doi: 10.1111/j.1420-9101.2008.01596.x

The rate of environmental change drives adaptation to an antibiotic sink

G. G. PERRON,* A. GONZALEZ† & A. BUCKLING*

*Department of Zoology, University of Oxford, Oxford, UK
†Department of Biology, McGill University, Montreal, QC, Canada

Keywords:

adaptation;
antibiotic resistance;
bacteria;
environmental change;
evolution;
migration;
Pseudomonas aeruginosa;
Source–sink dynamics;
temporal fluctuation.

Abstract

Recent accelerated trends of human-induced global changes are providing many examples of adaptation to novel environments. Although the rate of environmental change can vary dramatically, its effect on evolving populations is unknown. A crucial feature explaining the adaptation to harsh environments is the supply of beneficial mutations via immigration from a 'source' population. In this study, we tested the effect of immigration on adaptation to increasing concentrations of antibiotics. Using experimental population of *Pseudomonas aeruginosa*, a pathogenic bacterium, we show that higher immigration rates and a slow increase in antibiotic concentration result in a more rapid evolution of resistance; however, a high immigration rate combined with rapid increases in concentration resulted in higher maximal levels of resistance. These findings, which support recent theoretical work, have important implications for the control of antibiotic resistance because they show that rapid rates of change can produce variants with the ability to resist future treatments.

Introduction

One of the most important features of all organisms is their ability to persist in the face of rapid and sustained changes to their environment (Levins, 1969; Lenski *et al.*, 2006). Unless the environment quickly reverts to its initial condition, persistence requires that individuals migrate to track favourable conditions or the population must adapt *in situ* by expanding its niche (Gomulkiewicz & Holt, 1995). Adaptation to both novel biotic and abiotic environments often involves a suite of physiological, behavioural and genetic changes that interact to enhance population fitness and permit long-term persistence. Recent accelerated trends of human-induced global environmental change are providing many examples of adaptation to novel environments (Skelly *et al.*, 2007; Hendry *et al.*, 2008). For example, the release of industrial products into the environment, such as biochemical or pharmaceutical products, represents an increasingly significant environmental disturbance for all organisms (Kümmerer, 2001; Kolpin *et al.*, 2002). The release of antimicrobial compounds from medical or agricultural practices creates complex spatial and temporal gradients of selection that are now considered to be an important factor in the emergence and spread of antibiotic resistance (O'Brien, 2002; Levy & Marshall, 2004). The evolution of antibiotic resistance is a clear example of rapid and sustained adaptation that is of global concern to human health and the medical infrastructure upon which we depend (Palumbi, 2001).

Thus far, the majority of experimental and theoretical studies of adaptation have followed changes in phenotype in a novel constant environment (Elena & Lenski, 2003) or sequential environments (Gonzalez & Holt, 2002; Kassen, 2002; Holt *et al.*, 2004; Buckling *et al.*, 2007; Matthews & Gonzalez, 2007). However, few environmental changes outside of laboratories occur instantaneously, and conditions are unlikely to remain constant over the time needed for the fixation of beneficial mutations. Because of this discrepancy between the stability of environments used to study adaptation and that of natural environments, there is a growing concern that changing environments should be

Correspondence: Gabriel G. Perron, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.
Tel.: +44 1865 271263; fax: +44 1865 310447;
e-mail: gabriel.guimond-perron@zoo.ox.ac.uk

taken into account in experiments and models of adaptation (Wilson *et al.*, 2006; Collins *et al.*, 2007). For example, bacteria face directional change in concentrations of antibiotics over the course of chemotherapeutic treatments as antibiotics accumulate in the tissue of the treated individual (Nix *et al.*, 1991), or also in the environment with the build up of antimicrobial compounds released by clinical, veterinary or agricultural practices (Levy, 2002). The rate at which antibiotics accumulate in different environments (e.g. soil or body tissues) will fluctuate as the dose of antibiotics administered changes in the case of therapeutic treatment or the rate of chemical degradation in streams or farmlands in agricultural areas. In this paper, we study adaptation to environmental change using the microbial response to antibiotics as a model system.

Theory suggests that the difference in the rate of directional environmental change can have an important impact on the dynamics and outcome of adaptation (Pease *et al.*, 1989; Collins *et al.*, 2007). Lower rates of change are associated with weaker selection, and hence both larger population sizes (assuming selection is hard) and smaller effects of fixed beneficial mutation. Larger population sizes will result in more rapid adaptation, and many mutations of small effect have a higher probability of hitting the fitness optima than few mutations of large effect (Fisher, 1930; Orr, 2002), leading to lower between-population phenotypic variation. In the context of antibiotic treatment, we therefore predict that a slower increase in the concentration of antibiotic will be more likely to promote the evolution of resistance, whereas steeper increases will result in greater variance in resistance phenotypes. Furthermore, phenotypes which do not confer complete resistance to the antibiotic concentration in which the populations are grown will be selected against pushing the mean level of resistance towards the upper limit of the variance. Bacteria growing under steep increases in antibiotic concentration should thus have a higher mean level of resistance.

Immigration is another key factor influencing the adaptation and persistence of populations in the face of environmental change (Garant *et al.*, 2007). Source–sink models, which take into account the importance of immigration in population processes, have been used to study the effect of spatial heterogeneity on population dynamics (Pulliam, 1988; Holt *et al.*, 2004) and have been used more recently to explain the evolution of virulence (Sokurenko *et al.*, 2006; Chattopadhyay *et al.*, 2007) and antibiotic resistance (Perron et al., 2007a) in micro-organisms. In this framework, a population is defined as a source if it is found within its 'fundamental niche' – the set of environment conditions and resources that permit a population to persist, grow (i.e. growth rate exceeds death rate over some range of densities) and produce emigrants (Hutchinson, 1978). A sink population outside its fundamental niche (a harsh environment) has a mean fitness less than one (e.g. death rate exceeds birth rate), and cannot be sustained without passive (Holt, 1985) or active (Pulliam, 1988) immigration.

Recent source–sink theory (Holt *et al.*, 2004) predicts that given sustained immigration, organisms can adapt and expand their niche to incorporate harsh environments. Lineages can adapt to environmental change either by using standing genetic variation or by fixing novel beneficial mutation (Barrett & Schluter, 2008). If a quantitative character is responsible for the resistance, the probability of adaptation depends upon the rate and severity of environmental change and the amount of genetic variance present in the population (Holt *et al.*, 2004). If the sink population has little genetic variance, or is even isogenic, the probability of adaptation will depend upon genetic variance through mutation and immigration (Pease *et al.*, 1989). Furthermore, immigration can also act to increase the population size in the sink, thus making it more likely that a beneficial mutant will appear in the sink (Kawecki, 1995; Holt, 2003).

In this study, we experimentally addressed the interplay between immigration and rates of environmental change on the evolution of antibiotic resistance. Using a black hole sink scenario, where immigration is only allowed from the source to the sink (Kawecki & Holt 2002), we predicted that a slower increase in concentration of antibiotics would more readily result in the evolution of antibiotic resistance, especially under low immigration, and would result in lower mean resistance and lower between-population variation in resistance. On the other hand, we predict that only populations experiencing high immigration rates will adapt to rapidly increasing concentrations of antibiotics and will result in a greater mean and variance in resistance.

Materials and methods

Bacterial cultures

Ninety-six populations were initiated with approximately 10^4 cells of isogenic *Pseudomonas aeruginosa* PAO1 (Stover *et al.*, 2000). Cultures were grown at 37 °C in 150 μL of King's media B (KB) in 96-well microtiter plates. Every 24 h, 1% of culture, representing 10^6 cells of a fresh overnight culture of the ancestor, was transferred to a fresh microcosm. Growth was recorded as change in optical density measured with a spectrophotometer at (600 nm).

Immigration regimes

Immigration rates refer to the proportion of bacterial cells transferred from a fresh stationary phase culture of the ancestral clone grown overnight in unsupplemented KB to the selection lines. Four immigration treatments with six replicates each, i.e. 0% (no cell), 0.1% (10^5 cells), 1.0% (10^6 cells) and 10% (10^7 cells), were tested on each

environmental disturbance regime. The different immigration rates used in this experiment were prepared by serially diluting 15 μL of the overnight ancestral culture in 135 μL of KB. Fifteen microlitres of each 10-fold dilution was then added to the selection lines following the initial daily transfer.

Environmental change

We created antibiotic regimes of different severities by changing the rate of increase in the concentration of the antibiotic rifampicin. Rifampicin is a rifamycin antibiotic which targets the β-subunit of RNA polymerase. Resistance can be conferred by many different single-point mutations of different effect at the *rpoB* locus (Lambert, 2005; Trinh *et al.*, 2006). All microcosms were initiated with 62.5 μg mL^{-1} rifampicin and we increased the concentration of rifampicin to: (1) 500 μg mL^{-1} at first transfer; (2) 187.5 μg mL^{-1} at transfer one and 500 μg mL^{-1} at transfer two; (3) 125 μg mL^{-1} at transfer one, 250 μg mL^{-1} at transfer two and 500 μg mL^{-1} at transfer three; and (4) 93.75 μg mL^{-1} at transfer one, 125 μg mL^{-1} at transfer two, 187.5 μg mL^{-1} at transfer three, 250 μg mL^{-1} at transfer four, 375 μg mL^{-1} at transfer five and 500 μg mL^{-1} at transfer six. The initial concentration used completely inhibited the growth of the bacterial population as determined in Perron *et al.* (2007a, b). This is essential as this shows that the change in density observed in the bacterial populations is because of inheritable genetic changes as opposed to environmental effects. At the end of the experiment, we evaluated the potential for niche expansion by estimating the growth of each bacterial population on five high concentrations of rifampicin (i.e. 500, 750, 1000, 1500 and 2000 μg mL^{-1}). Bacterial cultures were also grown in KB for 3 days at the end of the experiment to test the heritability of antibiotic resistance.

Statistical analyses

To analyse the evolution of antibiotic resistance during the course of the experiment, we modelled the temporal dynamics of bacterial growth using a hierarchical linear mixed model (lme function of the nlme package using R 2.4 software). The response variable was optical density (OD_{600}). Time (days since the beginning of the experiment) was considered as a random variable and treatments (rate of chance = 2 levels, immigration = 4 levels, antibiotic = 5 levels) were considered fixed effects. To account for nonlinear growth dynamics, we also included a quadratic effect of time as a fixed effect. Because all replicates were started under similar conditions, we constrained the model to a unique intercept. Replicates were taken to be random effects and were nested within treatments. We began by fitting the full model that included all fixed effects and their interactions, and then simplified it by sequential backward selection. We used an *F*-test to compare the fit of different models. A variance function (varIdent of nlme library) that permits different variances for each level of a stratification variable (here treatment) was used to model heteroscedasticity when necessary. We also used the corAR1 function to model the autocorrelation structure in the time series. Significance of fixed effects was tested with *F*-tests. Differences between treatments were tested with pairwise comparisons using log-likelihood ratio tests. Model parameters and confidence intervals were estimated with restricted maximum likelihood methods (Pinheiro & Bates, 2000).

Finally, we looked at the mean and variance of resistance to a range of antibiotic concentration antibiotics (x, $1.5x$, $2x$, $2.5x$ and $3x$, where x is the final concentration of antibiotic the populations were selected on: 500 μg mL^{-1}) at the end of the experiment. To look at the mean level of resistance of all bacterial populations, we used a generalized linear model with the average growth (OD_{600}) of the selection lines on all concentrations of antibiotic as the response variable and immigration rate and rate of change level as fixed factors. To look at the between-population variance in resistance, we estimated the variance in average density (OD_{600}) among the 12 replicates of each treatment × antibiotic concentration combination (for model optimization, we eliminated immigration from this analysis as it was not significant at $P > 0.05$). We then modelled the function of variance in resistance using a generalized linear model with antibiotic concentration as a categorical variable and the order in the rate of environmental change as covariate. Because many experimental lines went extinct in the low immigration treatment, we only used lines under 1.0% and 10% in these analyses. All analyses and model assumptions were performed and verified using R 2.4 software (http://www.r-project.org/).

Results

We first considered the effect of the rate of environmental change and immigration on the probability of the evolution of resistance in experimental populations. The evolution of resistance was determined indirectly from changes in the density of bacterial populations over time: resistant bacteria increase in density in the presence of antibiotics. The effect of immigration on the density of the evolving populations was dependent on the rate of change of antibiotic concentration, and changed over time (immigration × rate of change × time: $F_{9,1115} = 5.575$, $P < 0.0001$; Fig. 1). Essentially, increasing immigration rates had a significantly positive effect on the growth of *P. aeruginosa* under all environmental change regimes (immigration: $F_{3,1115} = 639.684$, $P < 0.0001$; Fig. 1). More interestingly, the rate at which the concentration of antibiotic increased influenced the probability and speed of adaptation (treatment: $F_{3,1115} = 7.064$, $P = 0.0001$): slower increases in concentration allowed

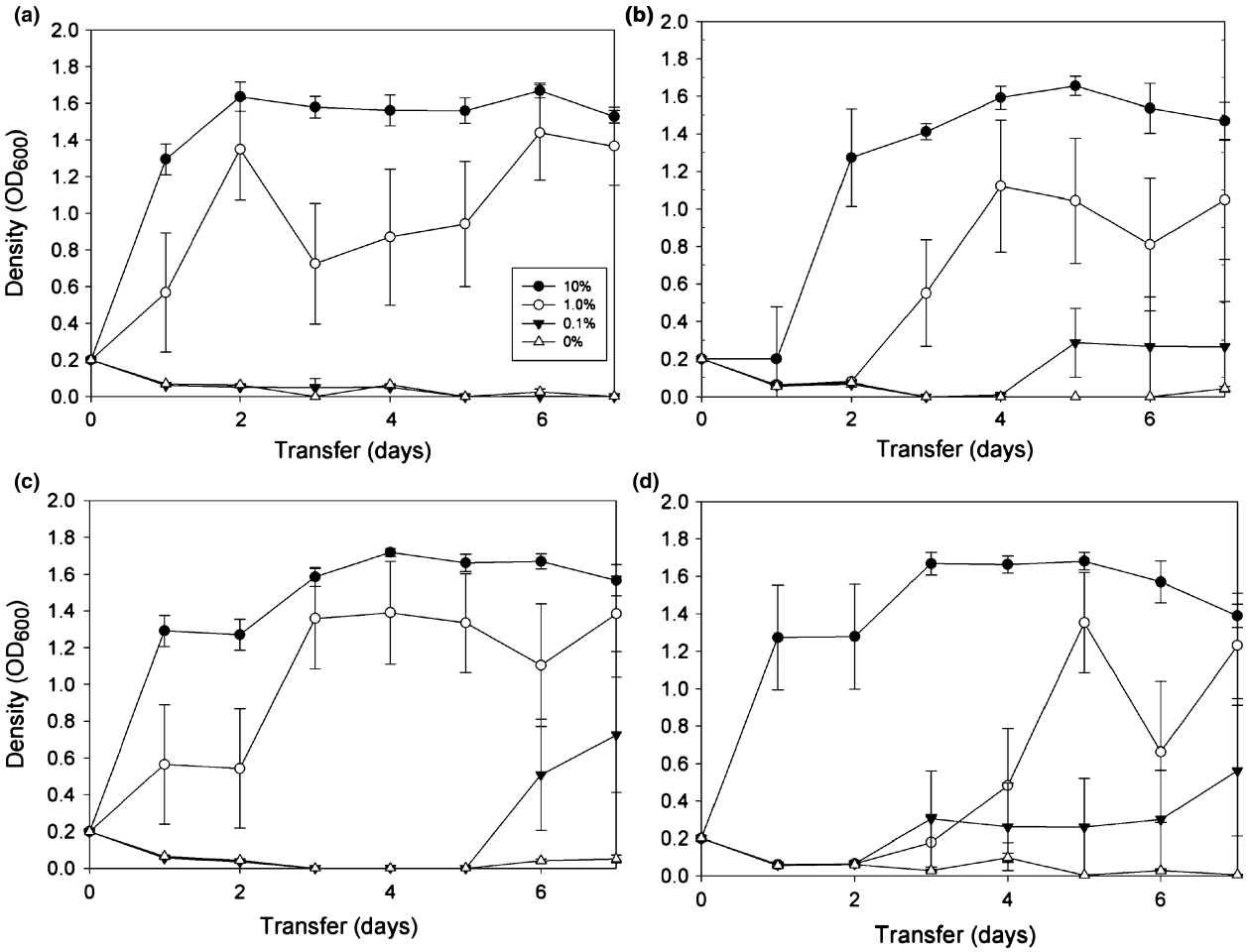

Fig. 1 Average density (±SE) of experimental lines of *Pseudomonas aeruginosa* over 70 generations growing in a source–sink scenario where the concentration of rifampicin was increased in the sink from 62.5 to 500 mg mL^{-1} by: (a) one step; (b) two steps; (c) three steps; and (d) six steps. The different migration rates used were 0% (open triangles), 0.1% (filled triangles), 1.0% (open circles) and 10% (filled circles).

for the evolution of resistance under all immigration rates (Fig. 1d), whereas populations went extinct (e.g. did not evolve resistance) under rapid increases in concentration at lower immigration rates (Fig. 1a). Populations exposed to an intermediate increase in antibiotic concentration evolved resistance under all dispersal rates, but this adaptation was delayed compared with that of slower increases in antibiotic concentration (treatment × time: $F_{3,1115} = 4.567$, $P < 0.01$; Fig. 1b,c).

We next determined the effect of the rate of environmental change and immigration on the level of resistance evolved by the different populations of bacteria at the end of the treatments. We estimated the level of evolved resistance by measuring the average density of each bacterial populations following re-inoculation at five different concentrations of antibiotic [x, 1.5x, 2x, 3x and 4x; x being the highest concentration (500 μg mL^{-1}) experienced during the selection experiment]. Growth at these five concentrations also shows the overall capacity of the populations to persist in the face of future change. The level of resistance was mainly affected by the rate at which the concentration of antibiotic was increased (rate of change: $F_{3,232} = 3.331$, $P = 0.02$; Fig. 2). Populations that had been selected under the steepest rate of increase in antibiotic concentration showed higher population density across a range of high concentrations of antibiotic; populations selected at slower rates produced lower bacterial growth on higher concentrations of antibiotics. Immigration had a positive effect on growth with higher immigration rates sustaining higher densities of bacteria (immigration: $F_{1,232} = 3.718$, $P < 0.05$; Fig. 2). We did not detect a significant interaction between immigration rate and antibiotic concentration, meaning that the trend was consistent over the different combinations of the treatments.

Finally, we determined how the variance in the level of resistance was affected by the rate of environmental change in antibiotic concentrations and immigration. We

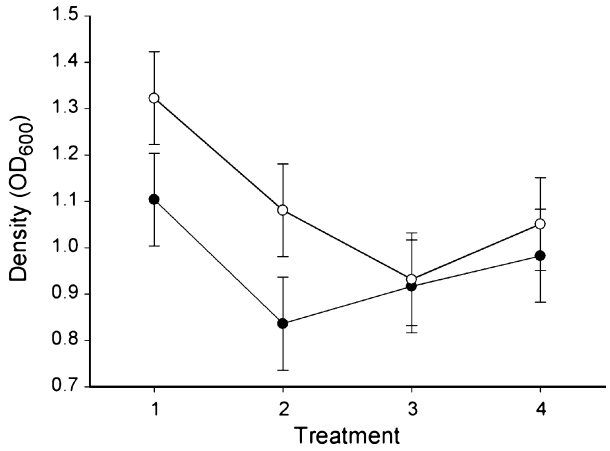

Fig. 2 The effect of immigration and the rate of environmental change on the level of resistance to rifampicin after selection experiment measured as the average density (±SE) of experimental populations *of Pseudomonas aeruginosa* grown on five concentrations of antibiotics (*x*, 1.5*x*, 2*x*, 2.5*x* and 3*x*, where *x* is the final concentration of antibiotic the populations were selected on: 500 μg mL^{-1}). Immigration rates tested were 1.0% (filled circles) and 10% (open circles), whereas the rate of environmental change are from 62.5 to 500 μg mL^{-1} in one step (1), two steps (2), four steps (3) and six steps (4).

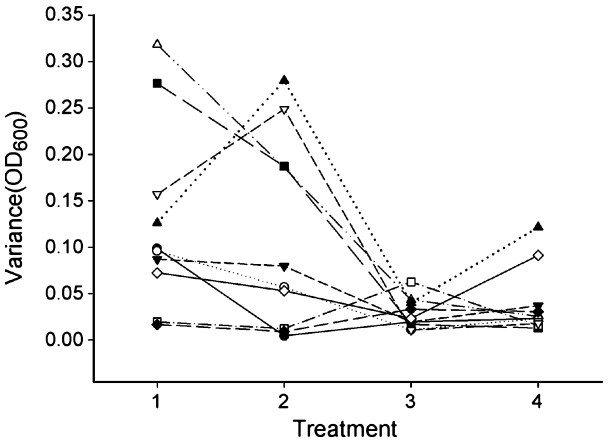

Fig. 3 The effect of the rate of environmental change on variance in the level of resistance to rifampicin in experimental population of *Pseudomonas aeruginosa* grown on five concentrations of antibiotics (*x*, 1.5*x*, 2*x*, 2.5*x* and 3*x*, where *x* is the final concentration of antibiotic the populations were selected on: 500 μg mL^{-1}). The rate of environmental change are from 62.5 to 500 μg mL^{-1} in one step (1), two steps (2), four steps (3) and six steps (4). Each line and dot combination is the result from a different replicate. Differences between immigration rates were small and are not shown here.

looked at the trend in variance in density among replicates (excluding any that did not evolve resistance) of each combination of treatments and antibiotic concentrations. When considering the rate of environmental change (or treatment) as a continuous variable, we found that the variance in the level of resistance decreased significantly as the rate of environmental change decreased, influenced by the level of antibiotic concentration tested (treatment × concentration: $F_{4,30} = 4.19$; $P < 0.01$; Fig. 3). Most notably, the variance in resistance in populations growing under a steep change in antibiotic concentration was much higher in the two highest concentrations of antibiotic (as seen in Fig. 3 with the four data points with the highest variance). The effect of change in the rate of environmental change was similar over the two different immigration rates considered for this analysis.

Discussion

We have shown experimentally that both the rate of environmental change and immigration had a significant effect on the adaptation of organisms facing novel environments. The results were consistent with the three key predictions outlined in the Introduction (Pease *et al.*, 1989; Holt *et al.*, 2004; Collins *et al.*, 2007). First, bacterial populations exposed to rapid increases in antibiotic concentration had a reduced probability of evolving resistance. Only populations under high immigration treatments evolved resistance. By contrast, given a minimal supply of genetic variation (or increase in population size) from immigration, bacteria exposed to gradual increases in concentration evolved resistance to levels of antibiotic concentration which initially completely inhibited their growth within 20 generations. Second and third, the bacterial populations faced with steep increases in antibiotics evolved the greatest level of antibiotic resistance and the greatest variance in resistance.

We suggest that the explanation for these results is the large decrease in fitness caused by the steep change in antibiotic concentration. Although any concentration of an antibiotic can impose selective pressures on a bacterial population, the strength of the selective pressure is positively related to the concentration of the antibiotic administered (Drlica, 2003). Stronger antibiotic-imposed selection resulted in smaller population sizes (hard selection), and hence a reduced probability of resistance mutations arising. When immigration was high enough to provide mutations of sufficiently large effect, different mutations were probably fixed in different populations, because the low mutation supply rate did not result in competition between mutations. This would therefore increase between population phenotypic variance both in resistance and pleiotropic effects of resistance, such as reduced growth rate (eg Andersson, 2006). This between-population variance would be further exacerbated by the fact that few mutations of large effect result in less precise adaptation (Fisher, 1930; Orr, 2002). Mean levels of resistance were probably increased because phenotypes which do not confer resistance to the

antibiotic concentration in which the populations are grown will go extinct, pushing resistance towards the upper limit of the variance. Where the antibiotic concentration changes more gradually, fitness will repeatedly decrease by small amounts, and hence mutations of smaller effect are likely to be fixed for each local optimum (Collins *et al.*, 2007). As such local phenotypic optima are more likely to be hit at each environmental change, fitness is expected to converge and thus to reduce between-population variance.

The mean and variance of resistance may also have been influenced by the distribution of fitness effects of mutations. Population genetic theory suggests that mutations of large effects are likely to be rarer than mutations of smaller effects (Orr, 2005). If this was the case for antibiotic resistance mutations, then the same mutations might more likely be fixed in different populations under rapid changes compared with gradual increases in antibiotic concentration, reducing between-population variance in the former. This effect, however, was not sufficiently important to compensate for the increased between-population variance resulting from imprecise adaptation. This is perhaps unsurprising given that more beneficial mutations of small (vs. large) effect are only explicitly predicted under relatively weak selection (Orr, 2005), hence may not be relevant to understanding the evolution of antibiotic resistance. Additional genetic data are however required to directly identify the mechanisms responsible for our results.

Our results suggest that the differential rate of accumulation of antibiotics in different environments could foster the evolution of resistance to concentrations of antibiotic which normally completely inhibit their growth. For example, clinical settings represent an ideal environment to select for highly resistant bacteria: therapy is provided at high drug concentrations, resulting in steep change in selective pressure (Barbosa & Levy, 2000; Levin *et al.*, 2000), whereas the transmission of bacteria between infected individuals results in a large supply of genetic variation (Festini *et al.*, 2006). Based on our work and recent theoretical predictions, such conditions would select for resistant bacteria which are likely to be resistant to subsequent treatment with higher concentrations of antibiotics. Selection acting on resistance is likely to differ where there is a slow build up of antimicrobial compounds into the environment, e.g. soil, farmlands, sewage water or stream water. Despite the absence of treatment, we can predict that the slow increase in antibiotic concentration in these environments will result in the evolution of bacteria resistant to clinically important doses of antibiotics at concentrations that would normally inhibit their growth. Important opportunistic pathogenic bacteria, such as *Escherichia coli*, *Salmonella* spp. or *P. aeruginosa*, are known to persist in agricultural environmental reservoirs (Côté & Quessy, 2005) or water supplies (Trautmann *et al.*, 2005) from where they can exchange resistance factors or infect human and animal hosts (Davison, 1999; Séveno *et al.*, 2002).

Although the overall effect of immigration may be similar to that of mutation supply rate (Levin *et al.*, 2000; Maisnier-Patin *et al.*, 2002) or to that of an elevated mutation rate (Oliver *et al.*, 2000; Perron *et al.*, 2006) in some circumstances, its effect on the potential for adaptation is important and needs further theoretical and experimental investigation (Perron *et al.*, 2007a). For example, although immigration can enable a population to persist in a fluctuating environment (Gonzalez & Holt, 2002; Matthews & Gonzalez, 2007), it may have a negative effect by swamping local adaptation (Lenormand, 2002; Garant *et al.*, 2007). Furthermore, the genetic background in which mutations appear will be different for immigrants and residents, potentially resulting in differential effects of mutation: mutations associated with antibiotic resistance are frequently epistatic (e.g. compensatory mutations; see Andersson, 2006.). Although immigration can affect the extent of the cost of resistance to antibiotic (Perron *et al.*, 2007a), this would not affect the conclusion pertaining to the rate of environmental change. As the conditions in this experiment allow the appearance of single mutations only within the immigrant population, a mutation in an immigrant's genetic background will not be affected by previous mutations that might have appeared in the resident population.

Our results may be of value to the application of general evolutionary principles to the problem of contemporary environmental change. Few environmental changes outside of laboratories occur instantaneously, and few natural environments remain constant over the time needed to fix beneficial mutations. In the light of recent studies (Collins *et al.*, 2007; Kopp & Hermisson, 2007) and our results, it becomes evident that the effect of the rate of environmental change must be considered not only from a theoretical perspective, but also if we wish to predict the evolutionary outcome of economically important phenomena, such as the utilization of antibiotics as the sole treatment for bacterial infections. The potential for resistant bacteria to resist future treatments, either to higher concentrations of the same drug or to new drugs, is of paramount importance for the future of clinical medicine and the prevention of infectious diseases. However, it would be valuable to validate these results in clinical settings to see if such findings can be directly applied to public health and to other antibiotic treatments. We have shown that gradual increases in the concentrations of an antibiotic can allow for a more rapid evolution of antibiotic resistance while limiting the extent of that resistance. On the other hand, abrupt increases in antibiotic concentration are less permissive to the evolution of resistance, but select for bacteria which are more likely to survive more severe treatments in the future. Our study highlights the importance of evolution and ecology in the emergence and spread of

diseases and emphasizes the need for more theoretical and experimental work on evolutionary consequences of environmental change.

Acknowledgments

The authors thank Ashleigh Griffin and Alex Hall for their comments on the manuscript as well as Rees Kassen for his helpful review. This work was funded by the Royal Society and the Leverhulme Trust. GGP is funded by the Clarendon Fund, the Fonds Quebecois pour la Recherche sur la Nature et les Technologies and the National Science Engineering and Research Council of Canada (NSERC); AG is funded by NSERC and the Canada Research Chair Program; and AB is funded by the Royal Society. The authors have no competing interests to declare.

References

Andersson, D.I. 2006. The biological cost of mutational antibiotic resistance: any practical conclusions*? Curr. Opin. Microbiol.* **9**: 461–465.

Barbosa, T.M. & Levy, S.B. 2000. The impact of antibiotic use on resistance development and persistence. *Drug Resist. Updat.* **3**: 303–311.

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

Buckling, A., Brockhurst, M.A., Travisano, M. & Rainey, P.B. 2007. Experimental adaptation to high and low quality environments under different scales of temporal variation. *J. Evol. Biol.* **20**: 296–300.

Chattopadhyay, S., Feldgarden, M., Weissman, S.J., Dykhuizen, D.E., Van Belle, G. & Sokurenko, E.V. 2007. Haplotype diversity in ''source–sink'' dynamics of *Escherichia coli* urovirulence. *J. Mol. Evol.* **64**: 204–214.

Collins, S., De Meaux, J. & Acquisti, C. 2007. Adaptive walks toward a moving optimum. *Genetics* **176**: 1089–1099.

Côté, C. & Quessy, S. 2005. Persistence of *Escherichia coli* and *Salmonella* in surface soil following application of liquid hog manure for production of pickling cucumbers. *J. Food Prot.* **68**: 900–905.

Davison, J. 1999. Genetic exchange between bacteria in the environment. *Plasmid* **42**: 73–91.

Drlica, K. 2003. The mutant selection window and antimicrobial resistance. *J. Antimicrob. Chemother.* **52**: 11–17.

Elena, S.F. & Lenski, R.E. 2003. Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. *Nat. Rev. Genet.* **4**: 457–469.

Festini, F., Buzzetti, R., Bassi, C., Braggion, C., Salvatore, D., Taccetti, G. & Mastella, G. 2006. Isolation measures for prevention of infection with respiratory pathogens in cystic fibrosis: a systematic review. *J. Hosp. Infect.* **64**: 1–6.

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford University Press, Oxford.

Garant, D., Forde, S.E. & Hendry, A.P. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. *Funct. Ecol.* **21**: 434–443.

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

Gonzalez, A. & Holt, R.D. 2002. The inflationary effects of environmental fluctuations in source-sink systems. *Proc. Natl. Acad. Sci. USA.* **99**: 14872–14877.

Hendry, A.P., Farrugia, T.J. & Kinnison, M.T. 2008. Human influences on rates of phenotypic change in wild animal populations. *Mol. Ecol.* **17**: 20–29.

Holt, R.D. 1985. Population dynamics in two-patch environments: some anomalous consequences of an optimal habitat distribution. *Theor. Popul. Biol.* **28**: 181–208.

Holt, R.D. 2003. On the evolutionary ecology of species' ranges. *Evol. Ecol. Res.* **5**: 159–178.

Holt, R.D., Barfield, M. & Gomulkiewicz, R. 2004. Temporal variation can facilitate niche evolution in harsh sink environments. *Am. Nat.* **164**: 187–200.

Hutchinson, G.E. 1978. *An Introduction to Population Ecology*. Yale University Press.

Kassen, R. 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. *J. Evol. Biol.* **15**: 173–190.

Kawecki, T.J. 1995. Demography of source-sink populations and the evolution of ecological niches. *Evol. Ecol.* **9**: 38–44.

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

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B. & Buxton, H.T. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. *Environ. Sci. Technol.* **36**: 1202–1211.

Kopp, M. & Hermisson, J. 2007. Adaptation of a quantitative trait to a moving optimum. *Genetics* **176**: 715–719.

Kümmerer, K. 2001. Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. *Chemosphere* **45**: 957–969.

Lambert, P.A. 2005. Bacterial resistance to antibiotics: modified target sites. *Adv. Drug Deliv. Rev.* **57**: 1471–1485.

Lenormand, T. 2002. Gene flow and the limits to natural selection. *Trends Ecol. Evol.* **17**: 183–189.

Lenski, R.E., Barrick, J.E. & Ofria, C. 2006. Balancing robustness and evolvability. *PloS Biol.* **4**: 2190–2192.

Levin, B.R., Perrot, V. & Walker, N. 2000. Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. *Genetics* **154**: 985–997.

Levins, R. 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bull. Entomol. Soc. Am.* **15**: 237–240.

Levy, S.B. 2002. *The Antibiotic Paradox*, 2nd edn. Perseus Publishing, Cambridge, MA.

Levy, S.B. & Marshall, B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* **10**: S122–S129.

Maisnier-Patin, S., Berg, O.G., Liljas, L. & Andersson, D.I. 2002. Compensatory adaptation to the deleterious effect of antibiotic resistance in salmonella typhimurium. *Mol. Microbiol.* **46**: 355–366.

Matthews, D.P. & Gonzalez, A. 2007. The inflationary effects of environmental fluctuations ensure the persistence of sink metapopulations. *Ecology* **88**: 2848–2856.

Nix, D.E., Goodwin, S.D., Peloquin, C.A., Rotella, D.L. & Schentag, J.J. 1991. Antibiotic tissue penetration and its relevance: models of tissue penetration and their meaning. *Antimicrob. Agents Chemother.* **35**: 1947–1952.

O'Brien, T.F. 2002. Emergence, spread, and environmental effect of antimicrobial resistance: how use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. *Clin. Infect. Dis.* **34**: S78–S84.

Oliver, A., Cantón, R., Campo, P., Baquero, F. & Blázquez, J. 2000. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* **288**: 1251–1253.

Orr, H.A. 2002. The population genetics of adaptation: the adaptation of DNA sequences. *Evolution* **56**: 1317–1330.

Orr, H.A. 2005. The genetic theory of adaptation: a brief history. *Nat. Rev. Genet.* **6**: 119–127.

Palumbi, S.R. 2001. Humans as the world's greatest evolutionary force. *Science* **293**: 1786–1790.

Pease, C.M., Lande, R. & Bull, J.J. 1989. A model of population growth, dispersal and evolution in a changing environment. *Ecology* **70**: 1657–1664.

Perron, G.G., Zasloff, M. & Bell, G. 2006. Experimental evolution of resistance to an antimicrobial peptide. *Proc. Biol. Sci.* **273**: 251–256.

Perron, G.G., Gonzalez, A. & Buckling, A. 2007a. Source-sink dynamics shape the evolution of antibiotic resistance and its pleiotropic fitness costs. *Proc. Biol. Sci.* **274**: 2351–2356.

Perron, G.G., Quessy, S., Letellier, A. & Bell, G. 2007b. Genotypic diversity and antimicrobial resistance in asymptomatic *Salmonella enterica* serotype typhimurium DT104. *Infect. Genet. Evol.* **7**: 223–228.

Pinheiro, J.C. & Bates, D.M. 2000. *Mixed-Effects Models in S and S-PLUS*. Springer, New York.

Pulliam, H.R. 1988. Sources, sinks and population regulation. *Am. Nat.* **132**: 652–661.

Séveno, N.A., Kallifidas, D., Smalla, K., VanElsas, J.D., Collard, J.-.M, Karagouni, A.D. & Wellington, E.M.H. 2002. Occurrence and reservoirs of antibiotic resistance genes in the environment. *Rev. Med. Microbiol.* **13**: 15–27.

Skelly, D.K., Joseph, L.N., Possingham, H.P., Freidenburg, L.K., Farrugia, T.J., Kinnison, M.T. & Hendry, A.P. 2007. Evolutionary responses to climate change. *Conserv. Biol.* **21**: 1353–1355.

Sokurenko, E.V., Gomulkiewicz, R. & Dykhuizen, D.E. 2006. Source-sink dynamics of virulence evolution. *Nat. Rev. Microbiol.* **4**: 548–555.

Stover, C.K., Pham, X.Q., Erwin, A.L., Mizoguchi, S.D., Warrener, P., Hickey, M.J., Brinkman, F.S.L., Hufnagle, W.O., Kowallk, D.J., Lagrou, M., Garber, R.L., Goltry, L., Tolentino, E., Westbrock-Wadman, S., Yuan, Y., Brody, L.L., Coulter, S.N., Folger, K.R., Kas, A., Larbig, K., Lim, R., Smith, K., Spencer, D., Wong, G.K.-., Wu, Z., Paulsen, I.T., Relzer, J., Saler, M.H., Hancock, R.E.W., Lory, S. & Olson, M.V. 2000. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* **406**: 959–964.

Trautmann, M., Lepper, P.M. & Haller, M. 2005. Ecology of *Pseudomonas aeruginosa* in the intensive care unit and the evolving role of water outlets as a reservoir of the organism. *Am. J. Infect. Control* **33**: S41–S49.

Trinh, V., Langelier, M.F, Archambault, J. & Coulombe, B. 2006. Structural perspective on mutations affecting the function of multisubunit RNA polymerases. *Microbiol. Mol. Biol. Rev.* **70**: 12–36.

Wilson, A.J., Pemberton, J.M., Pilkington, J.G., Coltman, D.W., Mifsud, D.V., Clutton-Brock, T.H. & Kruuk, L.E.B. 2006. Environmental coupling of selection and heritability limits evolution. *PloS Biol.* **4**: 1270–1275.

Received 10 April 2008; revised 3 June 2008; accepted 24 June 2008