ECOLOGY LETTERS

Ecology Letters, (2011) **14**: 282–288 doi: 10.1111/j.1461-0248.2010.01586.x

LETTER

Antagonistic coevolution limits population persistence of a virus in a thermally deteriorating environment

Quan-Guo Zhang[1]* and Angus Buckling[2]

[1]State Key Laboratory of Earth Surface Processes and Resource Ecology; MOE Key Laboratory for Biodiversity Science and Ecological Engineering, Beijing Normal University, Beijing 100875, China

[2]Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK; Biosciences, University of Exeter, Cornwall Campus, Penryn TR10 9EZ, UK

**Correspondence: E-mail: zhangqg@bnu.edu.cn*

Abstract

Understanding the conditions under which rapid evolutionary adaptation can prevent population extinction in deteriorating environments (i.e. evolutionary rescue) is a crucial aim in the face of global climate change. Despite a rapidly growing body of work in this area, little attention has been paid to the importance of interspecific coevolutionary interactions. Antagonistic coevolution commonly observed between hosts and parasites is likely to retard evolutionary rescue because it often reduces population sizes, and results in the evolution of costly host defence and parasite counter-defence. We used experimental populations of a bacterium *Pseudomonas fluorescens* SBW25 and a bacteriophage virus (SBW25Φ2), to study how host–parasite coevolution impacts viral population persistence in the face of gradually increasing temperature, an environmental stress for the virus but not the bacterium. The virus persisted much longer when it evolved in the presence of an evolutionarily constant host genotype (i.e. in the absence of coevolution) than when the bacterium and virus coevolved. Further experiments suggest that both a reduction in population size and costly infectivity strategies contributed to viral extinction as a result of coevolution. The results highlight the importance of interspecific evolutionary interactions for the evolutionary responses of populations to global climate change.

Keywords

Adaptation, environmental change, evolutionary rescue, experimental evolution, temperature elevation.

Ecology Letters (2011) 14: 282–288

INTRODUCTION

Population persistence in changing environments is a central concern of evolutionary biology and ecology (Maynard Smith 1989; Bradshaw 1991); and recently global climate change has motivated much research to understand the conditions under which rapid adaptation may rescue declining populations in changing environments (Davis *et al.* 2005; Orr & Unckless 2008; Bell & Gonzalez 2009; Chevin *et al.* 2010). A population exposed to a novel or changing environment to which it is maladapted is likely to decline rapidly. However, the population may escape the extinction by 'evolutionary rescue', where genotypes resistant to the environmental change increase in frequency and restore population growth sufficiently quickly to counter the population decline due to the environmental change (Maynard Smith 1989; Gomulkiewicz & Holt 1995).

Much theoretical work has investigated the potential for adaptive evolution in response to deteriorating conditions, emphasizing population characters such as effective population size (Gomulkiewicz & Holt 1995; Willi *et al.* 2006) and the quantity of genetic variation present in the original population (Bradshaw 1991; Lynch & Lande 1993; Lande & Shannon 1996; Barrett & Schluter 2008) or introduced by mutations and immigration (Burger & Lynch 1995; Kawecki & Holt 2002; Orr & Unckless 2008). This has recently been complemented by experimental evolution studies with microbes (Collins & Bell 2004; Bell & Collins 2008; Bell & Gonzalez 2009; Collins 2011).

However, most work to date has focused on the evolutionary adaptation of single populations to changing abiotic environments, with evolutionary interactions between species receiving little attention (but see Collins 2011 for a recent empirical study on interactions within trophic levels). This is particularly surprising given that interacting species have long been considered as crucial components of a population's environment. Evolutionary interactions between species may fundamentally influence the potential for a species to adapt to changing physical environment through changes in effective population size (Buckling & Hodgson 2007; Meyer & Kassen 2007; Forde *et al.* 2008), phenotypic alteration (Thompson 2005; Miura *et al.* 2006), or even the rate of mutations that ultimately supply genetic variation (Pal *et al.* 2007).

Antagonistic coevolution between hosts and parasites (i.e. the reciprocal evolution of host defence and parasite counter-defence) is likely to be particularly relevant to evolutionary rescue because (1) host–parasite coevolution is common (Thompson 2005; Burdon & Thrall 2009) and occurs very rapidly (Woolhouse *et al.* 2002; Schulte *et al.* 2010); and (2) both evolutionary and ecological interactions between hosts and parasites can relatively easily result in population extinction of either or both species in the absence of abiotic environmental change (McCallum & Dobson 1995; Boots & Sasaki 2003). Moreover, we can make some general predictions about the impact of an antagonistically coevolving partner species on the evolutionary rescue of a focal population in deteriorating environments. First, counter adaptation of the enemy species is likely to reduce population size (Buckling & Hodgson 2007; Forde *et al.* 2008), and hence both the number of beneficial mutations that occur and the fixation probability of these mutations (Wahl *et al.* 2002). Second, adaption to the enemy species is often traded off with survival, growth or reproduction (Bergelson & Purrington 1996; Kraaijeveld *et al.* 2001; Buckling *et al.* 2006) and these costs can be greater in more stressful environments (Janmaat & Myers 2005; Lopez-Pascua & Buckling 2008; Alto & Turner 2010). Antagonistic coevolution is therefore likely to reduce the chances of evolutionary rescue in deteriorating abiotic environments.

We tested this hypothesis using the bacterium *Pseudomonas fluorescens* SBW25 and a naturally associated lytic DNA phage SBW25Φ2. The virus binds to susceptible cells, injects in its DNA, replicates and then lyses the host cells to release phage progeny. Previous work has demonstrated a persistent coevolutionary arms race between the bacterium and the virus, with reciprocal evolution of elevated bacterial resistance and virus infectivity (Buckling & Rainey 2002). It is feasible to separate bacteria and phages so that the evolution of one partner can be suspended by replacing with the ancestral type at regular intervals while the other partner evolves (Morgan & Buckling 2006; Paterson *et al.* 2010). We created a deteriorating environment for the virus by a stepwise increase in temperature and examined whether coevolution with host bacteria affects viral adaptation to the thermally changing environment. We chose to manipulate temperature because high temperature often constitutes a significant stress for viruses (probably due to the lower thermostability of their simple viral proteins) but not their hosts (e.g. Ulmanen *et al.* 1983). We created a gradually, rather than abruptly, degrading thermal environment to maximize biological reality (e.g. Klironomos *et al.* 2005) as much as possible within a test tube.

METHODS

Strains and culture conditions

The bacterium *P. fluorescens* SBW25 (Rainey & Bailey 1996) and the bacteriophage virus SBW25Φ2 (Buckling & Rainey 2002) were used. Bacteria and phages were grown in 5 mL of M9 salt solution supplemented with 1 g L^{-1} glycerol and 2 g L^{-1} proteose peptone no. 3 (i.e. 0.1 times the standard KB nutrient), in 25 mL universal glass tubes with loose lids (microcosms), cultured in static incubators. Growth rate and stationary density of the bacterium do not change significantly with increasing temperature within the range of 28–33 °C (and the bacterium fails to grow near 35 °C). The phage can grow well below 29 °C but fails to reproduce above 30 °C: the increase of phage density (the ratio of final density to initial density) within a 2-day culture is only 1.9 (± 0.56 SE) at 30 °C and 1.1 (± 0.28 SE) at 31 °C, after a 2-day acclimation at the respective temperatures.

Selection experiment

We created 32 microcosms of the bacterium–phage system, cultured in two physical environments (temperature regimes): (1) temperature constant at 28 °C and (2) temperature elevated stepwise from 28 to 31 °C (0.2 °C per step for 15 steps over 32 days). In each environment, eight replicate microcosms were grown under each of the following two conditions: (1) evolution, where the phage was allowed to evolve and the bacterium was replaced with the ancestral type at each transfer (see below), and (2) coevolution, where the bacterium and the phage were allowed to coevolve.

Each microcosm was initially inoculated with 10^7 isogenic bacterial cells and 10^5 isogenic phage particles, and then cultures were propagated for 16 serial transfers (one transfer every 2 days). For the coevolution selection lines, 50 μL (1%) of culture from each microcosm was transferred to fresh media every 2 days; and for the evolution lines, the phage population from each microcosm was isolated, 50 μL (1%) of which plus 10^7 ancestral bacterial cells were transferred to a fresh microcosm (Paterson *et al.* 2010). At each transfer, the incubator for the temperature-elevated microcosms was set to increase in temperature by 0.2 °C (thus the transfer-1 cultures were grown at 28 °C and transfer-16 cultures were grown at 31 °C). Culture samples were frozen in 50% glycerol at −80 °C at every second transfer. To isolate the phage from the bacteria, we added 100 μL of chloroform to 900 μL of cultures, vortexed to lyse the bacterial cells, and then centrifuged at 15 800 *g* for 2 min to pellet the bacteria debris, leaving a suspension of phage in the supernatant. Phage population densities were measured every two transfers by plating phage dilutions onto soft agar plates containing the ancestral bacterial cells and counting the number of plaque forming units (PFUs) after 24 h culture at 28 °C.

Evolutionary changes

We tested for the heritability of phage adaptation to higher temperature (31 °C) of transfer-16 phages from the six (out of eight) evolution lines in the temperature-elevated environment that persisted until the end of the experiment. Note that in the temperature-elevated environment, all coevolution phage lines went extinct with the earliest extinction events occurring between transfer 8 (29.4 °C) and 10 (29.8 °C). The six putative high-temperature-adapted populations, and the ancestral phage as a control, were grown on the ancestral bacteria at 28 °C for one transfer (48 h), and then at 31 °C for two transfers. Density changes within the first transfer at 31 °C were used to estimate growth rate after benign-temperature acclimation; and density changes within the second transfer for estimating growth rate after high-temperature acclimation. The number of doublings per transfer was calculated as $\log_2 (N_f/N_0)$, where N_0 is the initial phage density and N_f the final density.

Measurement of coevolution and phage infectivity ranges

Coevolution was measured with respect to bacterial resistance and phage infectivity. We measured bacterial resistance and phage infectivity at the population level. Resistance of a bacterial population was determined by streaking 20 independent bacterial colonies across a line of phage (20 μL) that had previously streaked and dried on a KB agar plate. A colony was scored as resistant if there was no inhibition of growth by the phage. Bacterial resistance was measured as the proportion of resistant bacteria of the 20 colonies, and phage infectivity, the proportion of sensitive bacteria (Buckling & Rainey 2002).

To measure bacteria-phage coevolution in the coevolution lines, we estimated the resistance of bacterial populations at transfer 0 (ancestral bacteria), 4 and 8 to sympatric phages isolated from transfer 0 (ancestral phage), 4 and 8 (the coevolved phages in the temperature-elevated environment began to go extinct after transfer 8). If coevolution occurred, it is expected that future phages are better than contemporary phages, and contemporary phages better than past phages, at infecting contemporary bacteria, shown as a negative slope of bacterial resistance against the time when the phages were isolated. Similarly, future bacteria are expected to be better than contemporary, and contemporary bacteria better than past bacteria, at resisting contemporary phages, shown as a positive slope when bacterial resistance is plotted against the time when the bacteria were isolated (Brockhurst *et al.* 2003; Lopez-Pascua & Buckling 2008; Morgan *et al.* 2010).

To determine whether the phages evolved broader infectivity ranges in the coevolution selection lines than in the evolution lines

as observed in previous studies (Poullain *et al.* 2008), we compared the infectivity ranges of phages from the two types of selection lines at transfer 8 and 16 for the temperature-constant environment, and at transfer 8 for the temperature-elevated environment. The infectivity ranges were determined by measuring the infectivity of the phages from the two types of microcosms (coevolved and evolved) on the bacteria from the coevolution microcosms (Poullain *et al.* 2008). We randomly paired the phage populations (Morgan *et al.* 2010) from the eight coevolution and eight evolution microcosms, and measured phage infectivity against the coevolved bacteria.

The roles of effective population size and costs of increased infectivity

We carried out supplementary experiments to test two likely mechanisms underlying the impact of coevolution on phage adaptation: (1) a smaller population size of the coevolved phages (due to host resistance evolution) retards the evolutionary adaptation to the changing environment and (2) fitness cost of increased viral infectivity in the coevolved phages (a consequence of coevolution in this system) is greater at higher temperatures.

Population size-reduction experiment

When grown at 28 °C, the evolved phage lines had a stationary population size of *c.* 10^9 PFUs mL^{-1} and a bottleneck population size of *c.* 10^7 mL^{-1}. The coevolved phage lines had roughly 10-fold smaller stationary (*c.* 10^8 mL^{-1}) and bottleneck (*c.* 10^6 mL^{-1}) population sizes. To look at whether a reduction in population size alone could influence the phage population persistence in the temperature-elevated environment, we grew eight replicate microcosms, which resemble the 'temperature-elevated evolution' lines except for that the phage populations experienced 10^3-fold dilution at each transfer. Such a dilution rate, when the phage growing on the ancestral bacteria at 28 °C, would result in a stationary population size of *c.* 10^9 mL^{-1} and a bottleneck population size of *c.* 10^6 mL^{-1}.

Infectivity cost experiment

The transfer-16 phage populations of the evolved (with narrow infectivity ranges) and coevolved (with broad infectivity ranges) phage lines from the temperature-constant environment were grown on the ancestral bacteria at 28.0, 29.0, 29.4 or 29.8 °C (initially with 10^3 phage particles and 10^7 bacterial cells), and phage densities after 48 h (the transfer duration in the selection experiment) were estimated.

Statistical analysis

Duration of persistence (time to extinction) of the phage lines was analysed using survival analysis based on Cox proportional hazards model. Phage density data from the selection experiment were analysed using a general linear mixed model (GLMM), with the type of selection lines (evolution or coevolution) as a categorical explanatory variable, time (transfer number) as a continuous variable and selection line as a random factor. Phage density data from the infectivity cost experiment were analysed using GLMM, with selection type and growth temperature as categorical variables and selection line as a random factor. Population density data were log-transformed [$\log_{10}$ (1 + PFUs mL^{-1})], and proportion data (bacterial resistance and phage infectivity) arcsine-transformed.

RESULTS

Population persistence and evolutionary adaptation

All phage lines from the temperature-constant environment, under either evolution or coevolution selection, persisted until the end of the experiment (Fig. 1a). In the temperature-elevated environment, population extinction was observed earlier and more frequently under coevolution (Fig. 1b): time to extinction of the coevolved phages was significantly shorter than that of the evolved (survival analysis based on Cox proportional hazards model, $\chi = 3.82$, $P < 0.001$); and at the end of the experiment (transfer 16), all eight coevolved phage lines had gone extinct, whereas only two of eight evolved phage lines were extinct (Fisher's exact test, $P = 0.007$).

In the temperature-constant environment, the evolved phage lines had higher population densities than the coevolved lines, but both coevolved and evolved populations showed a similar decreasing trend over time (GLMM, selection type, $F_{1,14} = 162.6$, $P < 0.001$; time, $F_{1,110} = 36.0$, $P < 0.001$; time: selection type, $F_{1,110} = 2.68$, $P = 0.105$; Fig. 1a). In the temperature-elevated environment, the evolved phage had higher densities, and showed a slower decrease over time, than the coevolved phage (selection type, $F_{1,14} = 162.6$, $P < 0.001$; time, $F_{1,110} = 36.0$, $P < 0.001$; time: selection type, $F_{1,110} = 91.7$, $P < 0.001$; Fig. 1b).

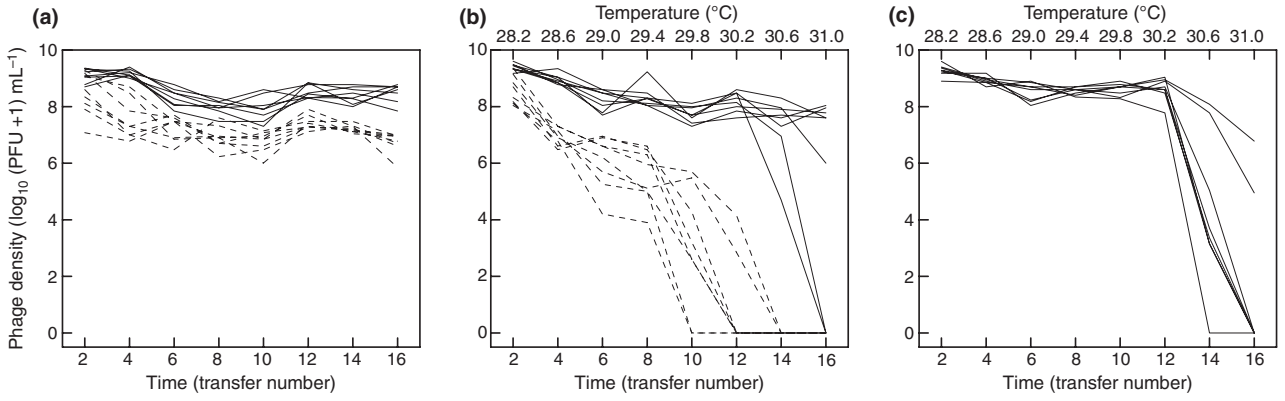

Figure 1 (a and b) Density of the evolved (solid lines) and coevolved phage populations (dashed lines) in the temperature-constant (a) or temperature-elevated environment (b). (c) Density of phage populations in the population size-reduction experiment. Each line represents a replicate population.

We determined whether there was a likely genetic (rather than just physiological) base to the high-temperature adaptation in the six phage lines that persisted until the end of the experiment in the temperature-elevated environment. All six populations had a significantly positive (one-sample *t*-test, $P < 0.05$) growth rate at 31 °C after a 2-day acclimation either at 28 °C (number of doublings per transfer: 2.3 ± 0.46) or at 31 °C (number of doublings: 9.6 ± 0.36). By contrast, the ancestral phage had a negative growth rate after 28 °C acclimation (number of doublings: −7.4 ± 0.90; $P = 0.014$) and a zero growth rate after 31 °C acclimation (number of doublings: 0.11 ± 0.22; $P = 0.654$). These results show that the positive growth at 31 °C of the evolved lines cannot be explained by temperature acclimation, but rather must result from genetic adaptation.

Bacteria-phage coevolution and phage infectivity ranges

Consistent with previous studies of coevolution between these organisms (Buckling & Rainey 2002; Brockhurst *et al.* 2003; Lopez-Pascua & Buckling 2008), bacteria-phage coevolution in both temperature-constant and temperature-elevated environments was escalatory, with bacteria becoming resistant to a wider range of phage populations and phage infective to a wider range of bacteria through time (see Figures S1 and S2). The rate of coevolution (estimated as the change in infectivity between ancestral phages and phages from transfer 8, measured against bacteria from an intermediate time point, transfer 4; Brockhurst *et al.* 2003) did not differ between temperature-constant and temperature-elevated microcosms (two-sample *t*-test, $t = 0.013$, d.f. = 14, $P = 0.990$). Consistent with previous work (Poullain *et al.* 2008; Paterson *et al.* 2010), coevolved phages had broader infectivity ranges than the evolved phages, under both temperature regimes (paired-samples *t*-test, $P < 0.05$; Fig. 2).

The effect of reduced population size

In the 'population size-reduction experiment', eight phage lines evolved on the ancestral bacteria in the temperature-elevated environment in the same way as for the main experiment, but with a reduced bottleneck population size (which approximated the bottleneck size in the coevolution lines in the main experiment). The duration of phage persistence was reduced in these population size-reduced lines, although the statistical difference is marginally non-significant ($\chi = 1.81$, $P = 0.0696$; Fig. 1), suggesting that a smaller effective population size may limit adaptive evolution. However, reductions in population size are unlikely to be the sole explanation for the reduced persistence duration in the temperature-elevated coevolved vs. evolved phages, as phage persistence in the population size-reduced lines was still longer than in the temperature-elevated coevolution lines ($\chi = 1.82$, $P = 0.0689$; Fig. 1).

The cost of phage infectivity

In the 'infectivity cost experiment', the evolved and coevolved phage lines, the latter having the greater infectivity ranges, from the temperature-constant environment were grown on the ancestral bacteria at a series of temperatures. The evolved phages had overall higher population sizes than the coevolved phages, and both types of phages showed lower performance at higher temperature (phage type, $F_{1,14} = 4.60$, $P = 0.050$; temperature, $F_{3,42} = 46.2$, $P < 0.001$).

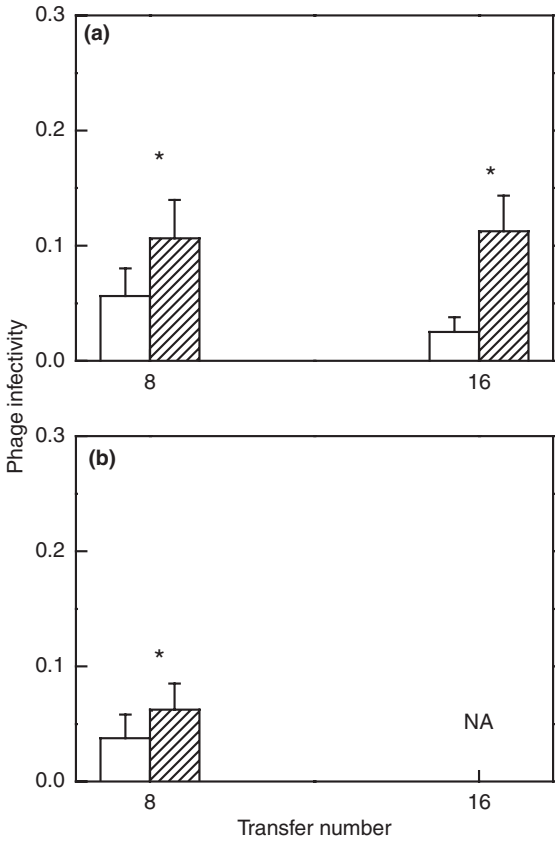

Figure 2 Infectivity ranges of phages from the evolution (unfilled bars) and coevolution selection lines (filled bars) in the temperature-constant (a) and temperature-elevated environment (b). Asterisks above the bars indicate significant difference between the evolved and coevolved phages (based on paired-sample *t*-test; $P < 0.05$). Data show mean ± SE.

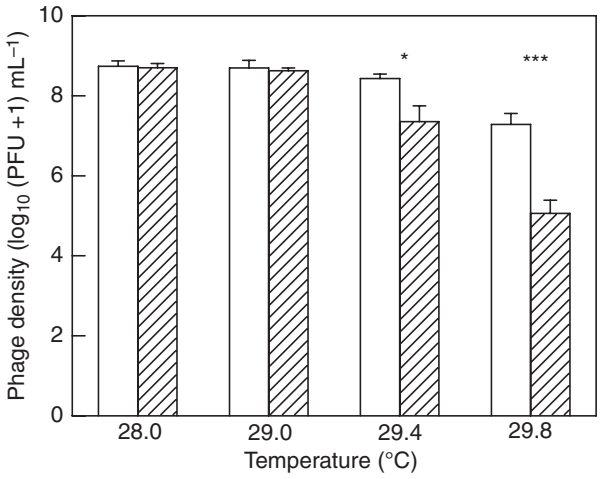

Figure 3 Density of phage populations of the evolution (unfilled bars) and coevolution selection lines (filled bars) from the temperature-constant environment growing on the ancestral bacteria at a series of temperatures. Asterisks above the bars indicate significant difference between the evolved and coevolved phages (based on two-sample *t*-test ; $*P < 0.05$; $***P < 0.001$).

Crucially, there was a significant interaction between growth temperature and phage type, demonstrating that the relative performance of the coevolved phages to the evolved phages was temperature dependent (phage type: temperature, $F_{3,42} = 7.69$, $P = 0.003$; Fig. 3). Specifically,

at 28 or 29 °C, the coevolved phage lines had non-significantly lower population densities than the evolved lines (two-sample *t*-test, $P > 0.700$), whereas at 29.4 or 29.8 °C, the coevolved lines had significantly lower densities ($P < 0.020$; Fig. 3). In other words, infectivity costs were elevated at high, but not low, temperatures.

DISCUSSION

Our results suggest that biotic interactions may play an important role in the evolutionary adaptation of populations to deteriorating environments. Specifically, host–parasite antagonistic coevolution between bacteria and phages reduced the likelihood of phage adaptation to environmental deterioration. The bacterium (*P. fluorescens* SBW25) has a broader temperature range than the phage virus (SBW25Φ2): the ancestral bacterium grows well below 33 °C, and the ancestral phage grows well below 29 °C but fails to reproduce at 30 or 31 °C (even following 2 days of physiological adaptation); an increase in temperature from 28 to 31 °C therefore constitutes a thermal stress on the virus but not the host bacteria. When grown in such a deteriorating environment, the virus was destined to go extinct in the absence of genetic adaptation; and we show here that such adaptation is more likely when phages evolved on a constant host genotype than when coevolving with the host.

There are several possible mechanisms underlying the negative influence of coevolving host bacteria on viral adaptation to high temperatures in our experiment, and subsequent experiments strongly suggest the operation of at least two. First, a reduction in population size of the virus: phage lines with smaller bottleneck population sizes were less likely to persist when faced with increasing temperature, indicative of slower rates of adaptation (Fig. 1). Second, a fitness cost of virus infectivity that becomes greater at higher temperatures: the relative population sizes reached by the coevolved (more infective) vs. the evolved (less infective) phages were reduced at higher temperatures (Fig. 3).

A reduction in population size may have two consequences that lead to slower rates of adaptation: a reduced supply of beneficial mutations and a lower fixation probability of beneficial mutations (genetic drift). The first is likely to have played an important role in our experiment, whereas the second should have little effect when the effective population size (N_e) is fairly large (e.g. Gillespie 1998), as in our experiment ($N_e > 10^6$ mL^{-1}). Considering both of these effects, Wahl *et al.* (2002) theoretically demonstrated that for experimental populations that experience repeated bottlenecks (with no death between bottlenecks), the total number of beneficial mutations to occur and survive is *c.* $2\mu N_0 (\ln D)^2/\alpha$, where μ is the beneficial mutation rate per replication, N_0 is bottleneck population size, D is the dilution ratio at bottlenecks and α is a coefficient used to define the exponential distribution of fitness advantage of beneficial mutations. This modelling estimate suggests that in our experiment the coevolved phage lines ($N_0 = \sim 10^6$ mL^{-1}, $D = 0.01$) should have 10-fold less beneficial mutations that occurred and survived compared with the evolved phages ($N_0 = \sim 10^7$ mL^{-1}, $D = 0.01$), and the population size-reduced phages ($N_0 = \sim 10^6$ mL^{-1}, $D = 0.001$) five-fold less than the evolved phages. Our experimental results are qualitatively consistent with these estimates, given that the evolved phages were most likely to adapt to temperature elevation, followed by the population size-reduced lines, and then the coevolved phages. However, the coevolved phages differed from the population size-reduced phages in adaptation probability more than expected based on these modelling estimates, suggesting that other mechanisms (such as growth rate costs of increased infectivity range) further decreased the chance of adaptation in the coevolved phages.

Another mechanism may also have contributed to the negative effect of coevolution: life history parameters of the phages may have changed, which then altered the fixation probability of beneficial mutations. An evolutionary change in burst rate (generation time) and death rate (phages may die between generations as a result of viral decay or clearance by bacterial cells; Dennehy *et al.* 2007) would alter the probability that a beneficial mutation will die before bursting, and thus the fixation probability of beneficial mutations (Hubbarde *et al.* 2007). It is possible that our coevolved phages had reduced burst rates (i.e. longer generation times) and increased death rates, and thus lower fixation probability of beneficial mutations; but we are unable to measure experimentally the death rate of the phages and hence quantify the potential importance of this mechanism.

Our results lend further support to the notion that host–parasite coevolution may fundamentally change the potential of adaptive evolution of the hosts or parasites (Pal *et al.* 2007; Forde *et al.* 2008; Alto & Turner 2010; Paterson *et al.* 2010), and highlight the importance of biotic interactions for the evolutionary responses of organisms to global climate change. As both mechanisms we experimentally demonstrated here (a reduction in population size and fitness costs resulting from coevolution) are likely to operate in many victim-exploiter systems, antagonistic coevolution may generally reduce the likelihood of evolutionary rescue in deteriorating abiotic environments.

With recent studies emphasizing the importance of ecological interspecific interactions for predicting species' responses to global climate changes (e.g. Maynard Smith 1989; Harmon *et al.* 2009; van der Putten *et al.* 2010), it is time to also develop general hypotheses on the role of evolutionary interactions in species' evolutionary adaptation to changing abiotic environments. It is likely that most between-species antagonistic interactions, both within- and between-trophic levels, will have a negative effect on adaptation by reducing the effective population size of a focal species and imposing selection for costly antagonistic adaptations. This suggestion is supported by our present work and other studies. First, recent modelling work showed that the presence of multiple competitor species may inhibit a focal species' evolutionary response to changing environment because the presence of more competitors increases the chance that at least one of them are pre-adapted to new conditions, which restricts the ecological opportunity for evolutionary adaptation in the focal species (de Mazancourt *et al.* 2008). Second, experimental work showed that competition between multiple strains of an alga limited their adaptation at elevated CO_2 probably because of an evolutionary trade-off between competitive ability and adaptation to abiotic environment (Collins 2011). By contrast to the evolutionary consequences of antagonistic interactions, mutualistic coevolution may facilitate adaptation to abiotic stress. For instance, certain secondary bacterial symbionts carried by aphids confer tolerance to heat shocks, which otherwise severely reduce the aphids' fecundity (Russell & Moran 2006). Aphids' fecundity resulting from the mutualism is likely to be increased by mutualistic coevolution, increasing the chance of adaptation to increased temperature. Incorporating the effect of evolutionary interactions is necessary for a more comprehensive understanding of the responses of species to future climate changes, and more rigorous theoretical and empirical work is badly needed in this field.

ACKNOWLEDGEMENTS

We thank three anonymous referees for their helpful comments. This work was funded by the National Natural Science Foundation of China (31070379 and 31030014) and the Fundamental Research Funds for the Central Universities of China (2009SAT-8 and 2009SC-4), and AB was funded by the European Research Council and the Leverhulme Trust.

REFERENCES

Alto, B. & Turner, P. (2010). Consequences of host adaptation for performance of vesicular stomatitis virus in novel thermal environments. *Evol. Ecol.*, 24, 299–315.

Barrett, R.D.H. & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends Ecol. Evol.*, 23, 38–44.

Bell, G. & Collins, S. (2008). Adaptation, extinction and global change. *Evol. Appl.*, 1, 3–16.

Bell, G. & Gonzalez, A. (2009). Evolutionary rescue can prevent extinction following environmental change. *Ecol. Lett.*, 12, 942–948.

Bergelson, J. & Purrington, C.B. (1996). Surveying patterns in the cost of resistance in plants. *Am. Nat.*, 148, 536–558.

Boots, M. & Sasaki, A. (2003). Parasite evolution and extinctions. *Ecol. Lett.*, 6, 176–182.

Bradshaw, A.D. (1991). The Croonian Lecture, 1991: genostasis and the limits to evolution. *Philos. Trans. R. Soc. B*, 333, 289–305.

Brockhurst, M.A., Morgan, A.D., Rainey, P.B. & Buckling, A. (2003). Population mixing accelerates coevolution. *Ecol. Lett.*, 6, 975–979.

Buckling, A. & Hodgson, D.J. (2007). Short-term rates of parasite evolution predict the evolution of host diversity. *J. Evol. Biol.*, 20, 1682–1688.

Buckling, A. & Rainey, P.B. (2002). Antagonistic coevolution between a bacterium and a bacteriophage. *Proc. R. Soc. B*, 269, 931–936.

Buckling, A., Wei, Y., Massey, R.C., Brockhurst, M.A. & Hochberg, M.E. (2006). Antagonistic coevolution with parasites increases the cost of host deleterious mutations. *Proc. R. Soc. B*, 273, 45–49.

Burdon, J.J. & Thrall, P.H. (2009). Coevolution of plants and their pathogens in natural habitats. *Science*, 324, 755–756.

Burger, R. & Lynch, M. (1995). Evolution and extinction in a changing environment: a quantitative-genetic analysis. *Evolution*, 49, 151–163.

Chevin, L.-M., Lande, R. & Mace, G.M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.*, 8, e1000357.

Collins, S. (2011). Competition limits adaptation and productivity in a photosynthetic alga at elevated CO_2. *Proc. R. Soc. B*, 278, 247–255.

Collins, S. & Bell, G. (2004). Phenotypic consequences of 1,000 generations of selection at elevated CO_2 in a green alga. *Nature*, 431, 566–569.

Davis, M.B., Shaw, R.G. & Etterson, J.R. (2005). Evolutionary responses to changing climate. *Ecology*, 86, 1704–1714.

Dennehy, J.J., Friedenberg, N.A., Yang, Y.W. & Turner, P.E. (2007). Virus population extinction via ecological traps. *Ecol. Lett.*, 10, 230–240.

Forde, S.E., Thompson, J.N., Holt, R.D. & Bohannan, B.J.M. (2008). Coevolution drives temporal changes in fitness and diversity across environments in a bacteria-bacteriophage interaction. *Evolution*, 62, 1830–1839.

Gillespie, J.H. (1998). *Population Genetics: A Concise Guide*. The Johns Hopkins University Press, Baltimore and London.

Gomulkiewicz, R. & Holt, R.D. (1995). When does evolution by natural selection prevent extinction? *Evolution*, 49, 201–207.

Harmon, J.P., Moran, N.A. & Ives, A.R. (2009). Species response to environmental change: impacts of food web interactions and evolution. *Science*, 323, 1347–1350.

Hubbarde, J.E., Wild, G. & Wahl, L.M. (2007). Fixation probabilities when generation times are variable: the burst death model. *Genetics*, 176, 1703–1712.

Janmaat, A.F. & Myers, J.H. (2005). The cost of resistance to *Bacillus thuringiensis* varies with the host plant of *Trichoplusia ni*. *Proc. R. Soc. B*, 272, 1031–1038.

Kawecki, T.J. & Holt, R.D. (2002). Evolutionary consequences of asymmetric dispersal rates. *Am. Nat.*, 160, 333–347.

Klironomos, J.N., Allen, M.F., Rillig, M.C., Piotrowski, J., Makvandi-Nejad, S., Wolfe, B.E. *et al.* (2005). Abrupt rise in atmospheric CO_2 overestimates community response in a model plant–soil system. *Nature*, 433, 621–624.

Kraaijeveld, A.R., Hutcheson, K.A., Limentani, E.C. & Godfray, H.C.J. (2001). Costs of counterdefenses to host resistance in a parasitoid of *Drosophila*. *Evolution*, 55, 1815–1821.

Lande, R. & Shannon, S. (1996). The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution*, 50, 434–437.

Lopez-Pascua, L.D.C. & Buckling, A. (2008). Increasing productivity accelerates host-parasite coevolution. *J. Evol. Biol.*, 21, 853–860.

Lynch, M. & Lande, R. (1993). Biotic interactions and global climate change. In: *Biotic Interactions and Global Change* (eds Kareiva, P.M., Kingsolver, J.G. & Huey, R.B.). Sinauer, Sunderland, MA, pp. 234–250.

Maynard Smith, J. (1989). The causes of extinction. *Philos. Trans. R. Soc. B*, 325, 241–252.

de Mazancourt, C., Johnson, E. & Barraclough, T.G. (2008). Biodiversity inhibits species' evolutionary responses to changing environments. *Ecol. Lett.*, 11, 380–388.

McCallum, H. & Dobson, A. (1995). Detecting disease and parasite threats to endangered species and ecosystems. *Trends Ecol. Evol.*, 10, 190–194.

Meyer, J.R. & Kassen, R. (2007). The effects of competition and predation on diversification in a model adaptive radiation. *Nature*, 446, 432–435.

Miura, O., Kuris, A.M., Torchin, M.E., Hechinger, R.F. & Chiba, S. (2006). Parasites alter host phenotype and may create a new ecological niche for snail hosts. *Proc. R. Soc. B*, 273, 1323–1328.

Morgan, A.D. & Buckling, A. (2006). Relative number of generations of hosts and parasites does not influence parasite local adaptation in coevolving populations of bacteria and phages. *J. Evol. Biol.*, 19, 1956–1963.

Morgan, A.D., Bonsall, M.B. & Buckling, A. (2010). Impact of bacterial mutation rate on coevolutionary dynamics between bacteria and phages. *Evolution*, 64, 2980–2987.

Orr, H.A. & Unckless, R.L. (2008). Population extinction and the genetics of adaptation. *Am. Nat.*, 172, 160–169.

Pal, C., Macia, M.D., Oliver, A., Schachar, I. & Buckling, A. (2007). Coevolution with viruses drives the evolution of bacterial mutation rates. *Nature*, 450, 1079–1081.

Paterson, S., Vogwill, T., Buckling, A., Benmayor, R., Spiers, A.J., Thomson, N.R. *et al.* (2010). Antagonistic coevolution accelerates molecular evolution. *Nature*, 464, 275–278.

Poullain, V., Gandon, S., Brockhurst, M.A., Buckling, A. & Hochberg, M.E. (2008). The evolution of specificity in evolving and coevolving antagonistic interactions between a bacteria and its phage. *Evolution*, 62, 1–11.

van der Putten, W.H., Macel, M. & Visser, M.E. (2010). Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. *Philos. Trans. R. Soc. B*, 365, 2025–2034.

Rainey, P.B. & Bailey, M.J. (1996). Physical and genetic map of the *Pseudomonas fluorescens* SBW25 chromosome. *Mol. Microbiol.*, 19, 521–533.

Russell, J.A. & Moran, N.A. (2006). Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proc. R. Soc. B*, 273, 603–610.

Schulte, R.D., Makus, C., Hasert, B., Michiels, N.K. & Schulenburg, H. (2010). Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. *Proc. Natl. Acad. Sci. USA*, 107, 7359–7364.

Thompson, J.N. (2005). *The Geographic Mosaic of Coevolution*. The University of Chicago Press, Chicago and London.

Ulmanen, I., Broni, B. & Krug, R.M. (1983). Influenza virus temperature-sensitive cap (m7GpppNm)-dependent endonuclease. *J. Virol.*, 45, 27–35.

Wahl, L.M., Gerrish, P.J. & Saika-Voivod, I. (2002). Evaluating the impact of population bottlenecks in experimental evolution. *Genetics*, 162, 961–971.

Willi, Y., Van Buskirk, J. & Hoffmann, A.A. (2006). Limits to the adaptive potential of small populations. *Ann. Rev. Ecol. Evol. Syst.*, 37, 433–458.

Woolhouse, M.E.J., Webster, J.P., Domingo, E., Charlesworth, B. & Levin, B.R. (2002). Biological and biomedical implications of the co-evolution of pathogens and their hosts. *Nat. Genet.*, 32, 569–577.

SUPPORTING INFORMATION

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

Figure S1 The evolution of phage infectivity and bacterial resistance through time in the temperature-constant microcosms.
Figure S2 The evolution of phage infectivity and bacterial resistance through time in the temperature-elevated microcosms.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer reviewed and may be re-organized for online delivery, but are not copy edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

Editor, Minus van Baalen
Manuscript received 24 September 2010
First decision made 25 October 2010
Second decision made 23 December 2010
Manuscript accepted 23 December 2010