

# Experimental adaptation to high and low quality environments under different scales of temporal variation

A. BUCKLING, M. A. BROCKHURST, M. TRAVISANO & P. B. RAINEY

Department of Plant Sciences, University of Oxford, Oxford, UK

## Keywords:

antagonistic pleiotropy;  
experimental evolution;  
generalist;  
mutation accumulation;  
*Pseudomonas fluorescens*;  
specialist.

## Abstract

We investigated the role of the scale of temporal variation in the evolution of generalism in populations of the bacterium *Pseudomonas fluorescens*. Replicate populations were propagated as batch cultures for approximately 1400 generations (192 days), in either high quality media only, low quality media only, or were alternated between the two at a range of temporal scales (between 1 and 48 days). Populations evolved in alternating media showed fitness increases in both media and the rate of alternation during selection had no effect on average fitness in either media. Moreover, the fitness of these populations in high quality media was the same as for populations evolved only in high quality media and likewise for low quality media. Populations evolved only in high or low quality media did not show fitness improvements in their nonselective media. These results indicate that cost-free generalists can evolve under a wide range of temporal variation.

## Introduction

Understanding the environmental and genetic factors that cause organisms to be ecological specialists or generalists is crucial to our understanding of biodiversity (Rosenzweig, 1995; Bell, 1997). The evolution of generalists is predicted to be constrained by both lack of exposure to particular environmental conditions and fitness tradeoffs between environments (Fututyama & Moreno, 1988; Whitlock, 1996; Kassen, 2002). Such tradeoffs can arise from two, not mutually exclusive, processes. First, antagonistic pleiotropy: genes that confer high fitness in one environment necessarily confer lower fitness in other environments (Fututyama & Moreno, 1988). Second, mutation accumulation: adaptation to one environment can allow the acquisition of mutations neutral in the environment in which the population is

being selected, but that are deleterious in other environments (Kawecki, 1994; Whitlock, 1996).

Both theory and data suggest that generalists are most likely to evolve under fluctuating environmental conditions (temporal heterogeneity) (Fututyama & Moreno, 1988; Gavrillets & Scheiner, 1993; Leroi *et al.*, 1994; Reboud & Bell, 1997; Turner & Elena, 2000; Kassen, 2002). The rate of fluctuation is likely to play a crucial role in the evolution of generalists (Kassen & Bell, 1998; Kassen, 2002). Most notably, if fluctuations occur very slowly, species are likely to sequentially evolve to be specialists, because adaptations to the previous selective environments will be eroded through time if there are any fitness tradeoffs between environments (whether caused by antagonistic pleiotropy or mutation accumulation) (Kassen, 2002). A number of previous studies have addressed the importance of the scale of temporal variation on generalist evolution, but no significant effects of the scale of temporal variation have been reported (e.g. Mackay, 1980; Kassen & Bell, 1998; Scheiner & Yampolsky, 1998). However, it is possible that the scales were not sufficiently different for differences in generalist evolution to be observed.

Here, we extend these studies by addressing how a wide range of temporal variation (between approximately seven and 350 generations) affects the evolution of experimental populations of the bacterium *Pseudomonas fluorescens* (Rainey & Travisano, 1998) adapting to

Correspondence: Angus Buckling, Department of Plant Sciences, University of Oxford, UK.

Tel.: 01865 271263; fax: 01865 310047; e-mail: angus.buckling@zoo.ac.uk  
Present addresses: Angus Buckling, Department of Zoology, University of Oxford, Oxford, UK.

Michael A. Brockhurst, School of Biological Sciences, University of Liverpool, UK.

Michael Travisano, Department of Biology & Biochemistry, University of Houston, Houston, Texas, USA.

Paul B. Rainey, School of Biological Sciences, University of Auckland, Auckland, New Zealand.

high and low nutrient growth media (Travisano & Rainey, 2000) over approximately 1400 generations. Previous short-term work (10s of generations) using this experimental system suggests that there is likely to be fitness tradeoffs between these environments. In static tubes containing high nutrient media, *P. fluorescens* rapidly diversifies into a variety of spatial niche specialists with distinct colony morphologies. The numerically most dominant niche specialist ('Wrinkly Spreader'; WS) forms a mat at the air-broth interface. Crucially, WS are not detected in low nutrient media and show a large fitness cost relative to broth-dwelling 'Smooth' morphotypes, which dominate in low nutrient media (Travisano & Rainey, 2000).

## Materials and methods

### Culturing conditions

Thirty millilitre glass tubes containing 6 mL of either high or low nutrient media (see below) were inoculated with  $10^7$  cells of an isogenic population of *P. fluorescens* strain SBW25 (isolated from sugar beet leaf; Rainey & Bailey 1996) or a pantothenic acid auxotroph (*panB*-) of SBW25 (Rainey & Travisano, 1998). (The isogenic population was produced by growing ancestral SBW25 for 18 h shaken at 200 r.p.m. and 28 °C). Cultures were propagated in a 28 °C static incubator for 48 h with loose lids. After this period, cultures were homogenized using a vortex mixer, and 60 µL of culture transferred to a fresh tube containing 6 mL of media. This process was repeated for a total of 196 transfers, with cultures frozen at -80 °C in 20 % glycerol every 24 transfers. Note that *P. fluorescens* SBW25 (and the isogenic mutant) has been adapted to laboratory media for no more than four transfers (approximately 28 generations) prior to the experiment.

Cultures were either constantly propagated in high or low nutrient media, or alternated between the two. We used eight scales of temporal variation: every 1, 2, 3, 4, 6, 12, 24 and 48 transfers. The transfer regimes were such that all populations in the temporal variation treatments spent the same amount of time in each media, which was half the amount of time of the two constant treatments. We established four replicates per treatment (two wild-type and two pantothenate auxotroph) resulting in a total of 40 evolving populations.

High nutrient media was M9 salts plus 10 g glycerol and 20 g of Proteose Peptone no. 3 (an enzymatic digest of meat) per litre; low nutrient media was identical but without the Proteose Peptone (Travisano & Rainey, 2000). All microcosms were supplemented with 0.0024 % pantothenic acid, to negate any growth rate disadvantage of the pantothenic acid auxotroph (Rainey & Travisano, 1998). Note that ancestral population densities after 24 h growth (the time between experimental transfers) are approximately an order of magni-

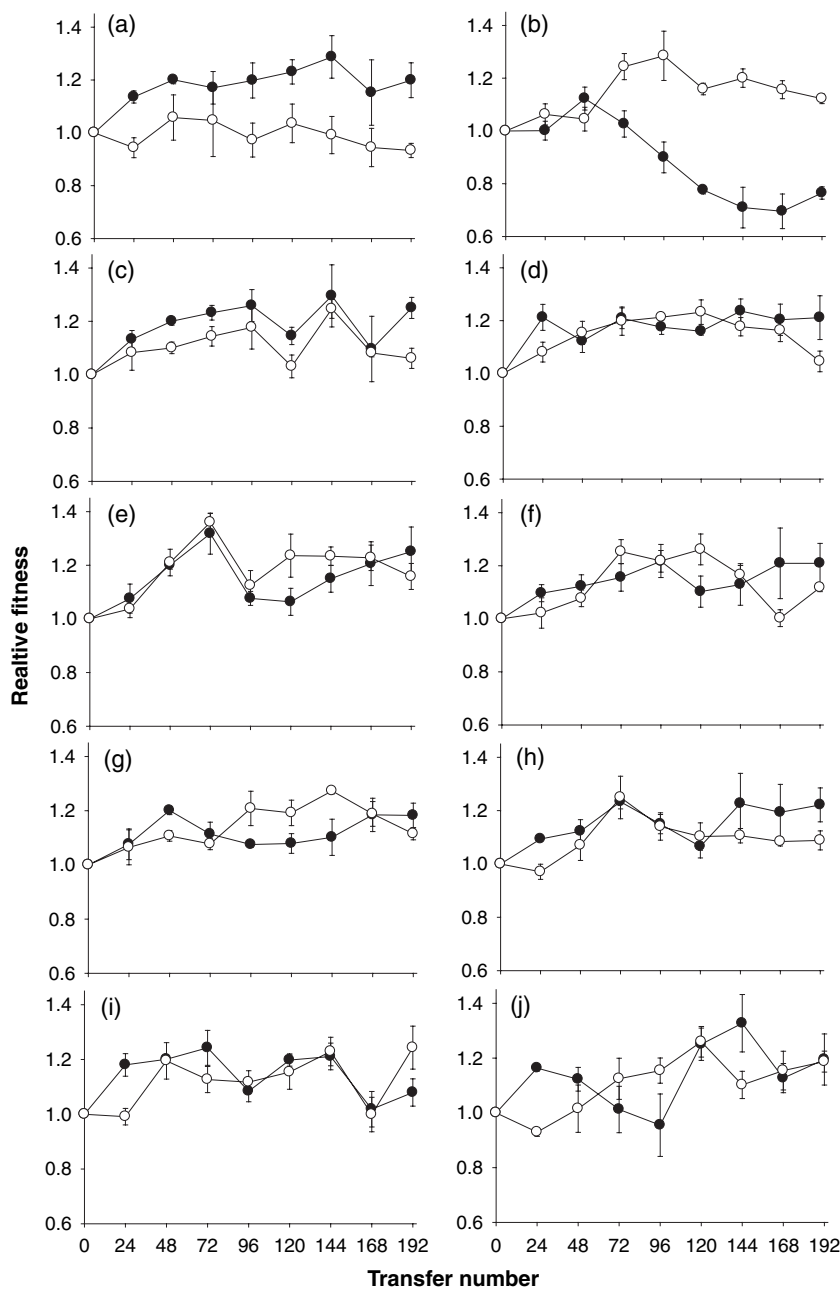
tude lower in low compared with high nutrient media (approximately  $10^8$  and  $2 \times 10^9$  cells mL<sup>-1</sup>); these differences were because of differences in both maximum carrying capacity and growth rate (Travisano & Rainey, 2000).

### Competition experiments

The fitness of evolving populations relative to the ancestor with the reciprocal genetic background was determined every 24 transfers. Bacteria were grown overnight in a shaker, as above, to ensure comparable physiological states, then  $10^7$  cells (1 : 1 ratio of competitors) were inoculated into either high or low nutrient media supplemented with pantothenic acid, negating any fitness cost of the *PanB* mutation. Competitions were carried out for 48 h under static conditions, with starting and final densities determined by plating cultures on vitamin-free KB agar supplemented with  $4.8 \times 10^{-6}$  % pantothenic acid, where the *PanB*-mutant is readily distinguished by its greatly reduced colony size (Buckling *et al.*, 2000). Relative fitness (*W*) was used to estimate the performance of the evolved populations relative to the ancestors, where *W* is the ratio of estimated Malthusian parameters (*m*) of the evolved populations and ancestor,  $m = \ln(N_t/N_0)$ , where  $N_0$  is the starting density and  $N_t$  the final density (Lenski *et al.*, 1991). All competitions were replicated twice.

## Results

The fitness of all population showed large fluctuations through time, such there was no change in average fitness between the first and last time points that fitness was measured (one-sample *t*-test of slopes against zero,  $n = 40$ ,  $P > 0.2$ , for both high and low nutrient media. This pattern held when the two constant environment treatments were excluded ( $n = 32$ ,  $P > 0.2$ , for both high and low nutrient media). The fitness of each population was therefore averaged through time (between transfers 24 and 196) for further statistical analyses. Average fitness in high nutrient media differed between treatments (one-way ANOVA with treatment fitted as a factor:  $F_{9,30} = 9.39$ ,  $P < 0.001$ ), with the constant low nutrient treatment having lower fitness than all others treatments (Fig. 1b;  $P < 0.05$  for all Tukey pairwise comparisons), which did not differ from each other ( $P > 0.2$ , in all cases). (Note that genetic background, wildtype or *PanB*- had no significant effect in any analyses ( $P > 0.2$ , in all cases), hence this factor was discarded from all statistical models). Populations within the treatments that did not differ in fitness (all but the constant low nutrient treatment) showed a fitness improvement relative to the ancestor (one-sample *t*-test:  $t = 16.19$ ,  $P < 0.0001$ ,  $n = 36$ ), and the constant low nutrient treatment showed a significant decline in fitness relative to the ancestor ( $t = 6.94$ ,  $P < 0.01$ ,  $n = 4$ ).



**Fig. 1** Mean ( $\pm$ SEM) fitness through time of evolving populations relative to the ancestor, in high (closed circles) and low (open circles) nutrient media. Populations were evolved in high quality media only (a), low quality media only (b), or alternated between high and low quality media, every 1 (c), 2 (d), 3 (e), 4 (f), 6 (g), 12 (h), 24 (i), or 48 days (j).

Average fitness in low nutrient media also differed between treatments ( $F_{9,30} = 6.74$ ,  $P < 0.001$ ): all treatments result in significantly greater fitness than the constant high nutrient media (Fig. 1a;  $P < 0.05$  for all Tukey pairwise comparisons), which did not differ from each other ( $P > 0.05$ , in all cases). Populations within all but the constant high nutrient treatment showed average fitness improvements relative to the ancestor ( $t = 17.66$ ,  $P < 0.0001$ ,  $n = 36$ ); the constant high nutrient treatment did not differ from the ancestor ( $t = 0.9$ ,  $P = 0.22$ ,  $n = 4$ ).

## Discussion

In this study we addressed how the scale of temporal variation affected adaptation to high and low nutrient media. Surprisingly, we found that the scale of temporal variation had no impact on the fitness of populations in the two environments: all showed equally good fitness improvements in both environments. Moreover, fitness of the 'generalist' populations in each environment was just as high as populations evolved only in single environments; despite the latter populations having

evolved in their single environments for twice as long. Such lack of costs of generalism are consistent with many previous studies (reviewed in Kassen, 2002). However, the scale of temporal variation was perhaps not sufficiently broad to detect differences in generalist evolution between the temporal variation treatments. In the one treatment where fitness declined below that of the ancestor (the constant low quality treatment assayed in good quality media), this decline occurred only by 96 transfers (one-sample *t*-test against ancestral fitness;  $P < 0.05$ ) – longer than the longest scale of variation. Despite this, the results demonstrate that generalists can be maintained when the rate of temporal variation is very slow (approximately 350 generations).

Measuring the fitness of whole populations provides a useful measure of the costs of generalists. Even if, at the extreme, 'generalist' populations consisted of two sub-populations specialized on the two different media, the population level assays would detect a reduction in fitness of these populations relative to pure specialists. However, if population level fitness costs of generalism had been detected, such population-level assays would have provided no insight as to whether the population consisted of specialists or generalists. Fitness assays of individual genotypes would have been subsequently carried out if costs of generalism in the alternating treatments had been detected.

Despite the ease with which apparently cost-free generalist populations can evolve, we did find evidence of a tradeoff between fitness in the two environments in the populations evolved solely in the low nutrient media. These populations showed a significant decrease in fitness from the ancestor in high quality media. That we did not observe any cost of being generalist in the temporal variation treatments demonstrates that antagonistic pleiotropy cannot be responsible for the reduction in fitness in high quality media. As such, the mechanism responsible for this tradeoff in fitness between the two environments must be mutation accumulation: the build up of mutations neutral in their selective (poor quality) environment but deleterious in the other (good quality) environment (Kawecki, 1994). Some studies of evolving populations of microbes have reported mutation accumulation as the mechanism responsible for loss of fitness in nonselective environments (Reboud & Bell, 1997; Funchain *et al.*, 2000; Giraud *et al.*, 2001), whereas others have reported antagonistic pleiotropy to be an important determinant (Travisano & Lenski, 1996; Cooper, 2000), at least in the short-term (MacLean *et al.*, 2004). Mechanisms responsible for tradeoffs are clearly very system specific and an understanding requires detailed knowledge of interactions between metabolic pathways (e.g. Pal *et al.*, 2005). It is interesting that the observed tradeoff was asymmetric: populations evolved in high nutrient media did not show a decline in fitness in the low nutrient media. Such asymmetries are not uncommon in selection experiments (Kassen, 2002).

A surprising feature of this study is the extent of fluctuations in fitness through time, and hence no net change in mean fitness throughout most of the experiment, relative to other experimental evolution studies (e.g. Lenski *et al.*, 1991). There are two likely explanations for this. First, large amounts of diversity are observed in populations of *P. fluorescens* evolving in spatially structured environments (Rainey & Travisano, 1998). Much of this diversity is maintained by negative-frequency dependent selection of niche specialist genotypes, but this results in fluctuating, rather than stable, genotype frequencies through time (Rainey & Travisano, 1998; Rainey & Rainey, 2003). Fitness assays were carried out at scale of whole populations, hence fitness relative to the ancestor is likely to fluctuate through time because of the temporal variation in the frequencies of different genotypes. Second, it is possible that selection is not directional in this system. Selection will favour genotypes that are fitter than contemporary coexisting genotypes, but this does not necessarily equate with consistent increases in fitness relative to the ancestor. Paquin & Adams (1983) reported such an effect in experimental populations the yeast, *Saccharomyces cerevisiae*. Competition experiments within evolving populations were not carried out in the present study, because of the difficulty in competing genotypes which do not have different genetic markers. Note that both these factors could have obscured the detection of antagonistic pleiotropy.

This study supports the growing body of theory and data that antagonistic pleiotropy does not necessarily constrain the evolution of generalists (Kawecki, 1994; Whitlock, 1996; Kassen, 2002). It further shows that generalists can evolve, even when fluctuations occur very slowly.

## Acknowledgments

We thank Nick Colegrave and two anonymous referees for useful comments. The work was supported by NERC (UK) and the Royal Society.

## References

- Bell, G. 1997. *Selection*. Chapman & Hall, London.
- Buckling, A., Kassen, R., Bell, G. & Rainey, P.B. 2000. Disturbance and diversity in experimental microcosms. *Nature* **408**: 961–964.
- Cooper, V.S. 2000. The population genetics of ecological specialisation in evolving *Escherichia coli* populations. *Nature* **407**: 736–739.
- Funchain, P., Yeung, A., Stewart, J.L., Lin, R., Slupska, M.M. & Miller, J.H. 2000. The consequences of growth of a mutator strain of *Escherichia coli* as measured by loss of function among multiple gene targets and loss of fitness. *Genetics* **154**: 959–970.
- Fututyama, D.J. & Moreno, G. 1988. The evolution of ecological specialisation. *Annu. Rev. Ecol. Syst.* **19**: 207–233.

- Gavrilets, S. & Scheiner, S.M. 1993. The genetics of phenotypic plasticity. V. Evolution of reaction norm shapes. *J. Evol. Biol.* **6**: 31–48.
- Giraud, A., Matic, I., Tenaillon, O., Clara, A., Radman, M., Fons, M. & Taddei, F. 2001. Costs and benefits of high mutation rates: adaptive evolution of bacteria in the mouse gut. *Science* **291**: 2606–2608.
- Kassen, R. 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. *J. Evol. Biol.* **15**: 173–190.
- Kassen, R. & Bell, G. 1998. Experimental evolution in *Chlamydomonas*. IV. Selection in environments that vary in time at different scales. *Heredity* **80**: 732–741.
- Kawecki, T.J. 1994. Accumulation of Deleterious Mutations and the Evolutionary Cost of Being a Generalist. *Am. Nat.* **144**: 833–838.
- Lenski, R.E., Rose, M.R., Simpson, S.C. & Tadler, S.C. 1991. Long-term experimental evolution in *Escherichia coli*. 1. Adaptation and divergence during 2,000 generations. *Am. Nat.* **138**: 1315–1341.
- Leroi, A.M., Lenski, R.E. & Bennett, A.F. 1994. Evolutionary adaptation to temperature. 3. Adaptation of *Escherichia coli* to a temporally varying environment. *Evolution* **48**: 1222–1229.
- Mackay, T. 1980. Genetic variance, fitness and homeostasis in varying environments: an experimental check of the theory. *Evolution* **34**: 1219–1222.
- MacLean, R.C., Bell, G. & Rainey, P.B. 2004. The evolution of a pleiotropic fitness tradeoff in *Pseudomonas fluorescens*. *Proc. Natl Acad. Sci. USA* **101**: 8072–8077.
- Pal, C., Papp, B. & Lercher, M.J. 2005. Adaptive evolution of bacterial metabolic networks by horizontal gene transfer. *Nat. Genet.* **37**: 1372–1375.
- Paquin, C.E. & Adams, J. 1983. Relative fitness can decrease in evolving asexual populations of *S. Cerevisiae*. *Nature* **306**: 368–371.
- Rainey, P.B. & Bailey, M.J. 1996. Physical and genetic map of the *Pseudomonas fluorescens* SBW25 chromosome. *Mol. Microbiol.* **19**: 521–33.
- Rainey, P.B. & Rainey, K. 2003. Evolution of cooperation and conflict in experimental bacterial populations. *Nature* **425**: 72–74.
- Rainey, P.B. & Travisano, M. 1998. Adaptive radiation in a heterogeneous environment. *Nature* **394**: 69–72.
- Reboud, X. & Bell, G. 1997. Experimental evolution of *Chlamydomonas*. III. Evolution of specialists and generalists types in environments that vary in space and time. *Heredity* **78**: 507–514.
- Rosenzweig, M.L. 1995. *Species Diversity in Space and Time*. Cambridge University Press, Cambridge.
- Scheiner, S.M. & Yampolsky, L.Y. 1998. The evolution of *Daphnia pulex* to a temporally varying environment. *Genet. Res.* **72**: 25–37.
- Travisano, M. & Lenski, R.E. 1996. Long-term experimental evolution in *Escherichia coli*. 4. Targets of selection and the specificity of adaptation. *Genetics* **143**: 15–26.
- Travisano, M. & Rainey, P.B. 2000. Studies of adaptive radiation using model microbial systems. *Am. Nat.* **156**: S35–S44.
- Turner, P.E. & Elena, S.F. 2000. Cost of host radiation in an RNA virus. *Genetics* **156**: 1465–1470.
- Whitlock, M.C. 1996. The red queen beats the jack-of-all-trades: the limitations on the evolution of phenotypic plasticity and niche breadth. *Am. Nat.* **148**: S65–S77.

Received 24 May 2006; revised 20 June 2006; accepted 23 June 2006