rpm), 3 mL from each culture was transferred into 15-mL centrifuge tubes and spun at 3000 rpm for 10 min. The resulting supernatants were passed through 0.22- μ m filters

to obtain bacteria-free lysates. These supernatants were diluted 10,000-fold (10^{-4}) in LB medium and 100 μ l was added to 10 mL fresh media (total dilution = 10^{-6}). Passaging of this sort has been shown to establish sink populations whereby extinction occurs within four passages unless the populations are rescued by migration (Dennehy et al. 2006). To these populations, 10^2 , 10^3 , 10^4 , 10^5 , or 10^6 phages were added from frozen ancestral stock populations of $\Phi 6h$ or $\Phi 6$, where each migration level was replicated three times. Except for the occasional mutant, $\Phi 6$ is unable to infect ERA, so these migrants were intended as controls for the experimental treatments receiving ancestral $\Phi 6h$ migrants. Initially we expected these populations to go extinct, but we were able to recover phage from these populations at the end of the experiment. We consider possible reasons for this result in the discussion. The remaining three flasks received no migrants to confirm that populations were indeed experiencing sink conditions. These populations were expected to go extinct.

Each of the 33 experimental flasks was maintained under their respective treatment conditions for 30 serial passages. As a compromise to increase replication and number of treatment levels, we did not explicitly measure total population size following each passage. Our justification for this stems from previous data for the same phage/host pair, which showed that the average population densities of populations undergoing serial passaging were remarkably stable (Results: Sustainability of Emerging Viruses on the Native and Novel Hosts, and Fig. 3 in Dennehy et al. 2006). We expected the addition of migrants to allow positive population growth in the populations receiving migrants. Moreover, these previous data allowed us to estimate total number of phage progeny following each passage for each population. From these estimates, we used mathematical simulations to estimate ratio of local to migrant phages added to fresh media and hosts for each treatment, iterated over 30 passages. These theoretical analyses are provided as Online Supporting Information. One salient result of our theoretical simulations was that treatments with more migrants do not necessarily have higher local/migrant ratios because of the complex interplay between density-dependent growth, dilution, and migration. We address this finding in greater detail in the Discussion and in the Online Supporting Information.

ABSOLUTE FITNESS ASSAYS

Following 30 passages of experimental evolution, purified phage lysates from each replicate were obtained by filtering and plating 1–1000 μ l from the final flask on ERA lawns. Some treatments (e.g., populations receiving migrants from $\Phi 6$ stock) produced very few plaques, thus multiple rounds of plating were required to recover the populations. Purified lysates were recovered from these plates as described above, and were titered to enumerate total phages per milliliter. To assay absolute fitness, 10^4 phages were added to 1.2×10^8 ERA cells in 10 mL LB medium and allowed

to grow for 18 h. Resulting phage progeny was then isolated and titered to determine total number of phages per milliliter. This number was multiplied by 10 to give the total number of progeny produced (N_f) in the 10 mL of LB medium. Absolute fitness was calculated as $W_{abs} = \log_{10}(N_f/N_i)$ where initial inocula, N_i , was 10^4 . The absolute fitness of each experimental population was measured three times, whereas that of the ancestor was measured six times.

Results

We determined the effects of migration from two different sources ($\Phi 6h$ stock able to infect ERA and $\Phi 6$ stock unable to infect ERA) on the adaptation of $\Phi 6h$ populations to ERA by regressing mean absolute fitness for each population against the \log_{10} number of migrants per passage. Prior to our experiments, we had expected populations receiving migrants from frozen $\Phi 6$ stock would go extinct because these migrants are not able to infect the host ERA and the dilution rate on passaging exceeded $\Phi 6h$'s growth rate. Contrary to our expectations, all of these populations survived. We pose possible explanations for these results in the Discussion section.

For both treatments, the data were best fit by second-order quadratic regressions. Migration from $\Phi 6$ stock had that no effect on the absolute fitnesses of the receiving populations after 30 passages (Fig. 1; $r^2 = 0.269$, $F = 2.211$, $df = 2$, $P = 0.1522$). Here, the quadratic regression failed to explain the variation in absolute fitness. By contrast, the quadratic regression fit for the populations receiving migrants from $\Phi 6h$ was best described by an upwardly concave curve (Fig. 1; $r^2 = 0.817$, $F = 26.726$, $df = 2$, $P < 0.0001$). These populations showed increasing absolute fitnesses with increasing numbers of migrants per passage until approximately 10^4 – 10^5 migrants per passage. Absolute fitnesses thereafter declined.

We independently compared absolute fitnesses at each level of migration for populations receiving migrants from $\Phi 6h$ stock with that of the ancestor using unpaired t -tests. All populations, except one, showed absolute fitnesses significantly greater than that of the ancestor (P -values < 0.01); the absolute fitnesses of populations receiving 10^6 migrants per passage were not significantly different than that of the $\Phi 6h$ ancestor (t -test; $df = 14$, t -value = 1.267, $P = 0.2260$).

From our absolute fitness measurements at the end of the experiments, we calculated whether the populations had “emerged” (i.e., achieved positive population growth despite 1,000,000-fold dilution between passages) using the equation $\log_{10} W_{em} = \log_{10}(N_f/N_i) - \log_{10}(10^6)$. Essentially this is \log_{10} of the ratio of progeny viruses to initial inoculum minus the \log_{10} of dilution rate imposed during serial passaging. By this definition, the populations receiving 10^4 and 10^5 migrants per passage successfully

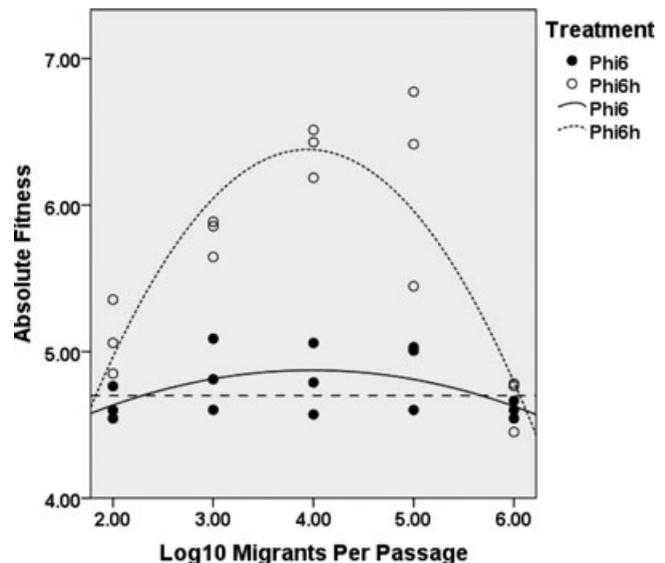


Figure 1. The absolute fitnesses of populations of $\Phi 6h$ phages following 30 passages on the novel host, *Pseudomonas pseudocaligenes* ERA, are plotted against the \log_{10} of the number of migrants per passage. Populations receiving migrants from $\Phi 6h$ stock are signified with open circles, whereas populations receiving migrants from $\Phi 6$ stock are depicted with solid circles. Each point is a pooled fitness estimate ($n = 3$) for that particular population. Data for both treatments are fitted with second-order polynomial regressions. The absolute fitness of ancestral $\Phi 6h$ on ERA is given by the dashed line.

emerged over the duration of the experiment. Following 30 passages, they showed per capita population growth rates $W_{em} > 1$ (data not shown). Thus, these evolved populations would be sustainable under our experimental conditions even if migration into the population ceased. This finding suggests that migration may be an important factor in the emergence of infectious viruses by permitting epidemic spread in the new host population.

Although we did not explicitly measure migration rate (i.e., ratio of locals/migrants at each passage) during the experiment, we include some crude theoretical simulations of these dynamics as Online Supporting Information. Although preliminary, these analyses reveal some interesting aspects of the relationship between migration rate and fitness among adapting populations. One interesting result of our analyses was that the \log_{10} ratio of local to migrant phages at each passage quickly tends to equilibrium, which supports our contention that, under source conditions, the population dynamics of host and phage tend toward an equilibrium. Although we did not explicitly demonstrate this, we presume that the populations receiving migrants experienced source conditions as evidenced by their survival until the end of the experiment.

Because these ratios were estimated at each serial passage, and because some of the treatment's first few initial ratios were

highly divergent from the rest (see Fig. S1), we calculated the harmonic mean of the ratios for each treatment. According to our simulations, the harmonic mean of the ratios of locals to migrants was 1.12:1 in the 10^2 migration treatment; 1.25:1 in the 10^3 migration treatment; 1.61:1 in the 10^4 migration treatment; 1.50:1 in the 10^5 migration treatment; and 1.15:1 in the 10^6 migration treatment. These estimates conform to our observations that fitness increased the most in the intermediate migration treatments. It is also clear that treatments with more migrants do not necessarily have higher local/migrant ratios because of a complex interplay between density-dependent growth, dilution, and migration. It should be noted that our theoretical simulations do not take into account adaptation by phage populations. We attempt to simulate the effects of adaptation in our simulations in the Online Supporting Information.

Discussion

The movement of individuals from one population to another is expected to have an inhibitive effect on adaptation in the receiving population because of trade-offs due to spatial heterogeneity (Antonovics 1976; Kirkpatrick and Barton 1997; Ronce and Kirkpatrick 2001; Lenormand 2002; Kawecki and Ebert 2004; Bolnick and Nosil 2007; Bridle and Vines 2007; Kawecki 2008; Yeaman and Guillaume 2009). However, theory by Holt and colleagues suggests that migrants, under certain circumstances, can also enhance adaptive evolution in receiving populations (Holt and Gomulkiewicz 1997; Gomulkiewicz et al. 1999; Holt et al. 2004). Other perspectives emphasize that migration and gene flow can have both positive and negative effects on local adaptation (Lythgoe 1997; Tufto 2000; Kawecki 2008; Lopez et al. 2009).

Empirical support for the positive influence of migration on evolutionary adaptation is available from serial passage experiments using microbes. For example, studies of *P. aeruginosa* adapting to antibiotic selection show that increasing the rate of migration increased the rate of resistance evolution and decreased the associated costs of resistance (Perron et al. 2007). Other studies using vesicular stomatitis virus reported a positive correlation between the migration rate and the magnitude of the mean fitness reached by the virus (Miralles et al. 1999). Studies on the effect of migration into coevolving populations of *P. fluorescens* and phage SBW25 Φ 2 reported significant associations between migration rate and evolutionary adaptation for both host and parasite (Brockhurst et al. 2007; Morgan et al. 2007). These latter studies observed that intermediate migration rates led to increased sympatric resistance of bacteria to phage infection.

Our results support the conclusions of the cited studies, and show that intermediate levels of migration resulted in the greatest fitness increases. In our experiments, serially passaged popula-

tions receiving migrants from an ancestral Φ 6h stock population showed significant increases in absolute fitness at every level of migration except the greatest. Where the number of migrants at each passage equaled 10^6 individuals, population absolute fitnesses on the novel host ERA were not significantly different from that of the ancestral Φ 6h population (see Fig. 1). We speculate that, in this treatment, the newly arrived migrants dominated the receiving population, thus hampered adaptive evolution in those lineages, an effect commonly called gene swamping (Lenormand 2002). The result of this extreme gene flow was that the sink population phenotypically, and likely genetically, closely resembled the source.

However, at migration rates of 10^2 – 10^5 individuals per passage, a strong positive relationship was observed between number of migrants and absolute fitness following 30 passages (Fig. 1). We hypothesize that two main effects, one genetic and one demographic, made such adaptive evolution possible. We were unable to distinguish between these two potential effects with our experiments.

Migration may have carried genetic variation into the evolving population. Although it is true that the ancestral source population did not change over the course of the experiment, it was genetically heterogeneous from the outset. The initial sample from this population (10^4 individuals) was likely small compared to the typical population size of a dsRNA bacteriophage. Repeated sampling over time is likely to carry the source's full genetic diversity into the sink, especially at the higher migration rates.

Because of epistasis, the fitness effects of any given gene depends on its genetic background (Weinreich et al. 2005). This fact leaves open the possibility that alleles in the source population, which were initially deleterious or neutral in the sink at the outset of the experiment, became advantageous after the population fixed one or more beneficial mutations. An example of such an effect comes from the resistance of *Escherichia coli* to the β -lactam antibiotic cefotaxime. Although a suite of five mutations together increase cefotaxime resistance approximately 100,000-fold, some combinations of these mutations are actually deleterious relative to the wild type (Weinreich et al. 2006).

An open question is how Φ 6 populations would have assimilated this genetic diversity. Φ 6 phages are somewhat unusual in that their genomes contain three segments of double-stranded RNA and are not believed to undergo homologous recombination (Mindich 1996, 2004). However, on coinfection, progeny phage containing segments from multiple "parents" can arise, a phenomenon called reassortment. Although coinfection is presumed to be rare among our experimental conditions because the MOI was usually well below 2. Nonetheless, over the course of phage growth in the experimental medium, the MOI will change as phage reproduces and hosts are killed off. In addition, these phages may

experience enhanced rates of coinfection (Turner et al. 1999). It is likely that our phage populations experienced at least occasional reassortment, and perhaps, although rare, these events had a large impact on evolutionary adaptation.

Coinfection and reassortment relates to our idea that genetic background can affect the direction of epistasis, and can influence the assimilation of genetic diversity into a population. Perhaps greater numbers of migrants would lead to more coinfection, which could result in greater numbers of haplotypes in these populations. If these populations are more genetically diverse than are the populations receiving fewer migrants, there may be more situations where some alleles disfavored in some backgrounds would be advantageous in others. Having more backgrounds and combinations of alleles to sample from would increase the probabilities of finding good combinations, leading to faster adaptation.

In addition, a known feature of this experimental system is that $\Phi 6h$ experiences positive density-dependent population growth at low densities (Dennehy et al. 2006). One hypothesis to explain this finding is that host entry by the virus is facilitated by attachment by more than one phage to the host. This contention is supported by evidence from studies showing that $\Phi 6$ attaches to infected cells significantly faster than to uninfected cells (Joseph et al. 2009). The presumed mechanism is upregulation of the $\Phi 6$ receptor, the host pilus, by infecting phages. It may be that pilus attachment by one phage causes pilus extension and increased opportunity for binding by other phages. Thus, by inflating initial population densities, migrants also increased the ability of phage to infect hosts. This phenomenon may enhance per capita population growth rates with concomitant effects on population genetic diversity. Increased population genetic diversity should be correlated with increased rates of evolutionary adaptation.

Positive density-dependent growth, also known as the Allee effect, was cited by Holt and colleagues as being a primary mechanism in promoting migration-enhanced evolutionary adaptation (Gomulkiewicz et al. 1999; Holt et al. 2004). An influx of migrants into a sink population may increase per capita growth rates, thus allowing the population to better access and fix beneficial mutations. These factors can act together in that migration increases population growth, which can lead to increased genetic diversity. With increased genetic diversity comes increased likelihoods of finding and fixing beneficial mutations, which increases fitness and leads to even greater population growth. The end result is that the population may eventually be able to sustain itself in the absence of migration, thus escaping the population sink, as we observed in the present experiments. This phenomenon has considerable relevance to the emergence of infectious diseases because the reduced fitness commonly experienced on new hosts may entail sink conditions (Dennehy 2009). If migration from pathogens persisting on original “source” hosts is sufficiently

large, it may lead to the emerging pathogen adapting to the novel host despite differential selection imposed by the two host types (Dennehy et al. 2010).

An alternate perspective is that negative density-dependence was the dominant factor in these experiments. As shown in Dennehy et al. 2006, under some conditions, increasing initial inocula leads to decreased overall productivity because bacterial hosts are depleted before they have an opportunity to reproduce. Overall, there may be fewer net progeny because there are fewer total number of host infections. In the context of the present experiments, a larger number of migrants may in fact depress population growth rates, resulting in lower net population sizes (and less genetic diversity) in the high-migration treatment. Additionally, negative density-dependence may explain why fitness has such a large effect on population size when the number of migrants is low as shown in our theoretical simulations in the Online Supporting Information.

It may be the case that migration rate (or the ratio of locals to migrants) is actually minimized in the intermediate migration treatments. This conclusion is supported by our theoretical simulations in the Online Supporting Information. In contrast to the quadratic regression fit of fitness against migrants per passage, which shows an upwardly concave curve, the harmonic means of the ratios of local to migrants at each migration treatment shows a downwardly concave curve when plotted against fitness achieved by the population after 30 passages. That is, the highest fitness populations experienced the highest ratios of locals to migrants, whereas the lowest fitness populations experienced the lowest ratios of locals to migrants. Perhaps the intermediate migration treatments actually minimize gene swamping in these experimental populations.

Remarkably, none of the populations receiving migrants went extinct, but all those not receiving migrants did. One would expect that some of the populations receiving migrants from ancestral $\Phi 6$ stock would have gone extinct because $\Phi 6$ is unable to infect ERA. Theoretically, these populations should have been almost indistinguishable from populations receiving no migrants.

Several possibilities may explain the observed results. First, $\Phi 6$ readily mutates to gain the ability to productively infect ERA. The $\Phi 6$ mutation rate has been conservatively reported to be on the order of 2.7×10^{-6} per locus per generation, but this may be an underestimate (Chao et al. 2002; Burch et al. 2007; Ferris et al. 2007). At least a dozen distinct single amino acid substitutions confer $\Phi 6$ the ability to infect ERA (Duffy et al. 2006; J. J. Dennehy, unpubl. data). The total equilibrium frequency of host range mutations in a source population growing on *P. phaseolicola* was estimated to be 3×10^{-4} (Ferris et al. 2007). Our own observations suggest that the total equilibrium frequency of host range mutations was between 1×10^{-4} and 1×10^{-5} , which

would seemingly preclude a jackpot effect (discussed below). As a consequence, considerable numbers of phages from a typical population possess the requisite mutation(s) to form plaques on a lawn of ERA (J. J. Dennehy, unpubl. data). Although these numbers may not seem relevant in the context of the migration scheme imposed on the experimental treatments (e.g., the highest level [10^6] of migration is expected to contain between 10 and 100 mutant phages able to infect ERA at each passage), it is noted that these numbers represent phages able to infect ERA and reproduce prolifically enough so as to make visible plaques. This standard may not account for mutant phages able to induce increased density-dependent growth, despite not being able to productively infect ERA. Perhaps substantial numbers of these mutants exist in $\Phi 6$ populations, but they are not recognized because they do not make visible plaques on ERA. These phages may be sufficient to sustain sink populations, but are unable to contribute population genetic diversity, therefore little adaptation is observed.

A second possibility is that our $\Phi 6$ stock has a greater than expected number of host range mutants due to a jackpot effect (Luria and Delbruck 1943). That is, by chance, the source plates from which the $\Phi 6$ stock was isolated contained greater numbers of plaques formed by host range mutants than would be expected from the equilibrium frequency. Following our experiments, we tested our $\Phi 6$ stock lysate for plating ability on ERA, but did not see a greater than expected number of plaques (data not shown). However, we note that we were only looking for the presence of visible plaques on lawns of ERA. Further experiments may shed light on these unexpected results.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Log₁₀ local to migrant phages over 30 passages.

Supporting Information may be found in the online version of this article.

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