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Abiotic heterogeneity drives parasite local adaptation in coevolving bacteria and phages

L. LOPEZ PASCUA*, S. GANDON† & A. BUCKLING*‡

*Department of Zoology, University of Oxford, Oxford, UK
†CEFE – UMR 5175, 1919 route de Mende, Montpellier, France
‡Biosciences, University of Exeter, Penryn, UK

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Abstract

Spatial abiotic heterogeneity can result in divergent selection, hence might increase the magnitude of host–parasite local adaptation (the mean difference in fitness of sympatric vs. allopatric host–parasite combinations). We explicitly tested this hypothesis by measuring local adaptation in experimentally coevolved populations of bacteria and viruses evolved in the same or different nutrient media. Consistent with previous work, we found that mean levels of evolved phage infectivity and bacteria resistance varied with nutrient concentration, with maximal levels at nutrient concentrations that supported the greatest densities of bacteria. Despite this variation in evolved mean infectivity and resistance between treatments, we found that parasite local adaptation was greatly increased when measured between populations evolved in different, compared with the same, media. This pattern is likely to have resulted from different media imposing divergent selection on bacterial hosts, and phages in turn adapting to their local hosts. These results demonstrate that the abiotic environment can play a strong and predictable role in driving patterns of local adaptation.

Introduction

Host–parasite antagonistic coevolution can result in parasites having greater fitness on their local compared with foreign hosts (or *vice versa*), and such parasite (or host) local adaptation plays a key role in parasite epidemiology as well maintaining genetic diversity (Kaltz & Shykoff, 1998; Kawecki & Ebert, 2004). However, studies in both natural and laboratory populations frequently show no consistent pattern of either parasite or host local adaptation (Kaltz & Shykoff, 1998; Greischar & Koskella, 2007; Hoeksema & Forde, 2008). One likely explanation for this variation in patterns of local adaptation is abiotic heterogeneity. Recent theory suggests that spatial variation in the abiotic environment can play a key role in shaping patterns of local adaptation, if such variation alters selection imposed by host and parasites on each other (a selection mosaic) (Gandon & Nuismer, 2009; Gavrilets & Michalakis, 2008; Nuismer, 2006). Specifically, abiotic heterogeneity can maintain polymorphisms in host and parasite populations (Gavrilets & Michalakis, 2008; Nuismer, 2006), a pre-requisite for local adaptation, and can alter the sign and magnitude of local adaptation relative to homogeneous environments (Gandon & Nuismer, 2009). However, the precise impact of abiotic heterogeneity on local adaptation is highly contingent on the specific biology, such as gene flow between patches, the genetic bases of host–parasite specificity and the extent of genetic drift (Gandon & Nuismer, 2009).

More general predictions about the impact of abiotic heterogeneity on local adaptation can be made if it is assumed that abiotic heterogeneity imposes divergent selection on host or parasite or both populations (Laine & Tellier, 2008; Koskella *et al.*, 2011): an assumption not made in the theory discussed earlier, where instead environmental heterogeneity has been modelled as spatial variation in the strength of parasite and host-imposed selection acting on hosts and parasites, respectively (Gandon & Nuismer, 2009; Gavrilets & Michalakis, 2008; Nuismer, 2006). Specifically, abiotic heterogeneity

Correspondence: A. Buckling, University of Exeter in Cornwall, Tremough, Penryn, Cornwall, TR10 9EZ, UK.
Tel.: +44 1326 371 800; fax: +44 1326 371 859;
e-mail: A.J.Buckling@exeter.ac.uk

is likely to increase local adaptation if different resistance (or infectivity) traits dominate in different environments as a result of direct or correlated selection imposed by the abiotic environment (Laine & Tellier, 2008; Koskella *et al.*, 2011). Although a number of empirical studies in both field and laboratory systems suggest the abiotic environment to be an important driver in host–parasite local adaptation (Brodie & Ridenhour, 2002; Laine, 2008; Thrall *et al.*, 2002; Toju & Sota, 2006; Zangerl & Berenbaum, 2003; Forde *et al.*, 2004, 2007, 2008; Laine, 2009), there has been no explicit test of the hypothesis that local adaptation is greater when measured between communities coevolved in different abiotic environments compared with the same abiotic environments. Here, we carry out such a test using experimentally coevolving populations of bacteria and bacteriophages.

We measured local adaptation in experimentally coevolved populations of the bacterium *P. fluorescens* and an associated lytic bacteriophage (Buckling & Rainey, 2002a), where communities were coevolved in either the same or different nutrient media. In this system, phages bind to susceptible bacteria, inject in their DNA, which then hijacks the bacterial cellular machinery to make multiple new phage particles. Phages then lyse the bacterial cell, and the life cycle starts again. Bacteria rapidly evolve resistance to phages, and phages in turn evolve counter-defence mechanisms to allow them to infect cells. This interaction results in a coevolutionary arms race, with bacteria and phages with increasing mean resistance and infectivity favoured by selection through time (Buckling & Rainey, 2002a; Morgan *et al.*, 2007; but see Hall *et al.*, 2011), with increased resistance and infectivity associated with fitness costs in the absence of phages (Buckling *et al.*, 2006; Hall *et al.*, 2011), and on ancestral bacteria (Poullain *et al.*, 2008), respectively. These coevolutionary dynamics are somewhat reminiscent of a multi-locus gene for gene model of specificity with costs (Sasaki, 2000) [or an inverse gene for gene model (Fenton *et al.*, 2009)]. Local adaptation of both bacteria and phages (as determined by the proportion of resistant/susceptible bacteria to a given phage population) has been detected under common garden conditions in both long-term (but not short-term) experimentally evolved (Buckling & Rainey, 2002a; Morgan *et al.*, 2005; Morgan & Buckling, 2006) and natural populations (Vos *et al.*, 2009; Koskella *et al.*, 2011), and we use a similar approach here. Although resistance and infectivity are only single components of host and parasite fitness, respectively (Laine, 2008), in this system the proportion of resistant hosts positively correlates with net population growth rate of bacteria, and negatively correlates with the net growth rate of phages (Benmayor *et al.*, 2009) in the media in which populations evolve. This is unsurprising, given that phages must infect hosts to replicate, and successful phage replication involves lysis of bacteria.

We chose to manipulate abiotic heterogeneity by altering nutrient concentration, for a number of reasons. First, variation in nutrient availability is ubiquitous in natural populations (Rosenzweig, 1995). Second, coevolutionary dynamics between bacteria and phages are affected by nutrient concentration, with mean infectivity and resistance evolving to higher levels in high- compared with low-nutrient concentrations (Forde *et al.*, 2004, 2007, 2008 Lopez Pascua *et al.*, 2010; Lopez Pascua & Buckling, 2008). Variation in mean infectivity and resistance levels could readily obscure patterns of local adaptation (e.g. if high nutrient concentrations result in the evolution of the most globally resistant and infective hosts and parasites, respectively; Thrall *et al.*, 2002), allowing for a relatively conservative test of the hypothesis that abiotic heterogeneity increases local adaptation. Third, experimental evolution of *P. fluorescens* in the absence of phage has revealed the operation of divergent selection between high- and low-nutrient media. Specifically, populations adapted to media with or without amino acids showed increased growth in their selective environment, but not in the alternative environment (Buckling *et al.*, 2007). Moreover, different frequencies of bacteria colony morphotypes are favoured under the different nutrient concentrations used in this study (Kassen *et al.*, 2000; Benmayor *et al.*, 2008), and these morphotypes differ in a range of fitness-related traits (Rainey & Travisano 1998; Rainey & Rainey 2003), including resistance to phages (Brockhurst *et al.*, 2004; Buckling & Rainey, 2002b).

Materials and methods

Culture conditions

Bacteria and phage were cultured in 6 mL of M9 salt solution supplemented with differing amounts of glycerol and proteose peptone (0.01, 0.1, 0.5, 1 and 5 times the standard concentration for King's Media B (KB) of 10 g L^{-1} glycerol and 20 g L^{-1} of proteose peptone no.3), in 25-mL glass universals with loose plastic lids (microcosms) (Lopez Pascua & Buckling, 2008). Bacteria were derived from a single *Pseudomonas fluorescens* SBW25 (Rainey & Bailey, 1996) clone grown overnight in KB broth, at 28 °C, in an orbital shaker at 200 r.p.m. (0.9 *g*).

Six replicate microcosms for each treatment were inoculated with 10^8 bacterial cells and 10^5 clonal phage particles, obtained from a single plaque of a clone of phage SBW25 ϕ2 (Buckling & Rainey, 2002a). The bacteria and phage were then allowed to coevolve in a static incubator at 28 °C for a 48-hour time period before 1% (60 μL) of the total population was transferred into a fresh microcosm, following vortex mixing to homogenize the culture. This process was repeated for a total of 16 transfers (approximately 120 generations). Every second transfer, samples (600 μL) of the total populations were frozen at −86 °C in 20% v : v glycerol : KB solution, and a sample of phage isolated from bacteria by adding

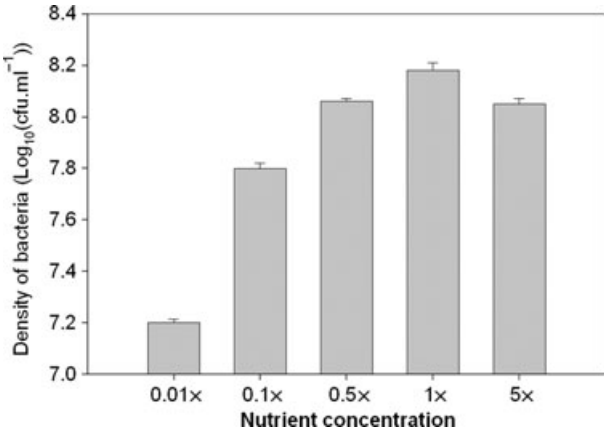

Fig. 1 Mean (± SEM) density of bacteria after 48 h growth in different media (nutrient concentrations; multiple of standard King's Media B).

100 μL of chloroform to 900 μL of the culture, and centrifuging at 13 000 r.p.m. (6 000 *g*) for 3 min, to lyse and pellet bacteria.

Measuring effect of media on bacterial growth

Three replicate microcosms of each media type were inoculated with 10^8 bacterial cells grown overnight at 0.9 g at 28 °C. Bacterial densities were estimated by plating culture onto KB agar and determining the number of colony-forming units (CFU) after 48 h growth at 28 °C. Media had a significant effect on bacterial densities, with bacteria in the 1× media achieving the greatest density, approximately 10-fold greater than bacteria in the 0.01× media, which was the lowest density (Fig. 1; $F_{4,10} = 36.77$; $P < 0.001$). Consistent with previous studies (Kassen *et al.*, 2000; Benmayor *et al.*, 2009), media at higher concentrations than normal KB inhibits growth to some extent.

Measurement of resistance

Resistance of a bacterial population against a given phage population was determined by streaking 20 independent bacterial colonies across a perpendicular line of phage (20 μL) that had previously been streaked and dried onto a KB agar plate. A colony was defined as resistant if there was no inhibition of growth; otherwise it was defined as sensitive. Population resistances were measured as proportions. At three time points (transfers 4, 8 & 12), we determined the resistance of each bacterial population to (i) its sympatric phages, (ii) to all other phages within the same experimental treatment from the same time point, and (iii) to a single phage population from each of the four other productivity treatments from the same time point (a single replicate from each of the different treatments was assigned to one of six independent blocks).

Statistical analyses

We first wanted to determine how nutrient concentration in which bacteria evolved affected the mean resistance of bacteria populations to phage populations evolved in the different nutrient concentrations, and whether there was an interaction between bacteria and phage evolution environment. This analysis was therefore carried out on sympatric and between-treatment bacteria–phage interactions only. We carried out a General Linear Mixed Model (GLMM) using Restricted Maximum Likelihood (REML) in JMP v8 (SAS, Cary, NC, USA), with angular-transformed (proportion of resistant bacteria in a population) fitted as the response variable with a normal error structure and identity link (standard least squares personality); nutrient concentration of both the bacteria and phage selection environment, and their interaction, as two 5-level factors; time as a 3-level factor; block as a random effect; and phage and bacteria populations as random effects, nested within phage and bacteria treatments, respectively, and block. A significant interaction term between phage and bacteria treatment would be consistent with local adaptation, but an explicit test requires orthogonal contrasts between sympatric and allopatric combinations (Thrall *et al.*, 2002). We therefore carried out a second analysis on this data set, replacing bacteria and phage treatments with a single 2-level factor, Sympatric/Allopatric, and fitting its interaction with time. Given that we found evidence for local adaptation when measured across all nutrient treatments, we repeated the latter analysis for all pairwise treatment combinations, controlling for multiple comparisons using a sequential Bonferroni correction (Rice, 1989). Model assumptions (normality and homogeneity of residuals) were confirmed.

We next determined whether there was any evidence of local adaptation within each nutrient treatment. We carried out a GLMM using REML as above with angular-transformed (proportion of resistant bacteria in a population) fitted as our response variable with a normal error structure and identity link; nutrient concentration of the bacteria and phage selection environment as a 5-level factor; time as a 3-level factor; an orthogonal contrast of sympatric vs. allopatric combinations as a 2-level factor; and phage and bacteria populations as random effects, both nested within nutrient concentration. It should be noted that these analyses of local adaptation simultaneously measure local adaptation from 'home vs. away' and 'local vs. foreign' perspectives (Kawecki & Ebert, 2004), as all possible pairwise interactions were measured within block (between-treatment analyses) or within treatment (within-treatment analyses).

Finally, we wanted to determine whether parasite local adaptation (proportion of resistant allopatric bacteria – proportion of resistant sympatric bacteria) showed any relationship with differences in global resistance (i.e. mean proportion of resistant bacteria to phages from all

environments) and infectivity (mean of 1-proportion of resistant bacteria from all environments) for each pairwise media combination. We therefore calculated the difference in global resistance (mean resistance$_x$ – mean resistance$_y$) and global infectivity (mean infectivity$_x$ – mean infectivity$_y$) for each pairwise (x and y) nutrient treatment combination. As a summary measure, we calculated the mean of these differences in infectivity and resistance, and then correlated this with parasite local adaptation for each pairwise nutrient treatment combination.

Results

The mean proportion of resistant bacteria for all pairwise combinations of bacteria and phage selection environments are shown in Fig. 2. Mean resistance varied with time ($F_{2,370} = 5.17$, $P < 0.01$), with the highest resistance at transfer 8 (compared with transfers 4 and 12), and also differed between nutrient concentrations in which bacteria and phages evolved. Specifically, mean resistance increased with increasing nutrient concentration in which bacteria evolved upto the 1× concentration, but then subsequently decreased as nutrient concentration further increased (Fig. 3a; $F_{4,18} = 18.7$, $P < 0.0001$). Likewise, the mean proportion of susceptible bacteria (a measure of phage infectivity) showed the same pattern with respect to the nutrient concentration in which phage evolved (Fig. 3b; $F_{4,23} = 8.6$, $P < 0.0002$). It should be noted that these patterns also mirror that of the maximum densities achieved by bacteria after 48 h growth in the absence of phages (see Materials and Methods and Fig. 1). As a result of treatment differences in mean resistance and infectivity, some nutrient treatments (notably the 1×) will simply result in universally more resistant bacteria and more infective phages than other treatments. This will inevitably obscure local adaptation to some extent, with bacteria from high-nutrient treatments less resistant to their sympatric phages than to phages from low-nutrient treatments; and bacteria from low-nutrient treatments more resistant to their sympatric phages than to phages from high-nutrient treatments (Fig. 2). Despite this, there was a strong interaction between phage and bacteria selection environment ($F_{16,366} = 3.54$, $P < 0.0001$), suggesting the possibility that parasites (or hosts) were on average locally adapted (have higher fitness with sympatric than allopatric enemies).

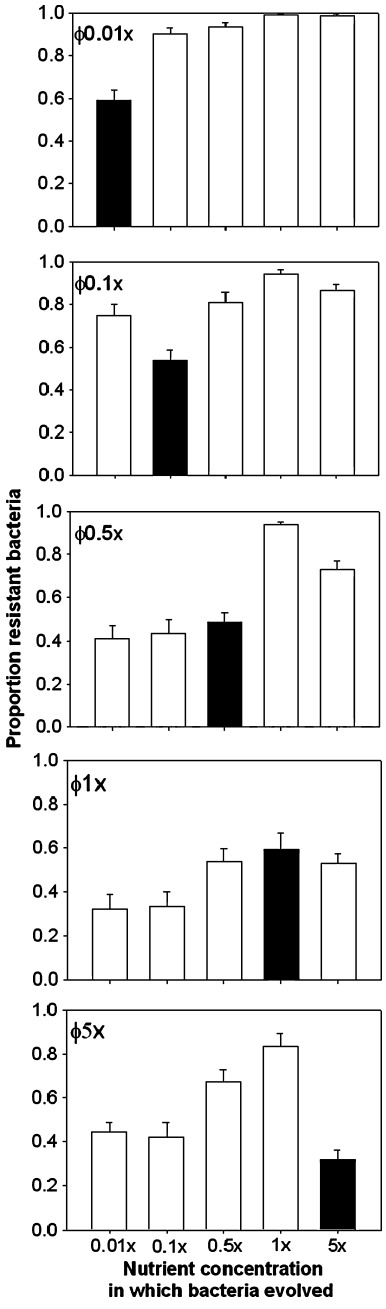

Fig. 2 Mean (± SEM) proportion of resistant bacteria evolved in different nutrient concentrations to phages evolved in different nutrient concentrations (from top to bottom: 0.01×, 0.1×, 0.5×, 1×& 5× standard concentration). Black bars indicate sympatric interactions.

Local adaptation

To further investigate whether phage (or bacteria) were locally adapted across selection environments, we determined whether mean resistance differed between sympatric and allopatric combinations of bacteria and phages. On average, 50% of bacteria were resistant to sympatric phages, whereas 69% were resistant to allopatric phages, demonstrating strong phage local adaptation ($F_{1,378.6} = 41.9$, $P < 0.0001$). This difference between resistance to sympatric and allopatric phages varied in time (time by sympatric/allopatric interaction: $F_{2,378.5} = 7.44$, $P < 0.001$), with no evidence of local adaptation at transfer 12, but large effects at transfers 4 and 8. All ten specific treatment combinations showed positive values

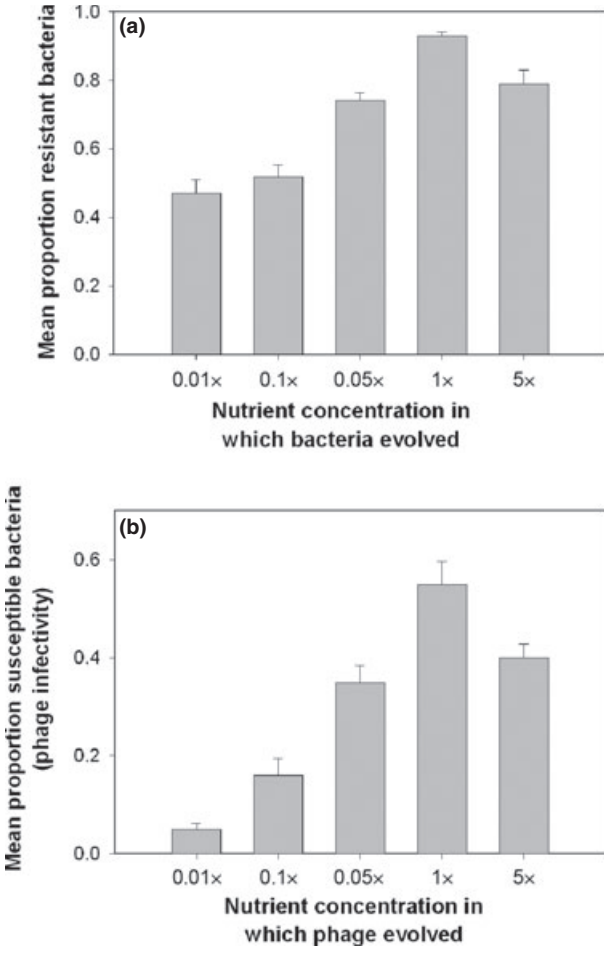

Fig. 3 Mean (± SEM) proportion of resistant bacteria (a) and phage infectivity (proportion of susceptible bacteria) (b) evolved in different nutrient concentrations.

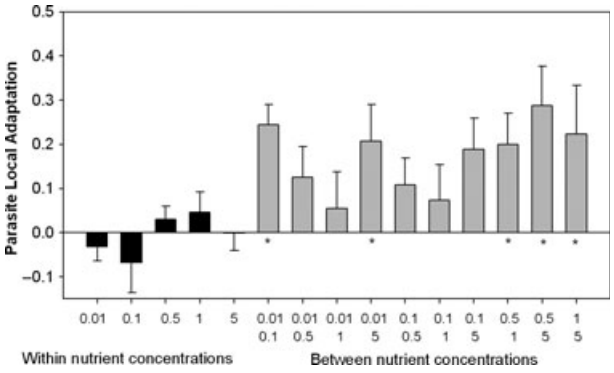

Fig. 4 Mean (± SEM) parasite local adaptation (allopatric–sympatric proportion of resistant bacteria) within each nutrient concentration (black bars), and between different nutrient concentrations (Gray bars), where *indicates significant local adaptation.

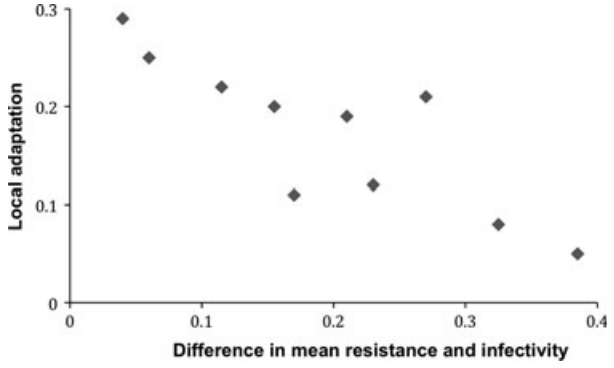

Fig. 5 The relationship between-nutrient concentration local adaptation (allopatric–sympatric proportion of resistant bacteria) and mean of the differences in infectivity and resistance between phages and bacteria evolved in the corresponding nutrient concentrations.

for parasite local adaptation, five of which were significant after correcting (using a sequential Bonferroni test; Rice, 1989) for multiple testing (Fig. 4).

By contrast, we found no evidence for local adaptation within selection environments across the whole data set (i.e. no difference in mean resistance between sympatric and allopatric combinations; Fig. 4, $F_{1,425} = 0.03$, $P > 0.2$), nor did local adaptation within selection environments differ between the nutrient treatments or time (interaction terms: $P > 0.2$, in both cases). Mean resistance however varied between time points ($F_{2,422} = 16.57$, $P < 0.0001$) and selection environment ($F_{4,\ 41} = 4.68$, $P = 0.003$).

The aforesaid data suggests that large differences in mean resistance and infectivity between particular treatment combinations obscured local adaptation. Consistent with this view, we found a highly significant negative correlation between mean parasite local adaptation and the difference in mean global resistance and infectivity for each pairwise treatment combination (Fig. 5; Spearman's rank correlation: $\rho = 0.85$, $P = 0.0017$). In other words, parasite local adaptation was greatest between two different nutrient treatments, when the different nutrient treatments resulted in similar mean levels of resistance and infectivity.

Discussion

We empirically investigated the importance of the abiotic environment in driving patterns of host–parasite local adaptation in coevolving populations of bacteria and parasitic viruses (phages). Specifically, we tested the hypothesis that local adaptation is greater between replicate communities that coevolved in different types of abiotic environment, compared with the same type of abiotic environment. Our results are consistent with this hypothesis: sympatric phages showed greater infectivity than allopatric phages when communities were

coevolved in media containing different concentrations of nutrients, whereas there was no evidence of local adaptation when communities were coevolved in the same media type. Whereas previous studies have similarly not shown local adaptation in the same media after short-term (as in this study) coevolution, longer-term coevolution of these communities can lead to local adaptation (Morgan *et al.*, 2005; Morgan & Buckling, 2006).

We found that mean resistance and infectivity increased with increasing nutrient concentration in which bacteria and phage evolved, up to the standard concentration for this media. This can be explained by the fact that rates of arm race (directional) coevolution have previously been shown to increase with increasing nutrient concentration (Lopez Pascua & Buckling, 2008; Lopez Pascua *et al.*, 2010), as a result of both increasing bacteria–phage encounter rates, increased mutation supply rate (through increased population sizes) and reduced costs of resistance (Lopez Pascua & Buckling, 2008). It should be noted that similar increases in mean resistance and phages with increasing nutrients have been reported in experimental studies of *Escherichia coli* and associated coevolving phages (Bohannan & Lenski, 2000; Forde *et al.*, 2004, 2007, 2008). The decline in resistance and infectivity between the 1× and 5× media probably reflects that this higher concentration of media is slightly toxic to bacteria, as shown by the lower density of bacteria after 48 h growth in the absence of phages (Fig. 1). As a result of this variation in mean resistance and infectivity, we found that phages evolved in high nutrient concentrations were often better at infecting bacteria from low nutrient media bacteria than their sympatric bacteria (i.e. phages were locally maladapted), whereas phages evolved in low nutrient concentrations were better at infecting sympatric bacteria than bacteria evolved in high nutrient concentrations (i.e. phages were locally adapted). These differences in global levels of resistance and infectivity obscured mean local adaptation across the different nutrient environments to some extent, because phages from high- and low-nutrient media have the opposite sign of local adaptation. Despite this, phages were still on average strongly locally adapted, when measured across all nutrient treatments. This result strongly contrasts the absence of local adaptation within-media, despite there being much less variation in global resistance and infectivity between populations evolved in the same, compared with different nutrient media (Figs 2–4).

What evolutionary mechanism is responsible for local adaptation in our study? Local adaptation occurs when there is variation in the frequency of host and parasite genotypes in different populations. This can result from random asynchronicity in genotype dynamics between populations driven by genetic drift or isolation by distance (Gandon & Michalakis, 2002; Gandon *et al.*, 1996; Gandon & Nuismer, 2009), or as a result of heterogeneity in abiotic selection pressures (Gavrilets & Michalakis, 2008; Gandon & Nuismer, 2009). Despite a lack of connectivity between replicate communities, local adaptation was not detected between communities evolved in the same nutrient concentration, which suggests asynchronicty in genotype dynamics is probably not driven by genetic drift in this study.

Recent theory has shown that abiotic heterogeneity can increase local adaptation, where abiotic heterogeneity results in spatial variation in the strength of host and parasite-imposed selection. Specifically, under a Gene for Gene model of coevolution, positive, compared with no, spatial covariance between the strength of selection on host and parasite populations can increase parasite local adaptation (Gandon & Nuismer, 2009). Our data are superficially consistent with this prediction in that we observe greater local adaptation between environments (where there is strong positive spatial covariance between host resistance and parasite infectivity) than within environments. However, our experimental set up differs markedly from the assumptions in these models, and similarities in results are therefore likely to be coincidental. Specifically, the model assumes gene flow, which is absent in our experiments, and the single-locus di-allelic bases of host–parasite specificity assumed in the model does not capture the complicated genetics involved in bacteria–phage specificity in this system (Paterson *et al.*, 2010; Scanlan *et al.*, 2011). Finally, in contrast to theoretical predictions, here we observed the strongest between-environment local adaptation where mean levels of resistance and infectivity are the most similar between environments, i.e. when spatial covariance is low.

We instead suggest that increased local adaptation between-nutrient selection environment resulted from divergent selection: different alleles were favoured in different nutrient concentrations, and this results in correlated (or direct) selection on phage-resistance traits. This in turn resulted in divergent selection on phage infectivity alleles between selection environments and hence, phage local adaptation. We have some evidence to support this view. First, in a study investigating experimental adaptation of *P. fluorescens* populations to media with or without proteose peptone (the same source of amino acids as manipulated in the current study), populations showed increased growth in their selective environment, but not in the alternative environment in the absence of gene flow (Buckling *et al.*, 2007). Second, different frequencies of bacteria colony morphotypes are favoured under the different nutrient concentrations used in this study (Kassen *et al.*, 2000; Benmayor *et al.*, 2008), and these morphotypes differ in a range of fitness-related traits (Rainey & Travisano 1998; Rainey & Rainey 2003), including resistance to phages (Brockhurst *et al.*, 2004; Buckling & Rainey, 2002b). We do not have a molecular mechanistic understanding of how phage specialization could increase as a result of

variation in nutrients, but we can speculate. Bacteria express membrane nutrient transporter proteins in response to resource availability (Gorke & Stulke, 2008). When resources are at high concentrations, they will typically express transporter proteins specific to the most nutritious resource, whereas when resources are less available they express receptors for multiple resources (Lendenmann *et al.*, 1996). We have evidence to suggest that ϕ2 binds to a part of lipopolysaccharide (LPS) membrane of SBW25 (P. Scanlan, A. Hall & A. Buckling, unpublished data), and it is possible that expression of different numbers and types of nutrient transport proteins somehow alters the confirmation of the LPS.

Where local adaptation occurred between populations evolved in different nutrient concentrations, it is the phages, rather than bacteria, that were locally adapted. We suggest this is because different resistant alleles dominated in different environments (rather than temporal asynchronicity of the same resistance alleles; Gandon *et al.*, 1996), and therefore, even a very slowly evolving parasite is likely to be better able to infect its own than foreign hosts. It should be noted, however, that parasite, rather than host, local adaptation might not always be expected in spatially heterogeneous environments if, for example, different parasite, rather than host, genotypes are selected by different abiotic conditions (Mitchell *et al.*, 2005; Laine, 2007).

The spatial pattern of adaption of the present study is in contrast to within-nutrient concentration local adaptation which occurs in this system if populations are left to diverge for sufficiently long (in excess of 50 transfers), where it is the bacteria that are typically locally adapted in the absence of migration (Buckling & Rainey, 2002a; Morgan *et al.*, 2005; Morgan & Buckling, 2006). (It should be noted that neither host nor parasite within-nutrient concentration local adaptation has been detected in previous studies where communities were coevolved for comparable amounts of time to the present study (Morgan *et al.*, 2005; Morgan & Buckling, 2006). Local adaptation is sometimes interpreted as informing which species has the evolutionary advantage in a coevolutionary arms race (Ebert, 1994; Greischar & Koskella, 2007; Hoeksema & Forde, 2008; Kaltz & Shykoff, 1998), but our data (and other recent work; Gomez & Buckling, 2011) suggests caution with this interpretation. There is much evidence to suggest that bacteria typically have the evolutionary advantage in this system (they are locally adapted, and it is phages that benefit most from elevated mutational supply via immigration (Morgan *et al.*, 2007, 2005), yet we observe strong parasite local adaptation with abiotic heterogeneity. Spatial heterogeneity in both the abiotic and biotic environment may therefore play a key role in explaining patterns of local adaptation in natural populations (Thompson 2005).

It is important to emphasize that our study might underestimate the importance of abiotic heterogeneity on local adaptation. First, host–parasite interactions were measured in a common garden (on agar plates), rather than in the selective environments (the different media), and this additional environmental influence can further increase (or decrease) local adaptation (Nuismer & Gandon, 2008). Second, abiotic environments are likely to vary in their propensity to generate genetic bottlenecks, and the resultant increase in genetic drift can further enhance local adaptation (Gandon & Nuismer, 2009). Third, variation in mean infectivity and resistance levels between environments (as observed here) readily obscure patterns of local adaptation.

In summary, we have shown that abiotic heterogeneity can increase parasite local adaptation in experimental populations of microbes. The suggested mechanism is that different resistance alleles dominate in different abiotic environments as a result of direct or correlated selection. How far can this result be generalized? There are a growing number of studies reporting genotype by environment interactions for both host resistance and parasite infectivity traits (Mitchell *et al.*, 2005; Laine, 2007; Laine & Tellier, 2008; Wolinska & King, 2009; Seppala & Jokela, 2010). Moreover, a recent study of natural bacteria–phage interactions on horse-chestnut trees (Koskella *et al.*, 2011) reported much stronger phage local adaptation to bacteria between trees than within trees, which is consistent with biotic heterogeneity increasing divergence in resistance traits. More studies are needed to determine whether spatial heterogeneity, both abiotic and biotic, is a predictable driver of parasite local adaptation.

Acknowledgments

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