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LETTER

Real-time microbial adaptive diversification in soil

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Abstract

Bacteria undergo adaptive diversification over a matter of days in test tubes, but the relevance to natural populations remains unclear. Here, we report real-time adaptive diversification of the bacterium *Pseudomonas fluorescens* in its natural environment, soil. Crucially, adaptive diversification was much greater in the absence of the established natural microbial community, suggesting that resident diversity is likely to inhibit, rather than promote, adaptive radiations in natural environments. Rapid diversification is therefore likely to play an important role in the population and community dynamics of microbes in environments where resident communities are perturbed, such as by agriculture, pollution and antibiotics.

Keywords

Adaptive radiation, bacteria diversity, competition, ecological niche, *Pseudomonas fluorescens*, soil, wrinkly spreader.

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INTRODUCTION

Adaptive radiations have played a major role in generating biodiversity (Gavrilets & Losos 2009). Within-niche competition and ecological opportunity play a major role in driving adaptive radiations (Schluter 2000; Losos 2010; Ricklefs 2010), both of which will inevitably be affected by the structure of the resident community. Resident diversity is typically thought to inhibit diversification of an invading lineage through niche packing and reduced population size (and hence supply of genetic variation): a view consistent with well-known adaptive radiations having occurred after mass extinctions or the colonisation of species-poor islands (Simpson 1953; Schluter 2000; Jablonski 2001; Losos 2010). However, other macroevolutionary patterns suggest that resident diversity can itself create novel ecological niches, hence promoting diversification (Whittaker 1977; Schluter 2000; Erwin 2008; Losos 2010). Moreover, the impact of diversity on diversification may be largely contingent on interactions with the specific ecologies of organisms and abiotic changes (Ezard *et al.* 2011).

Experimental studies of the role of resident diversity on adaptive radiations are also ambiguous. Using the bacterium *Pseudomonas fluorescens* SBW25, which can adaptively diversify into morphologically distinct niche specialists over a matter of days (Rainey & Travisano 1998), experiments have shown that prior niche occupation by evolved morphotypes constrains subsequent diversification (Brockhurst *et al.* 2007; Fukami *et al.* 2007). By contrast, the presence of a closely related species (*Pseudomonas putida*) had little impact on the diversification of *P. fluorescens* (Zhang *et al.* 2012), and a resident *Escherichia coli* genotype can open up novel ecological niches for other *E. coli* genotypes feeding on metabolic waste products (Rosenzweig *et al.* 1994; Friesen *et al.* 2004). Moreover, the presence of natural enemies of bacteria (predatory protists and viruses) can either promote or hinder evolutionary diversification *in vitro* depending on the ecological context (Buckling & Rainey 2002b; Brockhurst *et al.* 2004; Meyer & Kassen 2007; Benmayor *et al.* 2008). However, in all cases the resident 'community' consisted of only one species, which was sometimes the same as the invader. As a result, these studies tell us little about how resident diversity in natural communities will affect the ability of an invader to diversify. Here, we address this issue by determining how the presence versus absence of a natural microbial soil community affects the ability of an invading soil bacterium to adaptively diversify.

To determine the importance of the resident community for the evolution of diversity, we followed the real-time evolution of a focal strain of bacteria (*P. fluorescens* SBW25) (Rainey & Bailey 1996) in a natural environment, soil. After isolating the natural soil community, soil was sterilised and placed into replicate petri dishes. In a fully factorial design, the soil was inoculated or not with the natural microbial community and a viral parasite (lytic bacteriophage SBW25ϕ2) (Buckling & Rainey 2002a), which is known to play an important role in the diversification of SBW25 *in vitro* (Buckling & Rainey 2002b; Brockhurst *et al.* 2004; Benmayor *et al.* 2008). Using a 'mark-recapture' approach (Gómez & Buckling 2011), where the marker confers resistance to an antibiotic to which culturable members of the natural community were susceptible, we found that colony morphological diversity of initially clonal populations of *P. fluorescens* inoculated into the soil microcosms over 48 days evolved to be much greater in the absence of the natural community; bacteriophages had no impact on diversity. Crucially, catabolic profiling of evolved genotypes demonstrated that differences in colony morphology was indicative of possible differences in resource use. Finally, we show that selection played a much greater role in diversification in the absence compared with the presence of the natural microbial community; negative frequency-dependent selection operated between evolved genotypes in the absence, but not in the presence, of the natural community.

MATERIALS AND METHODS

Experimental design and soil sampling

A gentamicin-resistant strain of *P. fluorescens* SBW25 (Pal *et al.* 2007) was inoculated into 32 replicate sterile soil microcosms; 10 × 10 cm square petri dishes containing 100 g of twice autoclaved (unsieved) compost (John Innes no. 2), and, half the microcosms also inoculated with lytic bacteriophage SBW25ϕ2

(Buckling & Rainey 2002a), and half with a soil solution containing the resident microbial community (other treatments were inoculated with the same volume of M9 buffer), in a full factorial design, as described in Gómez & Buckling (2011). Soil microcosms were placed in an environmental chamber at 26 °C and 80% relative humidity. We followed *P. fluorescens* diversity every 4 days by scoring the frequencies of colony morphologies for 24 days and 24 days later. Soil samples (2 g) were collected using a sterile spatula and mixed with 10-mL sterile M9 buffer and glass beads, and vortexed for 1 min (Gómez & Buckling 2011). The resultant soil washes were diluted and plated onto King's media B (KB) agar supplemented with gentamicin (15 μg mL^{-1}) and incubated for 2 days at 28 °C.

We observed two distinct colony morphologies: the more common ancestral-like Smooth (SM) and Wrinkly Spreader (WS) (Rainey & Travisano 1998). We never observed within-replicate variation in WS, although WS morphology sometimes differed between replicates. We only observed within-replicate diversity in SM morphologies at day 48, where some SM colonies were more opaque than the normal ancestral-like SM. For this reason, our measure of within-population diversity is simply the proportion of WS.

Catabolic profiling

We used Biolog GN2 microplates, which contain 96 different carbon sources, to determine the relationship between colony morphology and possible resource use. We randomly isolated four WS and four SM colonies from evolved populations (after 48 days), one SM and one WS from each treatment, and then stored them in 20% glycerol at −80 °C. All bacterial clones were grown overnight in KB (28 °C at 200 rpm), and then, a 10^6-fold cell suspension diluted in M9 buffer was incubated for 2 h at 28 °C to starve the cells (MacLean *et al.* 2004). For each culture, each well of a Biolog microplate was filled up with 150 μL of culture suspension and incubated at 28 °C for 48 h, after which bacterial growth was assayed at 660 nm using an automated Bio-Tek synergy2 microplate reader. Biolog assays were not replicated. Absorbance data were used to generate qualitative growth scores for each substrate on a three point scale (MacLean *et al.* 2004), 0; corresponded to no detectable growth, 0.5; to weak growth and 1; to strong growth.

Competition experiments and growth assays

The eight isolates (four WS and four SM) described above were used in all subsequent competition experiments and growth assays described below. Competition experiments between independent pairs of WS and SM, where each pair was isolated from different replicates within the same experimental treatment, were carried out in both the presence and absence of the natural microbial community in soil microcosms, but also in glass bottles containing 6 mL of soil solution (3 g of soil/6 mL sterile water), shaken at 150 rpm. The WS and SM genotypes were competed in the following ratios (WS : SM): 1 : 1, 1000 : 1, 1 : 1000 within treatments, and all isolates were also grown independently. WS fitness (*W*) relative to SM was estimated from the ratio of the Malthusian parameter (*m*) (Lenski *et al.* 1991), where $m = \ln(N_f/N_0)$, and N_0 is the initial and N_f is the final density of the population after 10 days of competition. Bacterial population densities were determined from plating on KB agar.

Statistical analyses

The impact of the different treatments on WS frequency during the evolution experiment was determined using a Linear Mixed Effects Models fitted by restricted maximum likelihood (REML), where proportion of WS was $\log_{10}$-transformed, the presence or absence of phages and the natural microbial community fitted as two 2-level fixed effects, time fitted as a covariate and population fitted as a random factor. Log_{10}-transformed *P. fluorescens* density was analysed in the same way. A linear mixed model was fitted to the competition results, with community (present or absent) fitted as a factor, starting ratio fitted as a covariate and genotype pair fitted as a random effect. We also fitted selection environment as a two level factor (community present or absent). Note that 'phage present or absent' was not included in the model, given its lack of effect on diversification in the main evolution experiment. Analyses were carried out in JMP software. Hierarchical clustering analysis on the ancestral and evolved WS and SM genotypes was performed in SPSS v.18, using Euclidean distances with between-group linkage method. This method computes the smallest average distance between all group pairs and then they are clustered. A one-way ANOVA was conducted to evaluate differences in the mean number of substrates that could be used between clusters.

RESULTS

Diversification in the absence of resident community

We observed morphological diversification of *P. fluorescens* SBW25 in all four experimental treatments (Fig. 1), compromising two colony morphotypes: the more common ancestral-like SM and WS. These morphotypes resemble those previously identified from *in vitro* experiments (Rainey & Travisano 1998). No within-population diversity in WS was observed, and within-population diversity of SM was only observed at the final time point (day 48). The proportion of WS (our measure of diversity) was neither affected by the interaction between the presence of phages and the natural microbial community (Fig. 1; $F_{1,28} = 0.004$, $P > 0.2$) nor the presence of phages (Fig. 1; main effect of phages: $F_{1,28} = 1.155$, $P > 0.2$). By contrast, the proportion of WS was approximately threefold higher in the absence compared with the presence of the natural community (Fig. 1; main effect of the natural microbial community: $F_{1,28} = 25.283$, $P < 0.001$). Qualitatively similar results were obtained when diversity was calculated [as Simpson's diversity index (Simpson 1949)] at day 48, taking into account the additional SM variant present in some replicate communities [Generalized linear model (GLM): presence/absence of resident community $F_{1,28} = 17.78$, $P < 0.001$; effects of phage or phage by community interaction: $P > 0.2$, in both cases]. The resident community had no net effect on the density of *P. fluorescens* populations (main effect of resident community), although there was a strong interaction between the presence of the resident community and phage (Fig. 2; interaction between presence/absence of resident community and phages: $F_{1,28} = 60$, $P < 0.0001$), as described previously (Gómez & Buckling 2011).

Pseudomonas fluorescens diversify into catabolic specialists in soil

We measured the catabolic profiles of eight independent genotypes (four SM and four WS) and ancestral SBW25. Consistent with

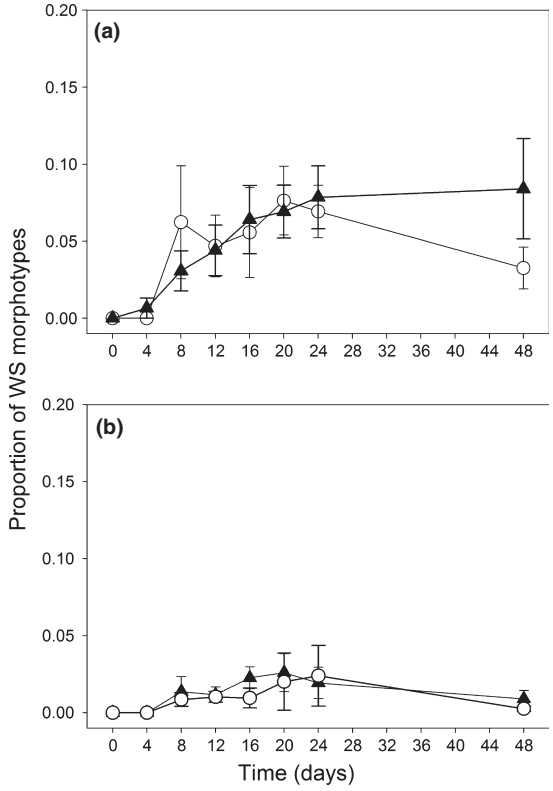

Figure 1 Evolutionary dynamic of Wrinkly Spreader (WS). Mean (± SEM) proportion of WS colony morphology through time for populations evolving with (▲) and without (○) phages, and in the absence (a) and presence (b) of the natural microbial community in soil microcosms.

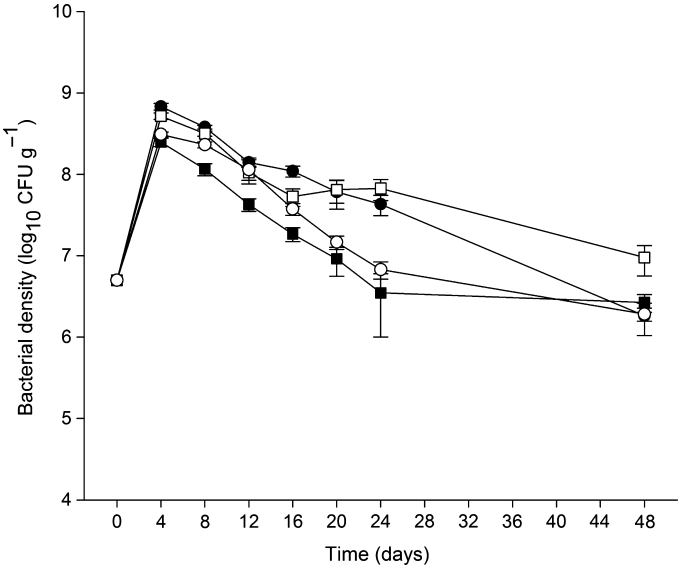

Figure 2 Density of *Pseudomonas fluorescens* through time. Mean (± SEM) density (colony forming units) of *P. fluorescens* in soil microcosms in the presence (open symbols) and absence (closed symbols) of the resident community, and the presence (circles) and absence (squares) of phages.

previous *in vitro* study (MacLean *et al.* 2004), evolved genotypes typically showed reduced catabolic activity on certain substrates, but in some cases had evolved the ability to catabolise certain substrates

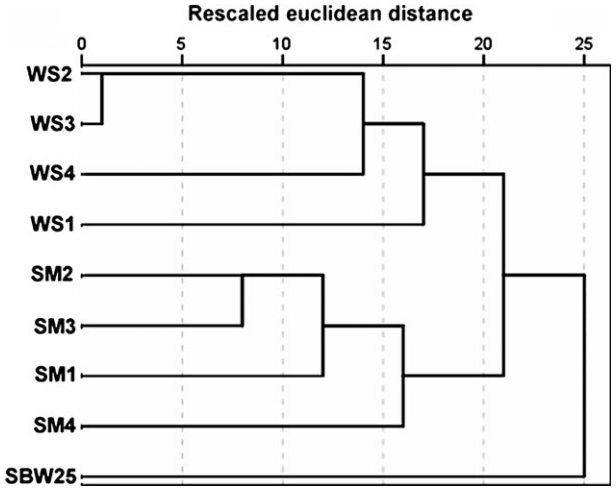

Figure 3 Hierarchical clustering tree based on Euclidean distances. Dendrogram for the ancestral *P. fluorescens* SBW25 and the four evolved WS and SM genotypes isolated after 48 days evolution in soil. WS and SM numbering refers to genotypes evolved in the absence (1 and 3) and presence (2 and 4) of phages and in the absence (1 and 2) and presence (3 and 4) of the resident community.

that the ancestral genotype could not (Fig. S1). Hierarchical clustering analysis on the growth data produced three clusters: SM, WS and ancestor (Fig. 3), with a very high cophenetic correlation coefficient (CP = 0.91; the correlation between actual pairwise differences and differences between pairs after clustering (Sokal & Rohlf 1962)). A key difference between the SM and WS clusters was the greater number of substrates that could be used by WS (one-way ANOVA; $F_{1,6} = 93.55$, $P < 0.001$). The lack of replication within treatments prevented further meaningful analyses of these data.

Selection drives evolutionary diversification

If diversification is driven by selection (i.e. it is adaptive), the different morphotypes should have a fitness advantage when rare because they occupy different ecological niches (Chesson 2000; Schluter 2000). To investigate this, one SM and WS genotype were isolated (from separate replicates) from each of the four treatments to determine their ability to reciprocally invade from rare, as well as their fitness at a 1 : 1 ratio, in both the presence and absence of the natural microbial community. SM and WS fitness was frequency independent in the presence of the microbial community, whereas both morphotypes had a large fitness advantage when rare in the presence (Fig. 4; frequency by community interaction: $F_{1,14} = 8.48$, $P < 0.01$). Note that fitness of SM and WS was equal at 1 : 1 ratios in both the absence and presence of the community (Fig. 4; $t_3 = 2.859$, $P < 0.065$; $t_3 = 0.808$, $P < 0.478$ respectively). Moreover, there was no evidence that SM and WS isolated from the treatments where the community was present or absent behaved differently (Fig. 4; frequency by community by selection–treatment interaction: $F_{1,14} = 0.32$, $P > 0.2$; frequency by selection–treatment interaction: $F_{1,14} = 1.9$, $P = 0.18$), ruling out the possibility that *P. fluorescens* had adaptively diversified in notably different way in the different treatments.

We also investigated the role of spatial heterogeneity in the maintenance of diversity of soil-evolved WS and SM by repeating the reciprocal invasion from rare assays in soil mixed with water and

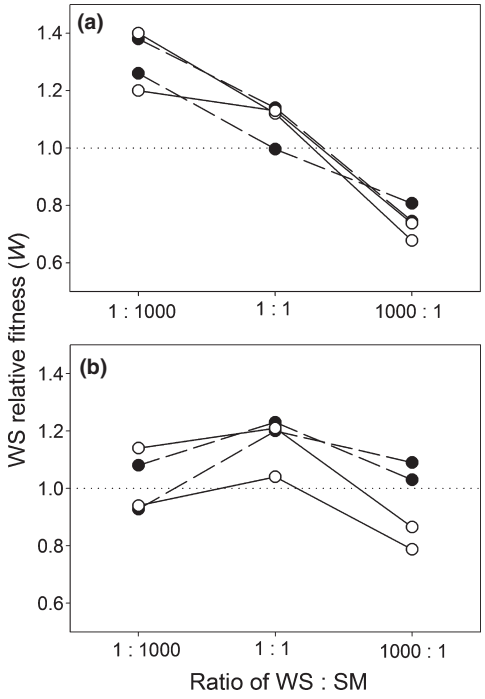

Figure 4 Competition between Smooth (SM) and Wrinkly Spreader (WS). Relative WS fitness at different WS : SM ratios in soil in the absence (a) and presence (b) of the natural microbial community in soil microcosms. Open symbols connected by solid lines represent each pair of evolved WS and SM genotypes isolated from populations evolved in the presence of the resident community. Closed symbols connected by dashed lines represent each pair from populations evolved in the absence of the resident community. When relative fitness = 1, WS and SM are equally fit. The four pairs of evolved WS and SM were independently competed under the different frequency and resident community conditions.

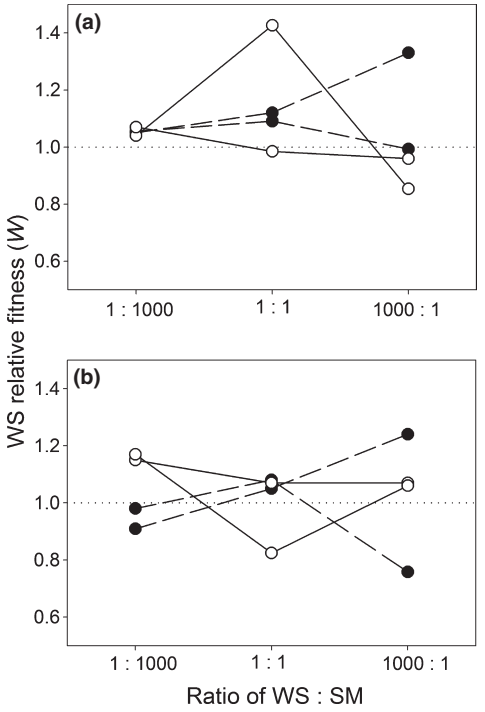

Figure 5 Competition between Smooth (SM) and Wrinkly Spreader (WS). Relative WS fitness at different ratios of WS : SM in shaken soil solution in the absence (a) and presence (b) of natural microbial community. Open symbols connected by solid lines represent each pair of evolved WS and SM genotypes isolated from populations evolved in the presence of the resident community. Closed symbols connected by dashed lines represent each pair from populations evolved in the absence of the resident community. When relative fitness = 1, WS and SM are equally fit. The four pairs of evolved WS and SM were independently competed under the different frequency and resident community conditions.

continually shaken. In this case, we observed no frequency-dependent selection in either the presence or absence of the resident community (Fig. 5; main effects and interaction: $P > 0.2$, in all cases), suggesting that the spatial structure of soil was important for the maintenance, and presumably the evolution, of diversity.

DISCUSSION

We have shown that the diversification of *P. fluorescens* population into two main morphological types (SM and WS) is constrained by the presence of a natural soil community (Fig. 1). Morphological diversity will of course inevitably underestimate genetic diversity. However, morphological differences correspond to differences in catabolic profiles, suggesting that colony morphologies are a useful measure of ecological diversity in this context, as is the case for this organism *in vitro* (Rainey & Travisano 1998) (Fig. 4). Our results are consistent with the observation that well-known adaptive radiations have occurred after major extinction events or on islands where there are few resident species to occupy existing niches (Schluter 2000; Jablonski 2001; Losos 2010).

Resident diversity is believed to impede diversification because of (1) reduced population size, and hence the supply of genetic variation required for adaption to novel ecological niches; and (2) reduced niche availability, which then reduces the selective advantage of escaping competition by adapting to a new ecological niche (Simpson 1953; Schluter 2000; Jablonski 2001; Losos 2010). Surprisingly, we found no net effect of the resident community (Fig. 2) on *P. fluorescens* density, suggesting this first mechanism plays a relatively unimportant role in this context. By contrast, we have good evidence that the resident microbial community reduced niche availability. Specifically, in the absence of the resident community competing, SM and WS morphotypes isolated from all treatments showed strong fitness advantages when rare (Fig. 4), demonstrating that competition within morphotypes was greater than between morphotypes, hence the morphotypes must have evolved to occupy different ecological niches (Chesson 2000; Schluter 2000). When the same genotypes were competed in the presence of the resident community, fitness was frequency independent, suggesting that WS and SM in the presence of the resident community were occupying the same ecological niche. Note that these competition experiments also measure frequency-dependent fitness of each morphotype with respect to the resident community, because the density of the resident community was held constant. The absence of frequency-dependent fitness in the presence of the resident community suggests that any diversifying selection mediated by interactions with the resident community or by intraspecific resource competition was much weaker than when the community was absent.

Why did populations still diversify to some extent in the presence of the natural community? First, diversifying selection may have

operated but our competition assays were not sensitive enough to detect it. Second, diversity may simply be the result of genetic drift. The SM and WS morphotypes were selectively neutral in the presence of the resident community (Fig. 4), and laboratory studies demonstrate that many different mutations can lead to the WS phenotype (Bantinaki *et al.* 2007). If WS morphotypes were continually being created by mutation, it seems plausible that the WS morphotype could have drifted to detectable frequencies in many of the replicates. It remains unclear to us how to distinguish between these two possibilities.

What ecological niches were WS and SM occupying in soil? The key feature of the WS morphotype *in vitro* is its ability to form a biofilm at the air–broth interface through over-expression of cellulose, allowing access to both broth nutrients and oxygen (Spiers *et al.* 2002, 2003; Koza *et al.* 2011). SM grows faster in the broth phase of the microcosm, as well as exploitatively colonising the WS biofilm once it has become established (Rainey & Rainey 2003). The importance of such niche differentiation remains unclear in soil, although our soil-evolved WS do have a greater propensity to form biofilms and attach to surfaces than soil-evolved SM. It is possible that the propensity of WS to form biofilms on solid surfaces, and hence potentially soil particles, could explain niche differentiation, if biofilm living conferred an advantage at the cost of reduced growth. However, the advantage of biofilm formation is typically most pronounced in stressful environments (Hall-Stoodley *et al.* 2004), hence if this trade-off governed niche differentiation we would expect greater selection for WS in the presence rather than the absence of natural communities. Previous studies using *in vitro* populations (MacLean *et al.* 2004) suggest that WS can use slightly different carbon sources than ancestral SM (Fig. 4), and the niche differentiation between soil-evolved SM and WS could be explained by this ability to use a range of carbon sources. These results suggest that *P. fluorescens* may have diversified into niche specialists that use slightly different nutrient resources.

While specialisation onto different resources in a homogeneous environment can promote adaptive diversification (Rosenzweig *et al.* 1994), spatial heterogeneity makes the process far easier by further reducing interspecific competition (Hedrick 1986). Our additional competition experiments suggest that diversification may have also been dependent on the spatial distribution of resources in soil. Specifically, we found that frequency-dependent selection was eliminated in both the presence and absence of the resident community when competition experiments were carried out in shaken, water-logged soil (Fig. 5). We cannot of course rule out the possibility that water logging and shaking soil is affecting the environment in numerous important ways over and above its impact on spatial structure.

We found no evidence for the presence of phages influencing diversification into different morphotypes, despite phages undergoing coevolution between resistance and infectivity traits in soil microcosms (Gómez & Buckling 2011). By contrast, coevolution in nutrient broth retards morphological diversification in spatially structured environments (Buckling & Rainey 2002b; Brockhurst *et al.* 2004). Nutrient broth and soil differ in numerous ways, but we highlight three obvious differences that may explain this discrepancy. First, the effects of phage on *P. fluorescens* diversity *in vitro* are greatly reduced in diluted nutrient media (Benmayor *et al.* 2008), which is presumably closer to soil nutrient concentrations than standard high nutrient media. Second, phage-imposed selection in nutrient media is primarily directional (i.e. bacteria evolved increase resistance through time), which purges diversity, whereas in soil coevolving phages impose fluctuating selection (Buckling & Rainey 2002b). Third, phages appear to cause larger reductions in population size in broth than in soil (Buckling & Rainey 2002b; Gómez & Buckling 2011).

These results raise general questions about the importance of interspecific interactions and the pace of adaptive evolution. It has long been argued (Van Valen 1973; Stenseth & Maynard-Smith 1984) that a population's adaptations are primarily driven by interactions with other organisms, because the biotic environment imposes both strong and continually changing selection pressures. As a result, the pace of adaptive evolution is predicted to be greater when adapting to biotic versus purely abiotic environments. While there is support for this prediction from studies that compare rates of evolution in the presence versus absence of limited numbers of enemies (e.g. Clark *et al.* 2007; Paterson *et al.* 2010), it remains unclear if this prediction holds in the context of interactions within whole communities (Stenseth & Maynard-Smith 1984). Our results suggest that adaptive evolution may in fact be constrained by community complexity, if this limits the potential to diversify into novel ecological niches.

The focus of this study is how the resident community influences the evolution of a focal, invading species. However, evolution of a focal species can in turn affect community structure (Harmon *et al.* 2009; Schoener 2011). It has recently been argued that prior adaption of a species can limit subsequent colonisation of other species, termed 'evolutionary priority effects' (Urban & De Meester 2009). Whether the ability to diversify in newly colonised habitats has further impact on subsequent community structure and function remains to be investigated.

Here, we have shown that the presence of a resident soil microbial community impedes adaptive diversification of an invading species. Of course, our resident communities were established from soil washes, so may not contain all community members. However, the results highlight the incredible ease at which bacteria can adaptively diversify in natural settings, which may have important implications in environments where resident communities are disturbed by agriculture and pollution (Hemme *et al.* 2010), and in clinical settings where antibiotics often remove much of the native microbial flora (Jernberg *et al.* 2010; Levert *et al.* 2010).

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AUTHORSHIP

PG and AB designed the research, analysed the data and wrote the manuscript and PG performed experimental analyses and collected data.

REFERENCES

Bantinaki, E., Kassen, R., Knight, C.G., Robinson, Z., Spiers, A.J. & Rainey, P.B. (2007). Adaptive divergence in experimental populations of *Pseudomonas fluorescens*. III. Mutational origins of wrinkly spreader diversity. *Genetics*, 176, 441–453.

Benmayor, R., Buckling, A., Bonsall, M.B., Brockhurst, M.A. & Hodgson, D.J. (2008). The interactive effects of parasites disturbance, and productivity on experimental adaptive radiations. *Evolution*, 62, 467–477.

Brockhurst, M.A., Rainey, P.B. & Buckling, A. (2004). The effect of spatial heterogeneity and parasites on the evolution of host diversity. *Proc. Biol. Sci.*, 271, 107–111.

Brockhurst, M.A., Colegrave, N., Hodgson, D.J. & Buckling, A. (2007). Niche occupation limits adaptive radiation in experimental microcosms. *PLoS ONE*, 2, e193.

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

Buckling, A. & Rainey, P.B. (2002b). The role of parasites in sympatric and allopatric host diversification. *Nature*, 420, 496–499.

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Ann. Rev. Ecol. Syst.*, 31, 343–366.

Clark, A.G., Eisen, M.B., Smith, D.R., Bergman, C.M., Oliver, B., Markow, T.A. *et al.* (2007). Evolution of genes and genomes on the Drosophila phylogeny. *Nature*, 450, 203–218.

Erwin, D.H. (2008). Macroevolution of ecosystem engineering, niche construction and diversity. *Trends Ecol. Evol.*, 23, 304–310.

Ezard, T.H.G., Aze, T., Pearson, P.N. & Purvis, A. (2011). Interplay between changing climate and species' ecology drives macroevolutionary dynamics. *Science*, 332, 349–351.

Friesen, M.L., Saxer, G., Travisano, M. & Doebeli, M. (2004). Experimental evidence for sympatric ecological diversification due to frequency-dependent competition in *Escherichia coli*. *Evolution*, 58, 245–260.

Fukami, T., Beaumont, H.J., Zhang, X.X. & Rainey, P.B. (2007). Immigration history controls diversification in experimental adaptive radiation. *Nature*, 446, 436–439.

Gavrilets, S. & Losos, J.B. (2009). Adaptive radiation: contrasting theory with data. *Science*, 323, 732–737.

Gómez, P. & Buckling, A. (2011). Bacteria-phage antagonistic coevolution in soil. *Science*, 332, 106–109.

Hall-Stoodley, L., Costerton, J.W. & Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. *Nat. Rev. Microbiol.*, 2, 95–108.

Harmon, L.J., B. Matthews, S., Des Roches, J., Chase, M., Shurin, J.B. & Schluter, D. (2009). Evolutionary diversification in stickleback affects ecosystem functioning. *Nature*, 458, 1167–1170.

Hedrick, P.W. (1986). Genetic-polymorphism in heterogeneous environments - a decade later. *Ann. Rev. Ecol. Syst.*, 17, 535–566.

Hemme, C.L., Deng, Y., Gentry, T.J., Fields, M.W., Wu, L., Barua, S. *et al.* (2010). Metagenomic insights into evolution of a heavy metal-contaminated groundwater microbial community. *ISME J.*, 4, 660–672.

Jablonski, D. (2001). Lessons from the past: evolutionary impacts of mass extinctions. *Proc. Natl. Acad. Sci. USA*, 98, 5393–5398.

Jernberg, C., Lofmark, S., Edlund, C. & Jansson, J.K. (2010). Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology*, 156, 3216–3223.

Koza, A., Moshynets, O., Otten, W. & Spiers, A.J. (2011). Environmental modification and niche construction: developing O2 gradients drive the evolution of the Wrinkly Spreader. *ISME J.*, 5, 665–673.

Lawrence, D., Fiegna, F., Behrends, V., Bundy, J.G., Phillimore, A.B., Bell, T. *et al.* (2012). Species interactions alter evolutionary responses to a novel environment. *PLoS Biol.*, 10, e1001330.

Lenski, R.E., Rose, M.R., Simpson, S.C. & Tadler, S.C. (1991). Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. *Am. Nat.*, 138, 1315–1341.

Levert, M., Zamfir, O., Clermont, O., Bouvet, O., Lespinats, S., Hipeaux, M.C. *et al.* (2010). Molecular and evolutionary bases of within-patient genotypic and phenotypic diversity in *Escherichia coli* extraintestinal infections. *PLoS Pathog.*, 6, e1001125.

Losos, J.B. (2010). Adaptive radiation, ecological opportunity, and evolutionary determinism. *Am. Nat.*, 175, 623–639.

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.

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

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.

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

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.

Ricklefs, R.E. (2010). Evolutionary diversification, coevolution between populations and their antagonists, and the filling of niche space. *Proc. Natl. Acad. Sci. USA*, 107, 1265–1272.

Rosenzweig, R.F., Sharp, R.R., Treves, D.S. & Adams, J. (1994). Microbial evolution in a simple unstructured environment: genetic differentiation in *Escherichia coli*. *Genetics*, 137, 903–917.

Schluter, D. (2000). *The Ecology of Adaptive Radiations*. Oxford University Press, Oxford.

Schoener, T.W. (2011). The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science*, 331, 426–429.

Simpson, E.H. (1949). Measurement of diversity. *Nature*, 163, 688.

Simpson, G.G. (1953). *The Major Features of Evolution*. Columbia University Press, New York.

Sokal, R.R. & Rohlf, F.J. (1962). The comparison of dendrograms by objective methods. *Taxon*, 11, 33–40.

Spiers, A.J., Kahn, S.G., Bohannon, J., Travisano, M. & Rainey, P.B. (2002). Adaptive divergence in experimental populations of *Pseudomonas fluorescens*. I. Genetic and phenotypic bases of wrinkly spreader fitness. *Genetics*, 161, 33–46.

Spiers, A.J., Bohannon, J., Gehrig, S.M. & Rainey, P.B. (2003). Biofilm formation at the air-liquid interface by the *Pseudomonas fluorescens* SBW25 wrinkly spreader requires an acetylated form of cellulose. *Mol. Microbiol.*, 50, 15–27.

Stenseth, N.C. & Maynard-Smith, J. (1984). Coevolution in ecosystems – Red Queen evolution or stasis. *Evolution*, 38, 870–880.

Urban, M.C. & De Meester, L. (2009). Community monopolization: local adaptation enhances priority effects in an evolving metacommunity. *Proc. Biol. Sci.*, 276, 4129–4138.

Van Valen, L. (1973). A new evolutionary law. *Evol. Theory*, 1, 1–30.

Whittaker, R.H. (1977). Evolution of species diversity in land communities. *Evol. Biol.*, 10, 1–67.

Zhang, Q.G. & Buckling, A. (2011). Antagonistic coevolution limits population persistence of a virus in a thermally deteriorating environment. *Ecol. Lett.*, 14, 282–288.

Zhang, Q.G., Ellis, R.J. & Godfray, H.C.J. (2012). The effect of a competitor on a model adaptive radiation. *Evolution*, 66, 1985–1990.

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