Evolutionary rescue can be impeded by temporary environmental amelioration

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39 **Abstract**

40 Rapid evolutionary adaptation has the potential to rescue from extinction populations
41 experiencing environmental changes. Little is known, however, about the impact of
42 short-term environmental fluctuations during long-term environmental deterioration,
43 an intrinsic property of realistic environmental changes. Temporary environmental
44 amelioration arising from such fluctuations could either facilitate evolutionary rescue
45 by allowing population recovery (a positive demographic effect) or impede it by
46 relaxing selection for beneficial mutations required for future survival (a negative
47 population genetic effect). We address this uncertainty in an experiment with
48 populations of a bacteriophage virus that evolved under deteriorating conditions
49 (gradually increasing temperature). Periodic environmental amelioration (short
50 periods of reduced temperature) caused demographic recovery during the early phase
51 of the experiment, but ultimately reduced the frequency of evolutionary rescue.
52 These experimental results suggest that environmental fluctuations could reduce the
53 potential of evolutionary rescue.

54

55 **Keywords:** adaptation, demographic recovery, environmental change, evolutionary
56 rescue, experimental evolution, microcosm, virus, warming.

57

INTRODUCTION

A biological population in decline due to environmental deterioration can recover in abundance if genotypes tolerant of the environmental change increase in frequency and restore population growth sufficiently quickly. This phenomenon is known as evolutionary rescue, and it has the potential to lessen the loss of biodiversity due to environmental changes, including those associated with global climate change (Maynard Smith 1989; Gomulkiewicz & Holt 1995; Bell 2013; Osmond & de Mazancourt 2013; Bourne *et al.* 2014; Carlson *et al.* 2014). Evolutionary rescue depends on the relative rate of demographic decline and evolutionary adaptation. Therefore, more severe stresses and higher rates of environmental changes, which cause higher rates of population decline, can decrease the chance of rescue (Burger & Lynch 1995; Collins *et al.* 2007; Perron *et al.* 2008; Bell & Gonzalez 2009; Collins & de Meaux 2009; Bell & Gonzalez 2011; Lindsey *et al.* 2013). Conversely, larger population sizes and higher genetic variation can promote evolutionary rescue by allowing more time, and more efficient selection, for adaptation (Gomulkiewicz & Holt 1995; Lande & Shannon 1996; Willi *et al.* 2006; Orr & Unckless 2008; Bell & Gonzalez 2009; Samani & Bell 2010; Ramsayer *et al.* 2013). Experimental studies of these questions have all involved monotonic environmental deterioration.

A more realistic scenario of environmental deterioration would show both a directional trend and short-term fluctuations (Karl *et al.* 1995; McLaughlin *et al.* 2002; Bell & Collins 2008). The impact of short-term environmental fluctuations on evolutionary rescue is now poorly understood. When environment fluctuates over

time, periods of extremely harsh environmental conditions can cause dramatic decline in population sizes and likely reduce the chance of evolutionary rescue (Bell 2013; Lindsey *et al.* 2013). The effect of episodes of environmental amelioration is, however, unclear. Temporary environmental amelioration may promote evolutionary rescue by acting as a temporal refuge and allowing populations to recover in abundance, as this can lead to slower demographic decline and thus increase the probability to obtain beneficial mutations before extinction (Wahl *et al.* 2002; Samani & Bell 2010). However, such environmental amelioration may also lead to a relaxation of selection for tolerance of environmental stress and thus reduce the probability of fixation of the beneficial mutations required for adaptation (Uecker & Hermisson 2011; Peischl & Kirkpatrick 2012; Alto *et al.* 2013; Kirkpatrick & Peischl 2013). This may be particularly important when progressive adaptation via fixation of multiple beneficial mutations is needed for populations to survive increasing magnitudes of stress, where later steps of adaptation are contingent on the success of earlier ones (Martin *et al.* 2013). In such cases, the negative effect of environmental amelioration on evolutionary adaptation may amplify through time (Fig. S1). With these antagonistic effects, the net impact of temporary environmental amelioration on evolutionary rescue is not easy to predict, and merits careful examination.

Here we report an experimental evolution study that addresses this question. We use populations of a lytic bacteriophage infecting the bacterium *Pseudomonas fluorescens*. In a previous study, we showed that evolutionary adaptation could

prevent this phage from extinction when temperature was monotonically elevated to a stressful level (Zhang & Buckling 2011). In the present study, populations of the phage were experimentally evolved in gradually warming environments, with or without periodic phases of environmental amelioration (lowered temperature). We also examined the effect of population size, both to confirm that this system behaves as expected (greater chance of evolutionary rescue for larger populations) and to test for an interaction with environmental amelioration in determining the chance of evolutionary rescue. We hypothesize that an interaction may arise, as the positive effects of environmental amelioration are likely to be more important for smaller populations, for which the risk of extinction from demographic stochasticity is higher and evolutionary adaptation is limited by the supply of beneficial mutations to a greater extent (Willi *et al.* 2006; Samani & Bell 2010; Ramsayer *et al.* 2013).

METHODS

Strains and culture techniques

The bacteriophage virus SBW25Φ2 (Buckling & Rainey 2002) and its host bacterium *Pseudomonas fluorescens* SBW25 (Rainey & Bailey 1996) were used in this study. The bacterium grows well at temperatures below 33 °C. The phage fails to reproduce above 30 °C, but may gain tolerance to higher temperature through evolutionary adaptation (Zhang & Buckling 2011). Bacteria and phages were grown in microcosms of 1.5 mL of 0.1KB medium (M9 buffer solution supplemented with 1

124 g L^{-1} glycerol and 2 g L^{-1} proteose peptone no. 3) in 24-well microplates, unshaken in
125 temperature-controlled incubators.

126 Phage population densities were measured by plating phage dilutions onto soft
127 agar plates containing the ancestral bacterial cells and counting the number of plaque
128 forming units (PFUs) after 24 h culture at 29 ºC. Isolation of phages from cultures
129 were achieved by mixing 900 µL of culture with 100 µL of chloroform, which was
130 then vortexed to lyse the bacterial cells, and centrifuged at 13,000 rpm for 2 min to
131 pellet the bacteria debris, leaving a suspension of phage in the supernatant.

132

133 **Evolutionary rescue experiment**

134 Phage populations were allowed to evolve through 20 serial transfers. Each
135 microcosm was initially inoculated with ~10^7 isogenic stationary-phase bacterial cells
136 and ~10^5 isogenic phage particles. Every two days, the phage population from each
137 microcosm was isolated, a portion of which was transferred into a fresh microcosm
138 with ~10^7 ancestral bacteria cells. As the biotic environment, host bacteria, was held
139 in its ancestral state, phage population persistence would be mainly determined by its
140 adaptation to the physical environment (and not result from coevolutionary dynamics
141 with its host).

142 The experiment followed a split-plot design and considered two factors:
143 environment and population size. Phage populations evolved in a deteriorating
144 environment with: (i) no periodic amelioration, temperature increasing in a stepwise
145 fashion from 29 to 31.4 ºC, six steps of 0.4 ºC change, one step per three transfers;

146 and (ii) periodic amelioration, which differed from the 'no periodic amelioration'
147 treatment in that, within each 3-transfer step, the second transfer experienced 0.4 ºC
148 lower temperature (Fig. 1). After the six steps of temperature increase (transfer 19),
149 phage populations were evolved at 31.4 ºC for one more transfer. This ensured that
150 phage persistence at the end of the experiment reflected adaptation to the environment
151 (by ruling out the possibility of transient occurrence of maladapted populations due to
152 the time lag between environmental change and extinction). Four replicate
153 incubators were used for each environmental change regime. Twelve phage lines
154 were grown in each incubator, six with large bottleneck population sizes (1% of phage
155 cultures transferred to new microcosms at each transfer), and six with small
156 bottleneck population sizes (0.1% of phage cultures transferred). Phage population
157 density of each line was measured at every transfer.

158 In addition, two replicate incubators were used for a constant environment,
159 where temperature was kept at 29 ºC. All phage lines in the constant temperature
160 environment persisted throughout the experiment, with highly stable population
161 dynamics (Fig. S2). This confirms that extinction events observed in the
162 deteriorating (temperature-elevated) environments were due to a failure to
163 evolutionarily adapt to higher temperature rather than instability arising from the
164 general lab conditions.

165

166 **Data analysis**

167 Duration of persistence (time to extinction) of phage lines was analyzed using

parametric survival regression model (with the default Weibull distribution). The status of populations (extinct or persistent) at certain points in time was analyzed with generalized linear mixed model (GLMM), with binomial errors for the response variable. In these analyses, evolution environment (no amelioration or amelioration) and bottleneck population size (large or small) were included as fixed effects, and incubator ID as a random factor. We examined whether the periodic environmental amelioration could cause a demographic recovery by calculating the change in population size for each phage line at each transfer, as $D_t - D_{t-1}$, where $D_t = \log_{10}(\text{PFU mL}^{-1} + 1)$, representing the population density at transfer t. We used the software R for data analyses (R Core Team 2014).

RESULTS

Population persistence and demographic responses to periodic environmental amelioration

Over the selection experiment, 76 out of 96 phage lines went extinct. Environmental amelioration and small bottleneck population size reduced the duration of persistence by 29% (6.9 transfers) and 39% (9.2 transfers), respectively (Fig. 1; survival regression model, amelioration, $z = -2.99$, $P = 0.003$; bottleneck size, $z = -5.62$, $P < 0.001$, interaction, $z = 0.109$, $P = 0.307$).

During the early stage of the experiment, periodic amelioration led to increased population size in most of the phage lines under the amelioration treatment. The

190 average change in population sizes during amelioration at transfer 3, 6, 9 and 12 for
191 large-bottleneck lines, and at transfer 3 and 6 for small-bottleneck lines, was
192 significantly positive (one sample *t* test, $P < 0.05$; Figs 1 & 2). During later episodes
193 of environmental amelioration, however, only some phage lines showed recovery in
194 population size, while most continued to decline and the average of population size
195 change was not positive (Figs 1 & 2).
196
197 **The possibility of progressive adaptation**
198 Evolutionary rescue under continuously increasing temperature may involve
199 progressive (multiple-step) adaptation. To infer this possibility, we grew phage
200 populations that evolved tolerance to a mildly stressful temperature (30.2 ºC; from
201 transfer 10) in a more stressful environment (31 ºC); these populations did not show
202 positive growth (Supporting Information Methods). This suggests that adaptation to
203 temperature above 31 ºC would need accumulation of additional, and therefore at least
204 two, beneficial mutations.
205 The observed pattern in population persistence is consistent with our hypothesis
206 that the negative effect of periodic amelioration on evolutionary rescue should be
207 stronger when accumulation of multiple beneficial mutations is required for
208 adaptation in progressively deteriorating environments (see Introduction and Fig. S1).
209 The amelioration treatment showed a significantly negative effect on the chance of
210 population persistence at the end of the experiment when the environment was
211 severely stressful (transfer 20, and 31.4 ºC; 76 extinction events in total; GLMM,

amelioration, z = -2.86, P = 0.004; bottleneck size, z = -3.53, P < 0.001, interaction, z = -0.003, P = 0.997), but did not have an impact at the mid-point of the experiment when the environment was only mildly harsh (transfer 10, and 30.2 ºC; 26 extinction events in total; GLMM, amelioration, z = -0.007, P = 0.994; bottleneck size, z = -0.008, P = 0.994, interaction, z = 0.007, P = 0.994).

The possibility of reversal of selection for beneficial mutations by periodic environmental amelioration

The negative effect of periodic amelioration on the spread of beneficial mutations would be stronger if the beneficial mutations incurred fitness costs in the benign environment. In this case, the episodes of environmental amelioration would select against the beneficial mutations needed for adaptation to the future stressful environment. We assayed population growth profiles for certain phage lines, and found that the finally rescued phage populations, regardless of their evolution environments, showed no reduction in growth performance at lower temperature compared with control populations evolved in the temperature-constant environment (Supporting Information Methods; Figs S3 & S4). This suggests that phages carrying mutations conferring tolerance to high temperature suffered no appreciable fitness costs in terms of reduced growth performance at lower temperature. While selection for beneficial mutations may have been relaxed during the amelioration periods, it would not have been reversed.

DISCUSSION

A large body of research suggests that increasing environmental variability associated with global climate change often, although not always, leads to negative demographic consequences for populations in the long run (McLaughlin *et al.* 2002; Drake 2005; Burgmer & Hillebrand 2011). Studies of evolutionary rescue have, however, paid little attention to the impact of environmental fluctuations, although a realistic scenario of environmental deterioration must observe fluctuations on short-term time scales (Karl *et al.* 1995; Bell & Collins 2008). In an environment with a deteriorating trend, fluctuations may lead to both phases of highly stressful conditions and episodes of more benign environmental conditions. Phases of extremely harsh environmental conditions are akin to very severe environmental deterioration, which is likely to reduce the chance of evolutionary rescue (Bell 2013; Lindsey *et al.* 2013). Our study shows that periodic environmental amelioration can also limit evolutionary rescue. Taken together, these results imply that the potential for rapid evolutionary adaptation to mitigate biodiversity loss might be limited in the face of increased climate variability.

In our experiment, temporary environmental amelioration could cause demographic recovery during the early stage of the experiment (Fig. 2), but ultimately reduced the chance of evolutionary rescue in the late stage (Fig. 1). It is likely that evolutionary rescue in this experimental system was mainly limited by the fixation, not the appearance, of beneficial mutations. Relaxed selection, during the episodes of environmental amelioration, could have reduced the chance of fixation of the

beneficial mutations that were later required for population survival in future, more stressful, environment. Thus the ecological (demographic) benefits of periods of environmental amelioration were outweighed by the evolutionary costs, wherein the failure of evolutionary adaptation diminished any positive effect of environmental amelioration on demography in the late stage of the experiment. The fact that the environmental amelioration treatment did not interact with the bottleneck population size treatment in affecting population persistence (see Results) also suggests that the positive effect of amelioration on demography had little influence on ultimate evolutionary rescue. It could be argued that periodic environmental amelioration can function as an 'evolutionary trap' (Ferriere & Legendre 2013; Carlson *et al.* 2014), and populations 'falling into the trap' fail to adapt to the future environmental change. The extent to which our findings were contingent on a relatively rapid environmental change combined with modest fluctuations (relative to the rate of the directional change) is unclear. Moreover, our experimental design and study organism preclude the possibility to examine the relevance of other important ecological and evolutionary processes such as recombination and dispersal (Bell 2013; Bourne *et al.* 2014; Carlson *et al.* 2014). More research is therefore required to explore the generality of these findings under a wider range of conditions and in other organisms.

Evolutionary adaptation to changing environments has attracted much interest in population genetics in the past several decades, where adaptation has usually been studied in a sense of relative fitness increase (but see Burger & Lynch 1995; Orr & Unckless 2008). It has been often suggested that lower levels of temporal

278 autocorrelation in environmental conditions could retard adaptation, and the
279 inconsistency in selection over time has been a major explanation (Lande & Shannon
280 1996; Lenormand *et al.* 2009; Alto *et al.* 2013; Chevin 2013; Kirkpatrick & Peischl
281 2013; Kingsolver & Buckley 2015). Interestingly, our periodic amelioration
282 treatment led to reduced temporal autocorrelation compared with the 'no amelioration'
283 environment. The consistency of our results with the earlier population genetics
284 studies implies that our conclusion should be fairly robust.

285 There has been a rich literature on how source-sink dynamics affects population
286 adaptation and persistence in spatially heterogeneous environments. Immigration
287 from benign habitats may help maintain the persistence of populations in harsh
288 habitats through a demographic rescue effect, and meanwhile affect the evolutionary
289 adaptation in the sink environment, either positively by increasing the mutation
290 supply, or negatively by interfering with the fixation of beneficial mutations (Holt &
291 Gomulkiewicz 1997; Perron *et al.* 2007; Venail *et al.* 2008; Bell & Gonzalez 2011;
292 Bourne *et al.* 2014). Similar logic may apply to adaptation in temporally
293 heterogeneous environments, where phases of relatively benign environmental
294 conditions function as temporal refuges that promote population survival but
295 meanwhile may retard the fixation of mutations required for adaptation to harsh future
296 environments. Meanwhile, fundamental differences also exist. For instance, in a
297 spatially heterogeneous environment, a realistic level of migration might not be very
298 high and the negative effect of immigration on the fixation of beneficial mutations in
299 a sink environment should be rather weak. In a temporally heterogeneous

environment, however, all individuals experience both benign and stressful conditions and the negative effect of environmental amelioration on the fixation of mutations for adaptation to harsh conditions is inevitable. Now a major gap in our understanding of what conditions favour evolutionary rescue is the combined effects of spatial and temporal variability (Bell & Gonzalez 2011).

Population persistence in increasingly deteriorating environments may require progressive adaptation via accumulation of multiple beneficial mutations and the chance for such multiple-step adaptation might be more limited (Weinreich *et al.* 2006; Toprak *et al.* 2012; Bell 2013). This seems to be the case in our experiment. Assays of phage growth performance suggested that tolerance to the highly stressful environment required multiple beneficial mutations (Supporting Information Methods). If the relaxation of selection during amelioration periods can delay the fixation of beneficial mutations (Peischl & Kirkpatrick 2012), this effect may accumulate through time and impact the later steps of adaptation to a larger extent (Fig. S1). This is consistent with our observations: environmental amelioration treatment did not show a significant effect on population survival at the mid-point of the experiment when the environmental stress was relatively mild, but showed a negative effect at the end of the experiment when the environment became very harsh. Therefore it will be helpful to explicitly consider evolutionary paths in future studies of evolutionary rescue (Lindsey *et al*. 2013).

The effect of evolutionary rescue in mitigating biodiversity loss can be particularly relevant to microbes, of which we may observe adaptive evolution in real

322 time. Studies of the causes and consequences of microbial extinction might greatly
323 benefit from taking account of evolutionary processes. For instance, while the
324 impacts of agricultural intensification and land use changes on soil microbial diversity
325 have been recognized (Postma-Blaauw *et al.* 2010; Wagg *et al.* 2014), it is unclear
326 whether environmental variability arising from those anthropogenic activities could
327 have reduced the potential for evolutionary adaptation in the microbes. The concept
328 of evolutionary rescue is also highly relevant for pathogen eradication, where reduced
329 chance of evolution of resistance to chemotherapeutic agents is desirable (Lagator *et*
330 *al.* 2013; Lindsey *et al.* 2013; Ramsayer *et al.* 2013; Alexander *et al.* 2014; Wu *et al.*
331 2014). Interestingly, our finding here is consistent with an early suggestion for drug
332 use practice, that temporary low-dose treatments may slow the rate of drug resistance
333 evolution (Read et al. 2011; Kouyos et al. 2014; Wu et al. 2014). The findings
334 highlight the importance of considering both the ecological and population genetic
335 consequences of environmental fluctuations for predicting the likelihood of
336 evolutionary rescue.
337
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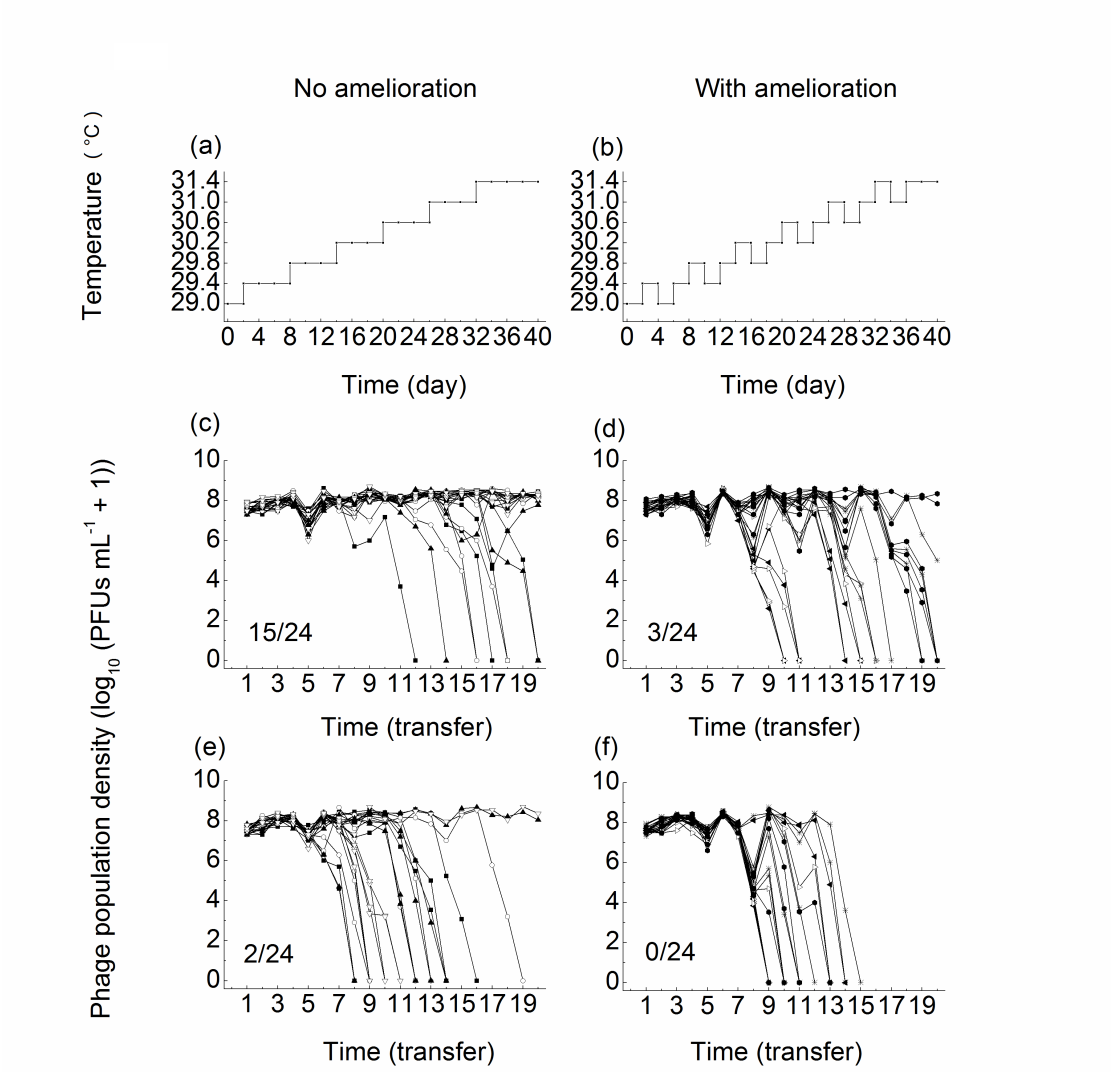
522 **Figure legend**



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524 **Figure 1** The trajectory of temperature change during the course of experiment (a-b),
525 and population density of each phage line through time under the large- (c-d) and
526 small-bottleneck size treatments (e-f). 'Day' was used as the *x*-axis for (a-b), and
527 'transfer' as the *x*-axis for (c-f). One transfer represents a period of two days; for
528 instance, transfer 1 is the period of day 0-2. Population density at one transfer
529 reflects the population growth performance during a 2-d period. Different symbols
530 in (c-f) indicate phage lines from different individual incubators. And the numbers

in panels indicate the frequency of phage lines that persisted throughout the experiment.

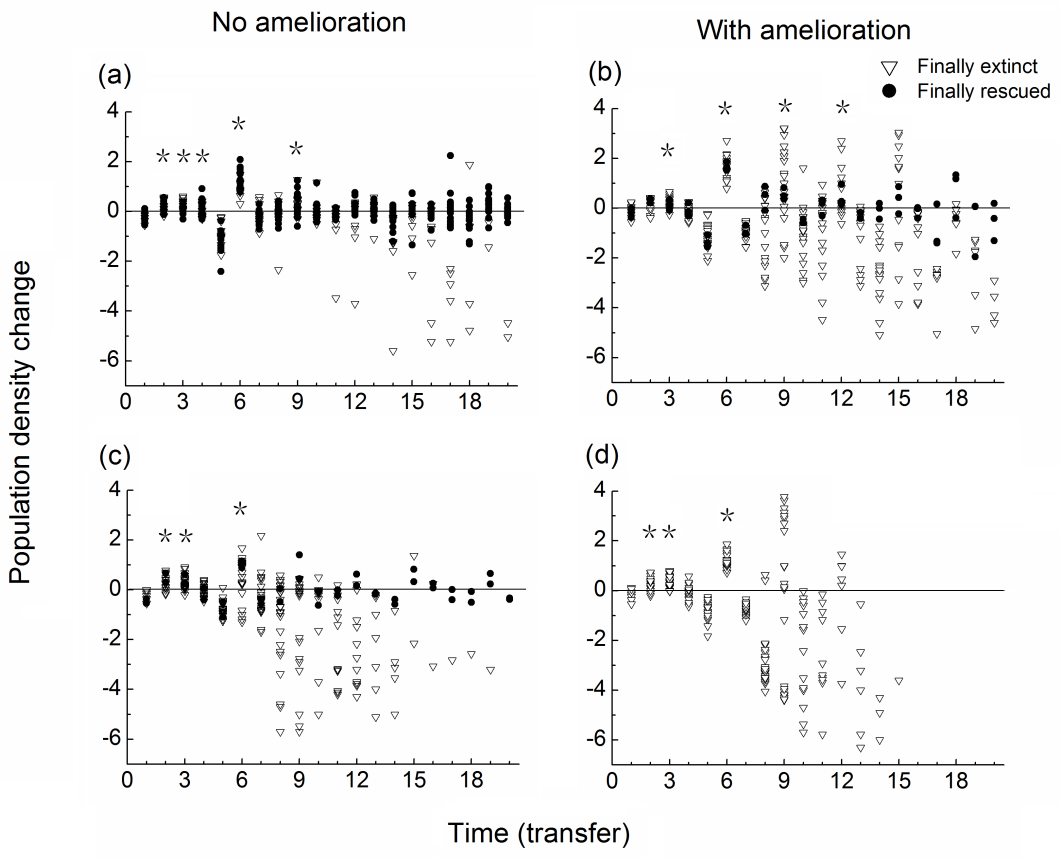


Figure 2 Change in population density at each transfer (D_t - D_{t-1}, where D_t = $\log_{10}$ (PFUs mL^{-1} +1), population density at transfer t) of each phage line through time under the large- (a-b) and small-bottleneck size treatments (c-d). Under the amelioration treatment, episodes of temporary temperature decrease occurred at transfer 3, 6, 9, 12, 15, and 18. Asterisks indicate significantly positive changes in average population densities (one-sample t test, $P < 0.05$).

Evolutionary rescue can be impeded by temporary environmental amelioration

Supporting Information

Methods

We examined whether progressive adaptation via accumulation of multiple beneficial mutations was needed for the persistence of our phage populations in the increasingly deteriorating environments. If phage populations that evolved tolerance to a mildly stressful temperature cannot grow at even higher temperature, it is suggested that multiple beneficial mutations would be required for further adaptation. Our ancestral phage can grow poorly at 29.8 ºC, and not above 30 ºC (Zhang & Buckling 2011), thus tolerance of temperature > 30 ºC would require at least one beneficial mutation. In our experiment, 45 out of 48 large-bottleneck phage populations survived at transfer 10 (30.2 ºC). Frozen stocks of these phage populations were reconditioned for 24 h at 30.2 ºC (grown with ~10^7 ancestral host bacteria cells); 1% of the phage extract was transferred to new microcosms with ~10^7 ancestral host bacteria cells, and grown for 48 h at 31 ºC. None of these phage lines showed positive growth at 31 ºC. This suggests that adaptation to temperature above 31 ºC need accumulation of at least two beneficial mutations.

We investigated whether adaptation to high temperature incurred fitness costs in terms of growth performance at lower temperature in our experimental system. Population growth profile at 29 ºC was assayed for the ancestral phage and eleven populations from transfer 20 (the end of the experiment), of which two were from the temperature-constant environment, three from the 'amelioration' treatment and six from the 'no amelioration' treatment. All of the eleven evolved populations were from large-bottleneck lines. Frozen stocks of these populations were first cultured for 24 h at 29 ºC; 1% of the phage extract was transferred to new microcosms with ~10^7 stationary-phase ancestral bacteria cells, and grown at 29 ºC. For each population, 30 replicate microcosms were initiated; three microcosms were randomly chosen at each of the following 10 points in time (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 8, and

48h), of which phage densities were measured. Mean density of the three replicates were used for assessing the population growth profiles. The log-transformed measurements were used to fit a three-phase linear model for microbial growth (Buchanan *et al.* 1997; Novak *et al.* 2006). The model assumes three distinct growth phases: lag phase with constant population density, log phase with exponentially increasing density, and stationary phase with constant density. Three parameters were obtained from this model: the length of lag phase, the maximum growth rate, and final yield. The goodness of fit of the model was very high: in all cases, the coefficient of determination was > 0.99. Note that this was an assay of population-level growth performance, but not an assay of 'one-step' growth curve. We examined phage populations, rather than individual phage isolates. There may be heterogeneity in growth performance within each population. Stationery-phase, but not exponential-phase, bacteria were used as host cells (consistent with our selection experiment), which may contain both active and less active cells and thus cause further asynchrony in growth within each phage population.

Figure S1 A graphical illustration of the hypothesis that temporary environmental amelioration during deterioration can slow the fixation of beneficial mutations. In asexual populations, as assumed here, favourable mutations need be established sequentially and thus later steps of adaptation are contingent on the success of earlier ones. Therefore the negative effect of environmental amelioration on the fixation of beneficial mutations may amplify through time. Note that panel (d) assumes a scenario of relaxed, but not reversed, selection for beneficial mutations during the amelioration phases.

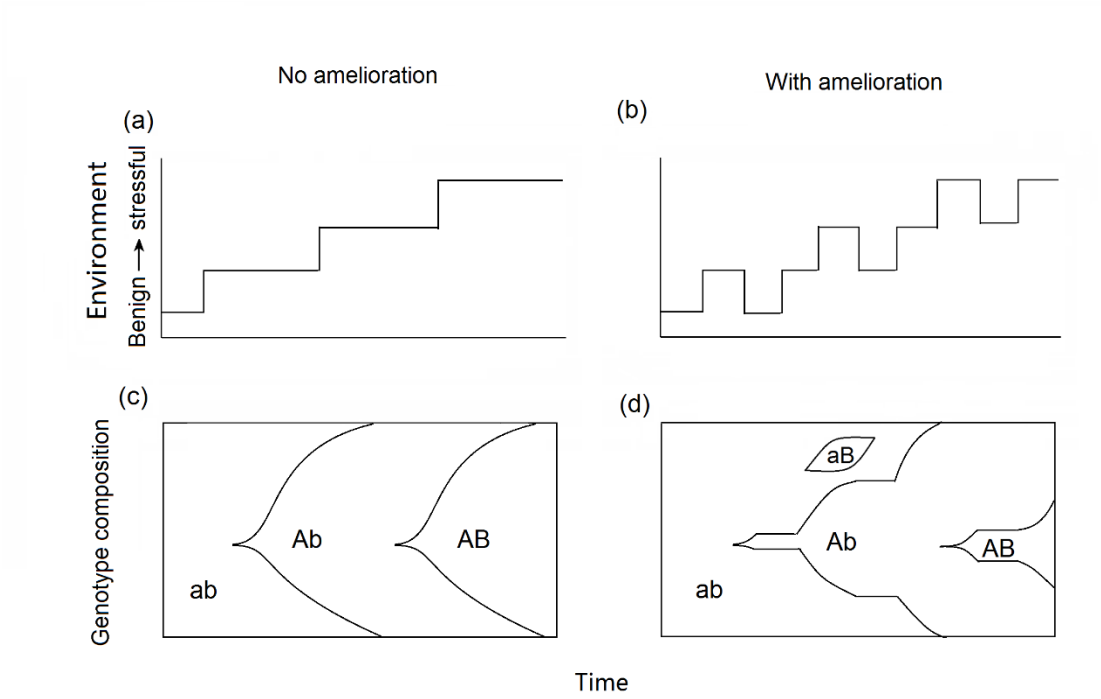
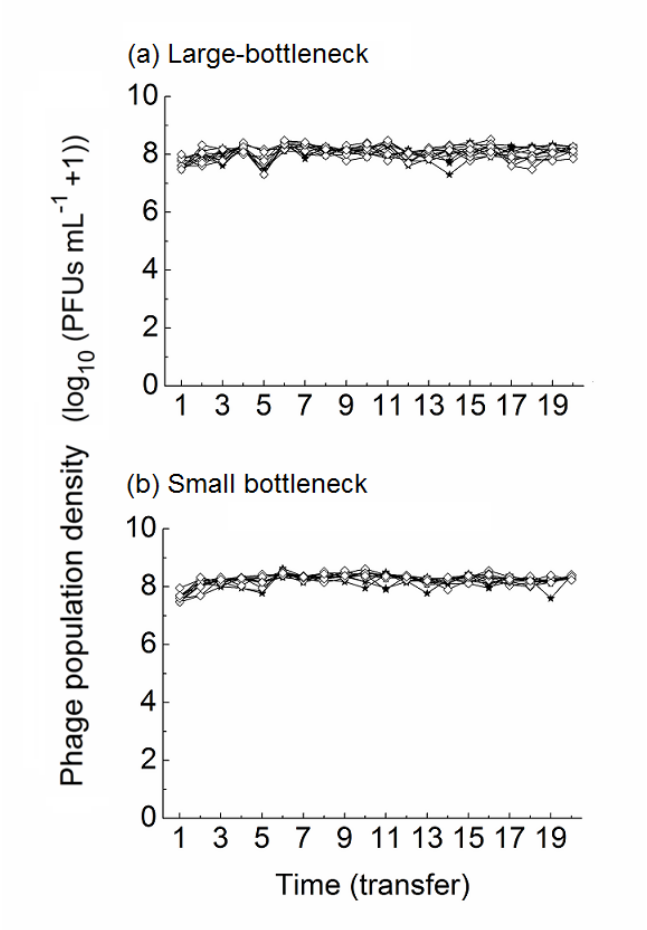


Figure S2 Population density of phage lines that evolved in the temperature-constant environment (29 °C). Two replicate incubators were used for this evolution environment; and within each incubator, twelve populations (six with large bottleneck population size and six with small bottleneck population size) were maintained. Different symbols indicate phage lines from different individual incubators.



609 **Figure S3** Growth profile at 29 ºC of the ancestral and eleven evolved populations,
610 either from the temperature-constant (control) or deteriorating environments. Each
611 line indicates an individual phage population.

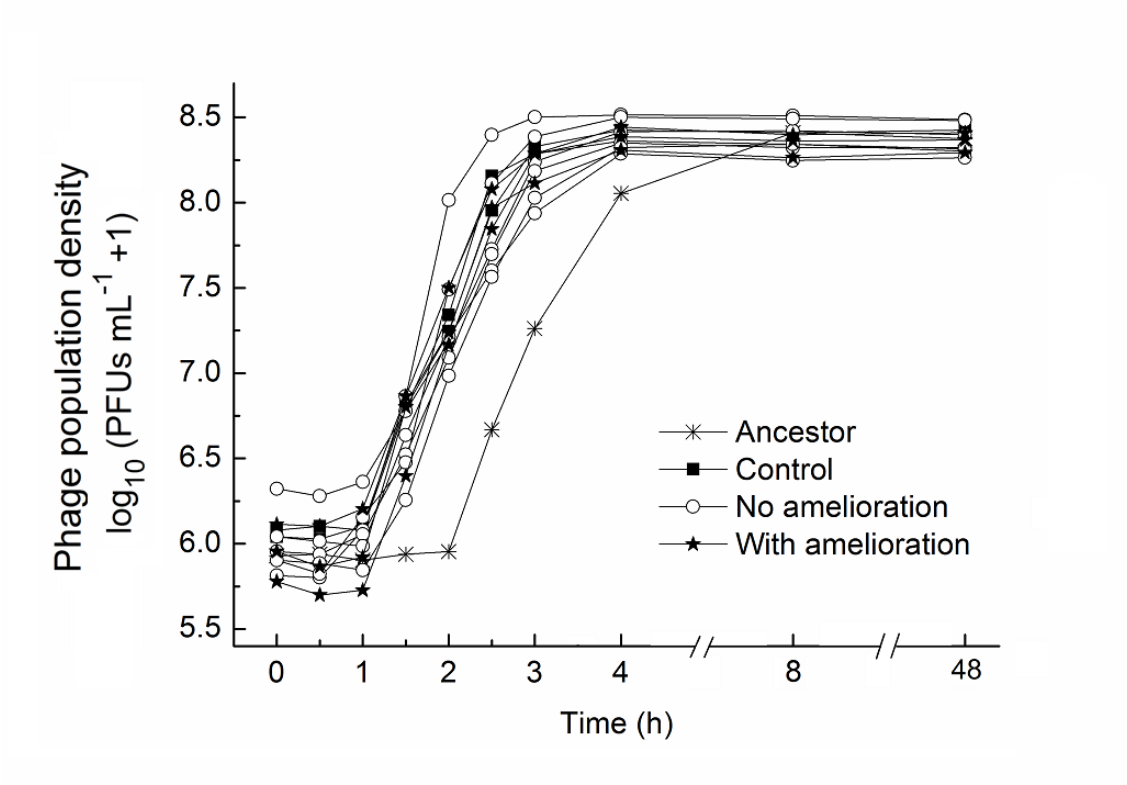


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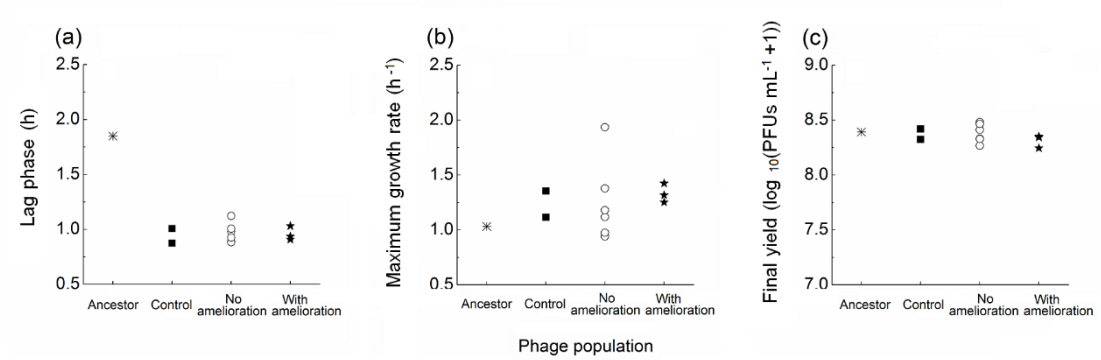
Figure S4 Phage population growth characteristics, length of lag phase (a), maximum growth rate (b), and final yield (c), of the ancestral phage and eleven evolved phage lines. There is no difference between the rescued lines from the deteriorating environments and those from the temperature-constant (control) environment in the growth characteristics (Mann-Whitney U-test, $P > 0.1$). This suggests tolerance to high temperature does not incur a cost in terms of growth performance at low temperature, and thus in our evolutionary rescue experiment selection for beneficial mutations was relaxed but not reversed during the amelioration periods. Note that the absence of such fitness costs in the finally rescued populations of the 'amelioration' environment may result from either (i) that the high temperature-tolerant mutations did not suffer a fitness costs, or (ii) that mutations with fitness costs existed but populations with such mutations went extinct due to the antagonistic selection arising from the environmental fluctuation. But only the first explanation is relevant to the 'no amelioration' evolution lines.



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