

MICROBIAL LOCAL ADAPTATION

The emergence of performance trade-offs during local adaptation: insights from experimental evolution

LISA M. BONO,¹ LENO B. SMITH JR, DAVID W. PFENNIG and CHRISTINA L. BURCH 
Department of Biology, University of North Carolina at Chapel Hill, CB# 3280, Chapel Hill, NC 27599, USA

Abstract

Environmental heterogeneity is considered a general explanation for phenotypic diversification, particularly when heterogeneity causes populations to diverge via local adaptation. Performance trade-offs, such as those stemming from antagonistic pleiotropy, are thought to contribute to the maintenance of diversity in this scenario. Specifically, alleles that promote adaptation in one environment are expected to promote maladaptation in alternative environments. Contrary to this expectation, however, alleles that underlie locally adaptive traits often fail to exhibit fitness costs in alternative environments. Here, we attempt to explain this paradox by reviewing the results of experimental evolution studies, including a new one of our own, that examined the evolution of trade-offs during adaptation to homogeneous versus heterogeneous environments. We propose that when pleiotropic effects vary, whether or not trade-offs emerge among diverging populations will depend critically on ecology. For example, adaptation to a locally homogeneous environment is more likely to occur by alleles that are antagonistically pleiotropic than adaptation to a locally heterogeneous environment, simply because selection is blind to costs associated with environments that are not experienced locally. Our literature review confirmed the resulting prediction that performance trade-offs were more likely to evolve during selection in homogeneous than heterogeneous environments. The nature of the environmental heterogeneity (spatial versus temporal) and the length of the experiment also contributed in predictable ways to the likelihood that performance trade-offs evolved.

Keywords: antagonistic pleiotropy, environmental heterogeneity, experimental evolution, local adaptation, trade-offs

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Introduction

Local adaptation – the evolution of features in separate populations that render the members of each such population better able to survive and/or reproduce in its particular environment (Williams 1966) – is common among natural populations (Hereford 2009), and it is thought to play a key role in the evolution of novel ecotypes and even reproductive isolation (Hedrick 1986; Kawecki & Ebert 2004; Rundle & Nosil 2005; Schluter & Conte 2009). Long-standing theory suggests that such

adaptation to one environment comes at the expense of slower adaptation (Whitlock 1996) and even maladaptation (Lynch & Gabriel 1987; Kawecki & Ebert 2004) to foreign environments, resulting in performance trade-offs across environments. Understanding the genetic bases of these trade-offs is therefore crucial for clarifying the conditions under which local adaptation and its consequences come about.

Performance trade-offs associated with local adaptation are generally presumed to arise when locally adaptive alleles are antagonistically pleiotropic (i.e. costly) or neutral with regard to fitness in an alternative (i.e. foreign) environment (Fry 1996; Kawecki 1997; Kawecki & Ebert 2004; Mitchell-Olds *et al.* 2007; Hall *et al.* 2010; Anderson *et al.* 2011; Ågren *et al.* 2013). However, adaptive alleles that are neutral with regard to fitness in

Correspondence: Christina L. Burch, Fax: 919-962-1625;

E-mail: cburch@bio.unc.edu

¹Present address: Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA

alternative environments (i.e. conditionally neutral) are not expected to contribute to polymorphism within populations (Tiffin & Ross-Ibarra 2014), nor to anything more than transient local adaptation between populations (Fry 1996; Kawecki 1997) unless gene flow is severely restricted. Instead, conditionally neutral alleles are expected to homogenize between local populations with even modest levels of gene flow and recombination (Mitchell-Olds *et al.* 2007; Hall *et al.* 2010; Fournier-Level *et al.* 2011).

Contrary to this conceptual expectation, investigations of the genetic basis of local adaptation in natural populations have more often found evidence for conditional neutrality than antagonistic pleiotropy (Weinig *et al.* 2003; Verhoeven *et al.* 2004, 2008; Gardner & Latta 2006; Latta *et al.* 2007, 2010; Latta 2009; Lowry *et al.* 2009; Hall *et al.* 2010; Anderson *et al.* 2013; Leinonen *et al.* 2013; Remington *et al.* 2013; Soudi *et al.* 2015), but see (Ågren *et al.* 2013; Anderson *et al.* 2013; Oakley *et al.* 2014). We lack enough evidence to make broad generalizations about the genetic basis of local adaptation, in part because of possible sources of bias in the data (Oakley *et al.* 2014). For instance, most of the studied populations are plants, several of those are predominantly selfing, and many do not exhibit reciprocal local adaptation. Another possible explanation for the bias against antagonistic pleiotropy is statistical (Ågren *et al.* 2013; Anderson *et al.* 2013). Antagonistic pleiotropy is more difficult to detect, because it requires both a statistically significant *benefit* in the *local* environment and a statistically significant *cost* in a *foreign* environment. Conditional neutrality, by contrast, requires only a statistically significant benefit in the local environment.

However, theory and laboratory evolution experiments suggest several plausible *biological* explanations for the bias against findings of antagonistic pleiotropy in local adaptation in nature. These explanations stem from the conceptual expectation (as in Fisher's Geometric Model; Fisher 1930; Schick *et al.* 2015) and empirical observation (Ostrowski *et al.* 2005; Duffy *et al.* 2006; Ferris *et al.* 2007; Lalić *et al.* 2011; Schick *et al.* 2015; Dillon *et al.* 2016) that the pleiotropic effects of adaptive mutations vary such that the universe of adaptive mutations includes both conditionally neutral and antagonistically pleiotropic alleles (Fig. 1). [Note that this view stands in contrast to exemplars in which the same morphological, physiological and/or behavioural adaptations that enable use of one resource necessarily prevent use of alternative resources (Benkman 1996; Robinson *et al.* 1996; Bolnick *et al.* 2003; Martin & Pfennig 2009; Ellerby & Gerry 2011).] If the pleiotropic effects among adaptive mutations do indeed generally vary from conditionally neutral to antagonistically pleiotropic, then the genetic basis of local adaptation is expected to depend on both

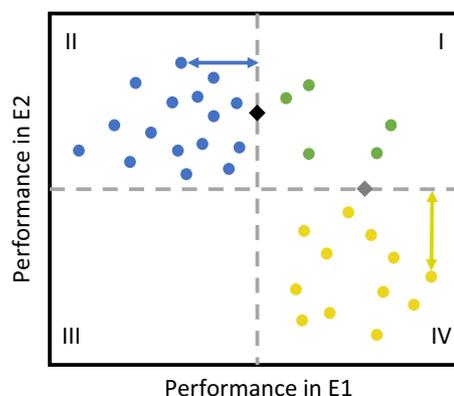


Fig. 1 Evolutionary consequences of variance in the pleiotropic effects of adaptive mutations. Dashed lines illustrate the performance of an ancestral genotype in alternative environments E1 and E2. Points illustrate the performance of a hypothetical collection of all the genotypes that are accessible by a single mutation and that achieve improved performance in at least one of the environments. The presence of mutant genotypes in all of quadrants I, II and IV is a common feature of empirical measures of adaptive mutations sampled from the bacteriophage $\phi 6$ (Duffy *et al.* 2006; Ferris *et al.* 2007) and other microbes (Dillon *et al.* 2016; Lalić *et al.* 2011; Ostrowski *et al.* 2005; Schick *et al.* 2015). Heterogeneous environments consisting of both E1 and E2 should favour the mutants in quadrant I (green circles and black and grey diamonds) that avoid costs in both environments. By contrast, homogeneous environments consisting of only E2 should favour mutants that are adaptive in E2 whether or not they are costly in E1 (green and blue circles and black diamond). In our review, we assessed the emergence of costs of adaptation by comparing the performance of evolved and ancestral populations in the alternative environments employed in each study (blue and yellow arrows). Adaptation via antagonistically pleiotropic mutations (blue and yellow circles) will generate costs measured in this way; adaptation via conditionally neutral mutations (black and grey diamonds) will not.

the ecological and evolutionary history of the adapted population.

Ecological history – specifically, the nature of the experienced environmental heterogeneity – should have a major effect on the genetic basis of local adaptation. Compared to adaptation to a locally *heterogeneous* environment, adaptation to a locally *homogeneous* environment is expected to be more likely to occur via alleles that are antagonistically pleiotropic, that is costly in the alternative environment (Fig. 1). The reason for this expectation is that selection is blind to costs associated with environments that are not experienced locally (as would be the case in a locally homogeneous environment). Environmental heterogeneity increases the number of environments that are experienced locally and thereby imposes selection against costs of adaptation for performance in the experienced environments. This effect should be particularly strong in temporally

heterogeneous environments, in which all individuals necessarily experience the full range of environmental variation. By contrast, populations evolving in spatially heterogeneous environments experience selection against costs of adaptation only to the extent that migration ensures exposure of each individual to the range of environmental variation. If migration between environments is restricted or if individuals influence their realized environmental heterogeneity through the expression of habitat preferences, then costs of adaptation should emerge more often in spatially than in temporally heterogeneous environments (Kassen 2002).

As with ecological history, *evolutionary* history can also affect the genetic basis of local adaptation. Evolutionary history can potentially do so in two ways. First, if adaptation derives from standing genetic variation instead of new mutations (Barrett & Schluter 2008), as may be likely for larger colonizing populations, conditionally neutral alleles should make a greater contribution. In particular, conditionally neutral alleles that are neutral in a past environment – but adaptive in the current environment – should be present in the pool of standing genetic variation at a higher frequency than antagonistically pleiotropic alleles (Lenski 1988a; Hermisson & Pennings 2005; Ferris *et al.* 2007; Matuszewski *et al.* 2015; Dittmar *et al.* 2016). Second, the contribution of antagonistic pleiotropy to local adaptation may change over evolutionary time. On the one hand, early adaptive steps in a new environment might be less often characterized by performance costs in alternative environments than later adaptive steps, because populations start out farther from the biochemical or physical constraints that generate antagonistic pleiotropy (Jasmin & Zeyl 2013; Satterwhite & Cooper 2015; Schick *et al.* 2015). On the other hand, the costs associated with antagonistically pleiotropic alleles fixed early may be compensated by mutations at interacting loci later in the adaptive walk (Lenski 1988b; Remold 2012; Bono *et al.* 2015).

Even among laboratory evolution experiments, where one can control the ecological and evolutionary history of adapting populations, direct tests of these biological explanations have been rare. More common are experiments that include adaptation to particular homogeneous and/or heterogeneous laboratory environments, followed by measures of the costs of adaptation for performance in alternative laboratory environments (e.g. Reboud & Bell 1997; Turner & Elena 2000; Cooper *et al.* 2001b). Although these experiments rarely dissect the genetic basis of adaptation and do not represent direct tests of the above explanations, they may exhibit patterns that are more consistent with some explanations than others.

Here, we evaluate our central hypothesis that the emergence of performance trade-offs among diverging

populations can be predicted from knowledge of their ecological and evolutionary history. To do so, we use two approaches. First, we review the experimental evolution literature to determine whether costs of adaptation are indeed more likely to emerge during adaptation: (i) to homogeneous than to heterogeneous laboratory environments; (ii) to spatially than to temporally heterogeneous laboratory environments; and (iii) over longer than over shorter evolutionary timescales. Second, we present an illustrative experiment in the bacteriophage $\phi 6$. Our evolution experiment used a design that was common in the literature review: populations of the bacteriophage $\phi 6$ were propagated either in a homogeneous (one host) environment or in a spatially heterogeneous (two host) environment. The literature review confirmed the generality of our anecdotal observation in $\phi 6$, both supporting the hypothesis that the emergence of performance trade-offs among diverging populations can indeed be predicted from knowledge of their ecological and evolutionary history.

Methods

Literature review

We searched the Web of Science database (<http://apps.webofknowledge.com>) for experimental evolution studies that tested for costs associated with adaptation to different environments. We chose search terms that struck a balance between including relevant articles and filtering out papers that did not include tests of evolved trade-offs. We used the Advanced Search option with the following Boolean search terms: (TS = 'experimental evolution' OR TS = 'evolution experiment' OR (TS = evolution AND TS = 'experimental test')) AND (TS = (generali*) OR TS = (speciali*)). We also included a small number of studies that were missed by this search but known to us or cited by other studies identified in the search. We retained only studies that report the results of natural selection, rather than artificial selection, and that allowed a comparison of evolved and ancestral performance in at least two environments. For each study, we recorded the species under selection, the type of selection (e.g. temperature, carbon source, or host range), the length of the evolution experiment in generations, the experimental design (homogeneous or heterogeneous environment), the type of environmental heterogeneity (spatial or temporal) when relevant, and whether trade-offs were detected (no, mixed, yes).

Our categorization of the existence and nature of environmental heterogeneity depended on the description of the authors in the study. It is worth noting that in some cases, the distinction is subtle. For example, a few studies examined the adaptation of bacteria to a

well-mixed liquid environment that contained both of the carbon sources glucose and acetate (Helling *et al.* 1987; Kurlandzka *et al.* 1991; Rosenzweig *et al.* 1994). Because the authors described that the concentration of glucose fluctuated from high to low as the bacteria reproduced during each transfer, we categorized this example as temporally heterogeneous, even though the heterogeneity was not imposed by the experimenter. In the case of spatial heterogeneity, alternative resources were required to be spatially segregated. Thus, the addition of both glucose and acetate to a well-mixed bacterial culture environment did not constitute spatial heterogeneity, whereas the addition of two host types to a well-mixed viral culture did. In the case of temporal heterogeneity, we required that a shift between environments was enforced in each serial dilution so that shifts were happening on the order of 1–10 generations. When shifts occurred on longer timescales, for example after thousands of generations (Satterwhite & Cooper 2015), we considered the periods of adaptation to each homogeneous environment separately, using the first and last measurements of performance in each period as the ancestral and evolved measure, respectively.

Trade-offs were measured as a cost of adaptation for performance in one or more alternative environments and were detected exclusively from comparisons between *evolved* and *ancestral* genotypes or populations (see Fig. 1), not from comparisons between different evolved populations. When experimental lineages were adapted to a single homogeneous environment, costs of adaptation were detected as statistically significant decreases in performance in at least one alternative environment. When experimental lineages were adapted to a heterogeneous environment consisting of two or more component environments, costs of adaptation were detected as significant decreases in performance on at least one of the component environments. We exclusively compared the performance of evolved populations to their ancestor because adaptation via antagonistically pleiotropic, but not conditionally neutral, alleles should cause evolved populations to exhibit reduced performance relative to their ancestor in at least one alternative environment. For studies that investigated n replicate lineages, our characterization of trade-offs depended on the number of lineages, k , that exhibited decreases in performance in at least one alternative or component environment as follows:

- $k = 0$: no;
- $0 < k < n$: mixed;
- $k = n$: yes.

We note that trade-offs have more often been identified by comparing different evolved genotypes to each

other, for example Kassen (2002). If we had used this test instead, we would not have been able to distinguish between antagonistic pleiotropy (compare a blue and a yellow circle in Fig. 1) and conditional neutrality (compare the black and grey diamonds in Fig. 1) because both types of mutations give the same pattern: the evolved genotype with the highest performance in one environment (e.g. E1 in Fig. 1) has the lowest performance in the alternative environment (e.g. E2 in Fig. 1).

The data were analysed in R version 3.1.2 (R Core Team 2013) using ordered logistic regression models, estimated using the *polr* function from the *MASS* package. Observed trade-offs were used as the dependent variable with ordered levels no < mixed < yes. Independent variables included taxon (virus, bacteria, unicellular eukaryote), environment (homogeneous, heterogeneous), heterogeneity type (spatial, temporal) and experiment length in generations.

Illustrative experimental evolution study

We used an evolution experiment in the RNA bacteriophage $\phi 6$ to illustrate both the strengths and weaknesses of using laboratory evolution experiments to address our main hypothesis: when the distribution of pleiotropic effects is variable, trade-offs are more likely to emerge in homogeneous than in heterogeneous environments. $\phi 6$ is ideal for this purpose, because it evolves rapidly to infect novel host bacteria (Cuppels *et al.* 1980), and the pleiotropic effects of adaptive mutations that expand host range vary from antagonistically pleiotropic to conditionally neutral (Duffy *et al.* 2006; Ferris *et al.* 2007). Previous evolution experiments in homogeneous host environments (Duffy *et al.* 2007) resulted in stronger trade-offs than those in heterogeneous environments (Bono *et al.* 2013, 2015), but the experimental designs differed in more than just environmental heterogeneity. Here, we compare evolution in homogeneous and heterogeneous environments directly.

The $\phi 6$ used in this study is a laboratory strain descended from the original isolate (Vidaver *et al.* 1973). The bacteria *Pseudomonas syringae* pathovar phaseolicola strain HB10Y (hereafter *phaseolicola*) and *P. pseudoalcaligenes* pathovar ERA (hereafter *ERA*) were used as alternative hosts. Both host strains were generously provided by Lin Chao (University of California, San Diego). Details of diluting, filtering, culture and storage of phage and bacteria appear in Duffy *et al.* (2006) and Ferris *et al.* (2007). All phage and bacteria were grown in LC medium (5 g/L yeast extract, 10 g/L bactotryptone, 5 g/L NaCl) at 25 °C.

Our experiments were initiated with a pair of generalist and specialist phage clones that evolved in a

previous experiment (Bono *et al.* 2013), in which the standard laboratory strain of $\phi 6$, a *phaseolicola* specialist unable to infect *ERA*, was serially diluted into microcosms containing both *phaseolicola* and *ERA*. All experimental populations in that study diversified into *phaseolicola* specialists and *phaseolicola* + *ERA* generalists. The clones used here were isolated from the single lineage in which the evolved specialists and generalists coexisted stably.

Our current experiment used a design that has often been implemented in other taxa to investigate the evolution of performance trade-offs in homogeneous or in heterogeneous environments. Our design (diagrammed in Fig. 2) created the two ecological scenarios in which local adaptation is expected to occur: (i) populations that experience divergent selection in different locally homogeneous environments and (ii) populations in a spatially heterogeneous environment in which competition imposes divergent selection for alternative resource use. Specifically, we compared the evolution of generalist $\phi 6$ in a homogeneous environment containing the bacterial host *ERA* only and in a heterogeneous environment containing both of the bacterial hosts *phaseolicola* and *ERA*. Phage populations evolving in the homogeneous experiments were founded by a *phaseolicola* + *ERA* generalist phage clone only. By contrast, phage populations evolving in the heterogeneous experiments were founded by a 1:1 mixture of *phaseolicola* + *ERA* generalists and *phaseolicola* specialists in an attempt to ensure that generalists experienced directional selection for improved performance on *ERA* in both environmental treatments. As in many of the studies in our literature review, our experiment was not designed specifically to test whether trade-offs are more likely to emerge in homogeneous than in heterogeneous environments. Thus, confounding factors may exist. In our case, the presence of specialist competitors in the heterogeneous (but not homogeneous) environment might have had an (unknown) impact. Note that we could not choose to leave the specialist competitors out of our heterogeneous environment experiments because the generalists would not have experienced directional selection for adaptation to *ERA* in that case. We suspect our inability to design a perfectly controlled $\phi 6$ experiment to test the hypotheses presented in this study also characterized many of the experiments we reviewed.

In both homogeneous and heterogeneous treatments, each transfer was initiated with a total of 10^6 phage and 2×10^8 exponentially growing host cells, consisting of *phaseolicola* only (homogeneous environment) or 1:1 *phaseolicola*:*ERA* (heterogeneous environment), in 1 mL LC medium. The resulting phage cultures were incubated shaking at 25 °C for 6 h, at which time phage were harvested by filtering the culture to remove host

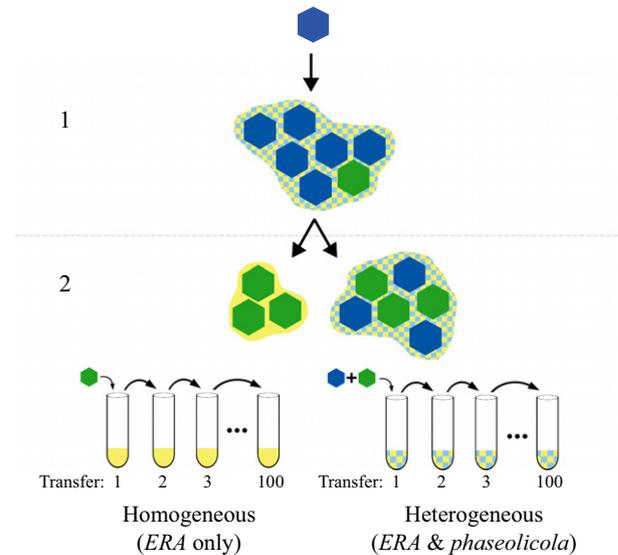


Fig. 2 Experimental design. (1) In a previous experiment (Bono *et al.* 2013), a specialist (blue hexagon) phage ancestor capable of infecting only the bacterial host *phaseolicola* was evolved in well-mixed microcosms containing both *phaseolicola* (light blue) and *ERA* (yellow) hosts. After several transfers, a generalist (green hexagon) evolved that was capable of infecting both hosts. Coexisting generalist and specialist clones were isolated from the end of that previous experiment and used as founders in the current experiment. (2) In the current experiment, we monitored the evolution of generalist phage in homogeneous microcosms containing only *ERA* (solid yellow) and in heterogeneous microcosms containing both *ERA* and *phaseolicola* (checked yellow and blue) for 100 serial transfers. Phage populations evolving in the homogeneous experiments were founded by a generalist clone only. Phage populations evolving in the heterogeneous experiments were founded by a 1:1 mixture of generalists and specialists to ensure that generalists experienced directional selection for improved performance on *ERA* in this treatment. Five replicate populations were evolved for each treatment.

cells, counted by plating on a two-host lawn (200 μ L *phaseolicola* + 2 μ L *ERA* taken from stationary phase cultures) and archived at -20 °C for later analysis. The next day 10^6 phage were used to initiate the next transfer. Hosts were revived from the freezer and were not allowed to evolve. Evolution of the phage population was monitored over 100 serial transfers (approximately 300 generations), and we conducted five replicate evolution experiments in each environmental treatment. We monitored the population dynamics by tracking the frequencies over time of the *phaseolicola* specialists and *phaseolicola* + *ERA* generalists, which form turbid and clear plaques, respectively, on the two-host lawn. We determined whether *ERA* specialists evolved by plating on one-host lawns of *ERA* and then toothpicking 100 of the resulting plaques onto lawns of *phaseolicola*. We did not observe in any replicate a phage capable of

infecting *ERA* that was not also capable of infecting *phaseolicola*.

We assessed evolved differences in performance on *phaseolicola* and *ERA* by measuring the rate of adsorption to each host. Adsorption rate is a major determinant of both phage growth rate and host range (Duffy *et al.* 2006; Ferris *et al.* 2007). To perform adsorption rate assays, 2000 phage were mixed with 1 mL of approximately 2×10^8 exponentially growing host cells and incubated shaking at 25 °C. Initially, and after 40 min, 500 µL of this mixture was centrifuged for >1 min at 2000 g to pellet the cells, and 200 µL of supernatant was plated on a lawn of *phaseolicola* to obtain a count of the free phage. The adsorption rate constant was calculated as $k = -\ln(P_{40}/P_0)/(40N)$ where N = concentration of host cells, and P_t is the number of free phage at time t , determined by colony and plaque assays, respectively.

As diagrammed in Fig. 1, we determined whether costs of adaptation were more likely to emerge in the homogeneous (*ERA* only) than in the heterogeneous (*ERA* + *phaseolicola*) environment by comparing the rate of adsorption to *phaseolicola* of generalists evolved in independent replicates of the two treatments to that of their generalist ancestor. The adsorption rate data were analysed in R version 3.1.2 (R Core Team 2013) using linear models, estimated using the *lm* function from the *STATS* package. Environmental treatment (heterogeneous or homogeneous) and date on which the assay was performed were treated as fixed effects. Type III tests of fixed effects are reported to account for the effect of the date on which the assay was performed.

Results

Literature review

Our search of the experimental evolution literature yielded 189 primary research articles, out of 210 total search results. Sixty-two of the 189 research articles included tests for performance trade-offs between environments following laboratory adaptation to either or both homogeneous and heterogeneous environments. Seventeen of the 62 research articles, including all of the experiments conducted in plants and insects, were excluded from our analysis because they assessed trade-offs using comparisons between different evolved populations rather than comparing independent evolved populations to an ancestral genotype or population. Five studies added new data or analysis to previously published work that was also in our collection. In these cases, we included only the most recently published article in our analyses. Details of experimental design and results of the 40 remaining research articles are shown in Table 1.

Starting by considering the literature review data set as a whole, we use an ordered logistic regression to model the likelihood of observing trade-offs (yes > mixed > no) as a function of the environmental treatment (homogeneous or heterogeneous), taxon (virus, bacteria, unicellular eukaryote) and the length of the experiment in generations (clustered into groups using the function $\text{floor}(\log_{10}(\text{generations})) = 0, 1, 2, 3$ or 4). We found that trade-offs were significantly more likely to emerge during selection in a homogeneous environment than in a heterogeneous environment (Fig. 3A, odds ratio = 3.507, $t = 2.172$, $P = 0.0149$). Neither taxon nor experiment length had a significant effect (at a $P < 0.05$ level) on the emergence of trade-offs in this analysis of the full data set.

We next investigated whether the type of environmental heterogeneity affected the emergence of trade-offs. Among experiments conducted in heterogeneous environments, trade-offs were more likely to emerge when the heterogeneity was spatial than when it was temporal (Fig. 3B, odds ratio = 6.750, $t = 1.884$, $P = 0.0298$ for a comparison of experiments that included only spatial or only temporal heterogeneity).

Finally, we were concerned that our timescale metric, the length of the experiment in generations, may have been a poor proxy for the length of the adaptive walk (i.e. number of adaptive mutations that fixed) in each experiment because the mutation rates of unicellular eukaryotes, bacteria and viruses differ dramatically. In an attempt to control for this difference, we investigated the effect of experiment length in each taxonomic group separately. For this analysis, we considered only the experiments conducted in homogeneous environments, where trade-offs were most likely to emerge. In bacteria, experiment length significantly increased the emergence of trade-offs (Fig. 3C, odds ratio = 3.940, $t = 2.148$, $P = 0.0158$, $n = 18$ observations). We did not observe a similar effect in either viruses or unicellular eukaryotes ($P > 0.2$ for both tests, $n = 11$ and 3 observations, respectively).

Illustrative experimental evolution study

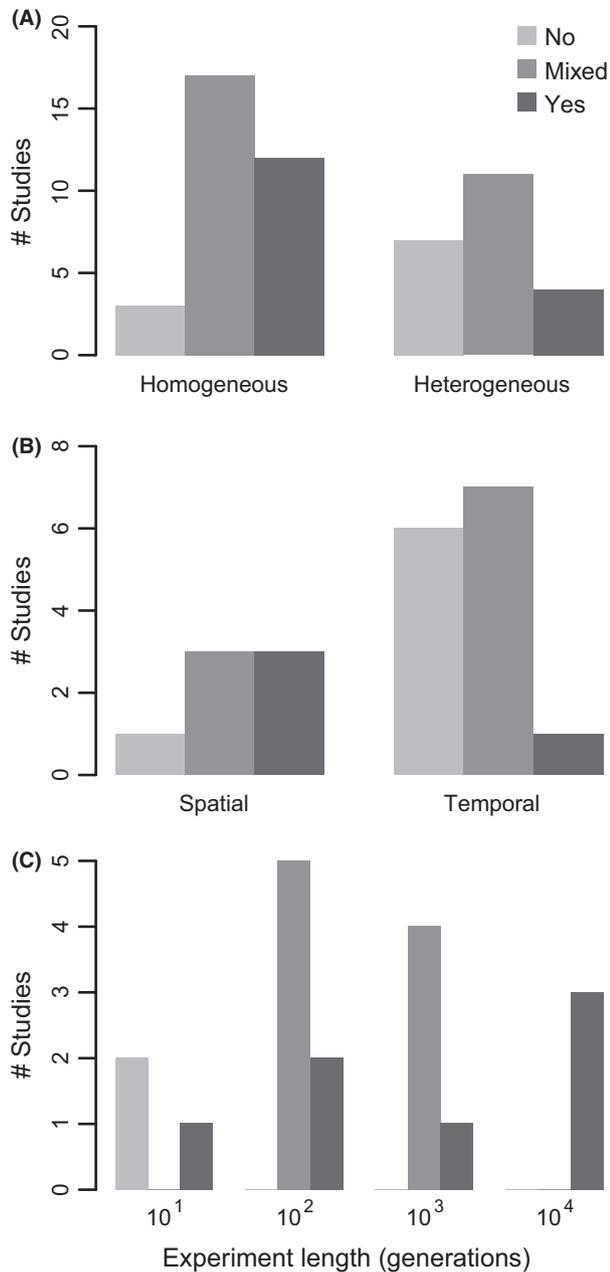
To illustrate the nature of the data contained in the literature review, we report the results of an experiment in which we compared the evolution of generalist $\phi 6$ in homogeneous (*ERA* only) and heterogeneous (*ERA* and *phaseolicola*) environments. Recall that phage populations evolving in the heterogeneous environment treatment were initiated with both *phaseolicola* + *ERA* generalists and *phaseolicola* specialists in an attempt to ensure that generalists experienced directional selection for improved performance on *ERA* in both environmental treatments. The frequency of generalists in

Table 1 Experimental evolution literature review

Citation	Taxon	Species	Selective environment	Minimum # generations	Homogeneous experiment	Heterogeneous experiment	Heterogeneity type	Homogeneous trade-offs	Heterogeneous trade-offs
Ostrowski <i>et al.</i> (2005)	Bacteria	<i>Escherichia coli</i>	Carbon source	400	Yes	No	NA	Mixed	NA
Tyerman & Doebeli (2010)	Bacteria	<i>Escherichia coli</i>	Carbon source	200	No	Yes	Temporal	NA	Mixed
Leiby & Marx (2014)	Bacteria	<i>Escherichia coli</i>	Carbon source	50 000	Yes	No	NA	Yes	NA
Satterwhite & Cooper (2015)	Bacteria	<i>Escherichia coli</i>	Carbon source	6000	Yes	Yes	Temporal	Mixed	No
Hughes <i>et al.</i> (2007)	Bacteria	<i>Escherichia coli</i>	pH	2000	Yes	No	NA	Mixed	NA
Philippe <i>et al.</i> (2009)	Bacteria	<i>Escherichia coli</i>	Protein binding	40 000	Yes	No	NA	Yes	NA
Bennett & Lenski (1999)	Bacteria	<i>Escherichia coli</i>	Temperature	2000	No	Yes	Temporal	NA	No
Cooper <i>et al.</i> (2001a)	Bacteria	<i>Escherichia coli</i>	Temperature	20 000	Yes	No	NA	Yes	NA
Bennett & Lenski (2007)	Bacteria	<i>Escherichia coli</i>	Temperature	2000	Yes	No	NA	Mixed	NA
Nidelet & Kaltz (2007)	Bacteria	<i>Holospira undulata</i>	Host range	400	Yes	No	NA	Mixed	NA
Ensminger <i>et al.</i> (2012)	Bacteria	<i>Legionella pneumophila</i>	Host Range	200	Yes	No	NA	Yes	NA
Lee & Marx (2012)	Bacteria	<i>Methylobacterium extorquens</i>	Carbon source	1500	Yes	Yes	Temporal	Yes	Mixed
Jasmin & Kassen (2007a)	Bacteria	<i>Pseudomonas fluorescens</i>	Carbon source	100	Yes	Yes	Both	Mixed	Mixed
Hall & Colegrave (2008)	Bacteria	<i>Pseudomonas fluorescens</i>	Carbon source	350	Yes	No	NA	Yes	NA
Friman & Buckling (2013)	Bacteria	<i>Pseudomonas fluorescens</i>	Predator defence	27	Yes	Yes	Spatial	No	Yes
Roemhild <i>et al.</i> (2015)	Bacteria	<i>Pseudomonas aeruginosa</i>	Antibiotic	100	Yes	Yes	Temporal	Not measured	Mixed
MacLean <i>et al.</i> (2004)	Bacteria	<i>Pseudomonas fluorescens</i>	Carbon source	100	No	Yes	Spatial	NA	Mixed
Jasmin & Kassen (2007b)	Bacteria	<i>Pseudomonas fluorescens</i>	Carbon source	600	Yes	Yes	Spatial	Mixed	Mixed
Buckling <i>et al.</i> (2000)	Bacteria	<i>Pseudomonas fluorescens</i>	Disturbance	10	Yes	Yes	Temporal	No	No
Buckling <i>et al.</i> (2007)	Bacteria	<i>Pseudomonas fluorescens</i>	Resource quality	1400	Yes	Yes	Temporal	Mixed	No
Nilsson <i>et al.</i> (2004)	Bacteria	<i>Salmonella typhimurium</i>	Resource range	60	Yes	No	NA	Yes	NA

Table 1 Continued

Citation	Taxon	Species	Selective environment	Minimum # generations	Homogeneous experiment	Heterogeneous experiment	Heterogeneity type	Homogeneous trade-offs	Heterogeneous trade-offs
Ketola & Saarinen (2015)	Bacteria	<i>Serratia marcescens</i>	Temperature	180	Yes	Yes	Temporal	Mixed	Mixed
Kassen & Bell (1998)	Unicellular Eukaryote	<i>Chlamydomonas reinhardtii</i>	Light/dark	80	Yes	Yes	Temporal	Yes	No
Jasmin & Zeyl (2013)	Unicellular Eukaryote	<i>Saccharomyces cerevisiae</i>	Carbon source	5120	Yes	No	NA	Mixed	NA
Wenger <i>et al.</i> (2011)	Unicellular Eukaryote	<i>Saccharomyces cerevisiae</i>	Resource abundance	250	Yes	No	NA	Mixed	NA
Duffy <i>et al.</i> (2006)	Virus	Bacteriophage $\phi 6$	Host range	1	Yes	No	NA	Mixed	NA
Duffy <i>et al.</i> (2007)	Virus	Bacteriophage $\phi 6$	Host range	100	Yes	No	NA	Mixed	NA
Bono <i>et al.</i> (2013)	Virus	Bacteriophage $\phi 6$	Host range	60	No	Yes	Spatial	NA	No
Bono <i>et al.</i> (2015)	Virus	Bacteriophage $\phi 6$	Host range	300	No	Yes	Spatial	NA	Mixed
This Study	Virus	Bacteriophage $\phi 6$	Host range	300	Yes	Yes	Spatial	Mixed	No
McGee <i>et al.</i> (2016)	Virus	Bacteriophage ID8	Growth/survival	30	Yes	Yes	Temporal	Not measured	Mixed
Sacristán <i>et al.</i> (2005)	Virus	Cucumber mosaic virus	Host range	10	Yes	No	NA	No	NA
Vasilakis <i>et al.</i> (2009)	Virus	Dengue virus	Host range	10	Yes	Yes	Temporal	Yes	No
Kubinak & Potts (2013)	Virus	Friend virus complex	Host range	10	Yes	No	NA	Yes	NA
Ciota <i>et al.</i> (2014)	Virus	St. Louis encephalitis virus	Host range	20	Yes	No	NA	Mixed	NA
Lalić <i>et al.</i> (2010)	Virus	Tobacco etch potyvirus	Host range	1	No	Yes	Spatial	NA	Yes
Bedhomme <i>et al.</i> (2012)	Virus	Tobacco etch potyvirus	Host range	15	Yes	Yes	Temporal	Yes	Mixed
Hillung <i>et al.</i> (2014)	Virus	Tobacco etch potyvirus	Host range	15	Yes	No	NA	Mixed	NA
Preslold <i>et al.</i> (2008)	Virus	Vesicular stomatitis virus	Host range	25	Yes	Yes	Temporal	Yes	Yes
Deardorff <i>et al.</i> (2011)	Virus	West Nile Virus	Host range	20	Yes	Yes	Temporal	Mixed	Mixed



heterogeneous environments fluctuated over the course of the experiment between 0.35 and 0.61, but generalists coexisted with the *phaseolicola* specialists for the duration of the experiment in all replicates (Fig. S1, Supporting information).

We compared the evolution of trade-offs in homogeneous versus heterogeneous environments by measuring the adsorption (binding) rates of the ancestral and evolved generalist phage on both *phaseolicola* and *ERA* (Fig. 4). Generalists that evolved in the homogeneous environment exhibited a stronger cost of adaptation in the form of reduced adsorption rates to *phaseolicola* ($k_{\text{phaseolicola}}$) than generalists that evolved in the

Fig. 3 The detection of trade-offs as costs of adaptation in laboratory evolution experiments depends on the ecological treatment and on the evolutionary timescale. (A) Among the full set of experiments which compared the performance of evolved and ancestral populations in alternative environments, costs of adaptation were more often detected following selection in homogeneous than in heterogeneous environments. (B) Among the set of experiments conducted in heterogeneous environments, costs of adaptation were more often detected when the heterogeneity was spatial than when it was temporal. (C) Among the set of bacterial experiments conducted in homogeneous environments, costs of adaptation were more often detected in longer than in shorter experiments. In all panels, bars show the number of studies in which zero (trade-offs detected = no), some (trade-offs detected = mixed) or all (trade-offs detected = yes) of the replicate evolved populations exhibited worse performance than the ancestor used to found the experiment in at least one of the alternative experimental environments.

spatially heterogeneous environment. Comparisons of the adsorption rates of evolved generalists isolated from each replicate population to the ancestral generalist phage confirmed a statistically significant cost of adaptation ($P < 0.05$ for a fixed effect of phage genotype on $k_{\text{phaseolicola}}$) in two of five replicates from the homogeneous treatment and in 0 of 5 replicates from the heterogeneous treatment. Treating our experiment as we did the others in our literature review, we described the emergence of trade-offs in our homogeneous and heterogeneous environments as 'mixed' and 'no', respectively. Note that in our statistical tests for costs of adaptation, both here and in our literature review, we did not first remove from our data set lineages that failed to exhibit significant adaptation (i.e. a significant increase in k_{ERA}). We made this choice to ensure we handled all of the reviewed literature equitably. In some of the reviewed literature, adaptation to the selected environment was assumed and not measured. In other cases, as in our illustrative example, the data are noisy and we did not feel justified ruling out adaptation when the statistical test had only failed to confirm it.

Because we know from previous work in $\phi 6$ that the pleiotropic effects of adaptive mutations are variable (Duffy *et al.* 2006; Ferris *et al.* 2007), the results of our experiment provide an anecdotal observation that when the pleiotropic effects of adaptive mutations are variable, trade-offs are more likely to emerge in homogeneous than in heterogeneous environments.

Discussion

As noted in the Introduction, performance trade-offs – such as those stemming from antagonistic pleiotropy – have long been proposed as being essential for

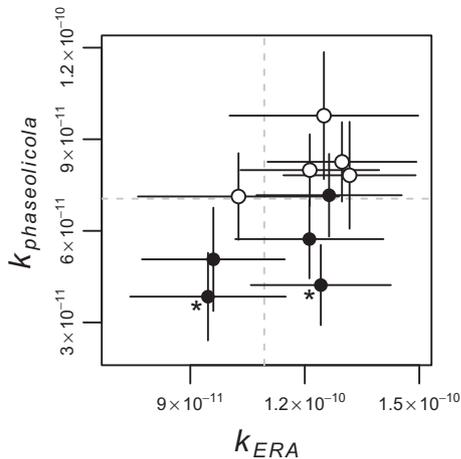


Fig. 4 Adsorption rates (k) of the evolved generalists on the bacterial hosts *ERA* and *phaseolicola*. Data are means \pm SED of evolved generalist clones isolated from populations that were propagated in homogeneous (*ERA* only; filled circles) and heterogeneous (*ERA* and *phaseolicola*; open circles) environments for 100 serial transfers. As in Fig. 1, the adsorption rates of the ancestral generalist are shown using the dashed grey lines. Asterisks indicate evolved generalists that exhibit a significantly ($P < 0.05$) slower adsorption rate to *phaseolicola* than the ancestral phage. As there was a significant effect of the date on which the adsorption assay was performed, the plotted adsorption rates have been corrected for effects of measurement date.

explaining the evolutionary maintenance of locally adapted ecotypes. The crux of the idea is that alleles that promote adaptation to one environment should promote maladaptation to alternative environments. However, contrary to this expectation, alleles that underlie local adaptation have rarely been shown to exhibit fitness costs in alternative environments, suggesting that the sort of antagonistic pleiotropy that is thought to promote local adaptation is rare. We sought to explain this paradox. Specifically, because the pleiotropic effects of adaptive mutations often vary from antagonistically pleiotropic to conditionally neutral (Ostrowski *et al.* 2005; Duffy *et al.* 2006; Ferris *et al.* 2007; Lalić *et al.* 2011), we hypothesized that the emergence of performance trade-offs during local adaptation should be predictable based on the evolutionary and ecological history of diverging populations.

Our main prediction was that *ecological* history – specifically, the amount and nature of the experienced environmental heterogeneity – should have a major effect on the genetic basis of local adaptation. Our test of this prediction built on the results of an earlier review (Kassen 2002) that proposed environmental heterogeneity as a general explanation for differences among natural populations in genetic variation and niche width. That review similarly aimed to explain the

ecological conditions under which local adaptation (specialized niche width) was more or less likely to evolve. It made two seemingly contradictory observations that motivated the deeper analysis we conduct here. Although selection in alternative homogeneous environments most often resulted in a negative genetic correlation for fitness across those environments, selection in a heterogeneous environment rarely resulted in a generalist with lower mean fitness (averaged across the component environments) than the mean of specialists selected separately in the homogeneous component environments. Because that review exclusively compared the performance of different evolved populations to each other (rather than to an ancestor), it could not distinguish the relative contributions of antagonistic pleiotropy and conditional neutrality to the observed negative genetic correlation. Nonetheless, the stronger than expected performance of evolved generalists was consistent with a smaller contribution of antagonistic pleiotropy in heterogeneous than in homogeneous environments.

Here, we reviewed the experimental evolution literature to provide an explicit test of the above prediction. Using a different test that compared the performance of evolved and ancestral populations, we confirmed both: (i) that the negative genetic correlation observed by Kassen (2002) did indeed often result from the fixation of antagonistically pleiotropic mutations in homogeneous environments and (ii) that the stronger than expected performance of evolved generalists resulted because antagonistic pleiotropy often made smaller contributions to adaptation in heterogeneous than in homogeneous environments.

Kassen (2002) also attempted to determine whether spatial and temporal heterogeneity in the environment had different effects on niche width (i.e. the emergence of local adaptation). At that time, there were only two direct comparisons of evolution from a common ancestral virus in spatially versus temporally varying environments (Gottschal *et al.* 1979, 1981; Reboud & Bell 1997) and their outcomes differed: stronger trade-offs evolved under spatial heterogeneity in one study (Reboud & Bell 1997) and under temporal heterogeneity in the other study (Gottschal *et al.* 1979, 1981). Our power to make the comparison was improved, both because of the large number of studies that have accumulated since 2002 and because we decided not to restrict our analysis to paired comparisons of evolution from a common ancestral virus in spatially versus temporally varying environments. Instead, we compared the full set of experiments conducted in spatially heterogeneous environments to the full set of experiments conducted in temporally heterogeneous environments. Although this comparison is less controlled, it

does confirm that costs of adaptation are more likely to emerge during adaptation to a spatially heterogeneous environment than to a temporally heterogeneous environment. This is exactly the pattern expected: the rate of migration between patches (m) in spatially heterogeneous experimental environments is necessarily at least somewhat restricted ($m \leq 1/2$ in all spatially heterogeneous experimental designs). In systems like $\phi 6$, where host preferences evolve and enable evolving populations to reduce their realized environmental heterogeneity, spatially heterogeneous experimental treatments may even evolve to resemble homogeneous environment treatments. Indeed, among the reviewed literature, there was not a significant difference in the emergence of trade-offs between experiments conducted in spatially heterogeneous and homogeneous environments (compare the left hand bar plots in Fig. 3B, A).

We also predicted that *evolutionary* history should affect the genetic basis of local adaptation. Specifically, we predicted that the contribution of antagonistic pleiotropy to local adaptation: (i) should be greater when adaptation occurs from new mutation than from standing genetic variation and (ii) might change over evolutionary time. We had only mixed success testing these predictions. A test of the effect of standing genetic variation turned out not to be possible because nearly all of the experiments initiated with genetically variable populations (rather than with clones) did not include a comparison of the evolved and ancestral populations. These included all of the studies in plants and insects, where (unlike with microbes) it is not possible to archive ancestral populations in the freezer. Although we could not measure the impact of standing genetic variation to the outcomes of laboratory evolution experiments, we note that *natural* populations will almost always contain more standing genetic variation than the founding clones employed in the experiments analysed here. This difference is consistent with the larger contribution of antagonistic pleiotropy to local adaptation in the laboratory (reviewed here) than what has been observed in natural populations (Weinig *et al.* 2003; Verhoeven *et al.* 2004, 2008; Gardner & Latta 2006; Latta *et al.* 2007, 2010; Latta 2009; Lowry *et al.* 2009; Hall *et al.* 2010; Ågren *et al.* 2013; Anderson *et al.* 2013; Leinonen *et al.* 2013; Remington *et al.* 2013; Oakley *et al.* 2014; Soudi *et al.* 2015).

We were able to provide a strong test of the effect of evolutionary timescale only among the set of experiments conducted in homogeneous environments in bacteria, where we had the largest collection of studies and the highest variance in experiment length. Among these experiments, costs of adaptation emerged more often in longer than in shorter experiments, suggesting that clones used to found laboratory evolution experiments

tend to start far from both the laboratory optimum and any constraint underlying antagonistic pleiotropy (Fig. 3C). We cannot speculate on whether the differences between local environments in nature tend to be larger or smaller than between local environments in the laboratory. However, our finding that the contribution of antagonistic pleiotropy to local adaptation in the laboratory resembles its contribution in nature – only over short timescales – is consistent with natural populations existing similarly far from local optima (e.g. see Kingsolver and Pfennig 2007).

The results of our review highlight the importance of including the variance in pleiotropic effects among adaptive mutations in models of local adaptation and ecological speciation. In our own work, we have found that the use of exemplars like beak size adaptation to alternatively sized seeds (as in Dieckmann & Doebeli 1999) – in which antagonistic pleiotropy is guaranteed by a physical constraint – has repeatedly caused us to incorrectly predict results of experiments like the one described here. Contrast such exemplars with the many systems that can evolve without such physical constraints. For instance, a virus might evolve to infect a novel host by attaching to a surface protein unique to the novel host at the cost of attaching to the surface protein of the standard host. Alternatively, it can avoid this cost altogether by evolving to attach to a protein that is shared between the novel and standard hosts. We leave that as a cautionary tale for future investigators of local adaptation, both in the laboratory and in the wild. Indeed, there is now a preponderance of evidence suggesting that pleiotropy is variable among adaptive mutations. Consequently, the emergence of performance trade-offs depends critically on evolutionary ecology.

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Data accessibility

Adsorption rate data: DRYAD entry doi:10.5061/dryad.b1301

The concept was developed by all of the authors. The evolution experiment was executed by L.M.B. The literature was surveyed by L.M.B., L.B.S. and C.L.B. Data analyses were conducted by L.M.B. and C.L.B. All authors contributed to the writing.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Generalist and specialist $\phi 6$ coexisted for the duration of experiments conducted in spatially heterogeneous environments containing both *phaseolicola* and *ERA* hosts.