Patterns of Local Adaptation in Space and Time among Soil Bacteria

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ABSTRACT: Our understanding of microbial biogeography has been governed by the dictum "Everything is everywhere, but the environment selects." In other words, the distribution of microbes is thought to occur in a regime of extensive dispersal and strong selection, generating local adaptation. However, direct tests of these assumptions are rare. Here, we investigate the extent of local adaptation in space and time of a collection of soil-derived microbial isolates, most belonging to the genus *Pseudomonas*, across a growing season from a deciduous forest in western Quebec, Canada, using a reciprocal transplant design. Average performance of all clones varied substantially in both space and time, in line with the expectation of strong selection in both dimensions. The behavior of genotype-by-environment variance in fitness and its components, responsiveness and inconsistency, in space and through time suggests that the strength of divergent selection increases as sites become more distant from each other in both dimensions. However, divergent selection was not strong enough to maintain different specialized types across the environments studied, which suggests that *Pseudomonas* and their close relatives are not locally adapted to the prevailing conditions of growth.

Keywords: local adaptation, microbial biogeography, time shift, soil bacteria, natural isolates.

Introduction

The principles governing the diversity and distribution of genotypes or species in space and time are, by and large, well understood. Genetic diversification (either through mutation or speciation) and dispersal add diversity to a community, whereas natural selection (or, equivalently, competition) and drift, which are the stochastic effects associated with finite population size, remove it (Vellend 2010; Nemergut et al. 2013). Less well understood, however, is which process or combination of processes are most important for determining the composition and structure of any given natural community. This is the task of biogeography (Martiny et al. 2006; Ramette and Tiedje 2007; Hanson et al. 2012).

In this light, the interpretation of the biogeography of microbes is particularly interesting. Microbes are easily dispersed, and so it is often presumed that there is a constant "rain" of every possible bacterial genotype or species on every ecosystem. A second assumption is that microbes can occupy a wide range of highly contrasted habitats or niches, which implies that, at most spatial scales, divergent selection, which occurs when different optimal phenotypes are favored in different conditions, will generate local adaptation and result in distinct genotypes being recovered from different environments (Fierer and Jackson 2006; Martiny et al. 2006; Van der Gucht et al. 2007). This hypothesis has come to be known as the Baas-Becking hypothesis, which states that "Everything is everywhere, but the environment selects" (Baas-Becking 1934).

The Baas-Becking hypothesis thus interprets the contemporary distributions of microbes as being determined almost entirely by selection generated by the prevailing abiotic and biotic conditions of growth. Dispersal limitation and drift, on the other hand, are expected to have little influence on the composition and structure of microbial communities. Efforts to study the biogeography of microbes typically use genetic markers such as multilocus sequence typing or, more recently, whole genomes to track the presence and absence of distinct genotypes across multiple spatial or temporal scales (e.g., Vogel et al. 2003; Fuhrman et al. 2006; Vos and Velicer 2008; Keymer et al. 2009; Kraemer and Velicer 2011; Gilbert et al. 2012). Although most studies find a substantial degree of environmental structure to microbial communities consistent with the Baas-Becking hypothesis (reviewed in Martiny et al. 2006 and Nemergut et al. 2013), the weight of evidence points to some degree of dispersal limitation as well (Oda et al. 2003; Papke et al. 2003; Vos and Velicer 2008). Thus, although the Baas-Becking hypothesis may be a useful first

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approximation to interpreting the biogeography of microbial communities, it clearly does not tell the whole story.

A complementary approach to investigating the structure of microbial communities, or any natural community for that matter, is to estimate the degree of local adaptation directly via experiment. For microbial communities in particular, the Baas-Becking hypothesis predicts that bacterial species or genotypes (we note that there is no formal distinction between genotypes and species that need be made when genotypes are asexual; Horner-Devine et al. 2004; Jessup et al. 2004) will be locally adapted to the prevailing conditions of growth. Local adaptation here refers to the fact that the genotypes or species isolated from that habitat will have highest fitness there and lower fitness in other habitats.

Local adaptation appears to be quite common in nature, with recent reviews of studies in macroorganisms (Hereford 2009; Fraser et al 2011; Sanford and Kelly 2011) and parasites (Greischar and Koskella 2007; Hoeksema and Forde 2008) showing evidence for local adaptation in 60%–80% of studies surveyed. Moreover, trade-offs in fitness across environments, a hallmark of local adaptation, readily evolve in laboratory selection experiments with microbes (Kassen 2014). Interestingly, the degree of specialization in these studies is often quite modest, with higher fitness in a "home" environment being associated with only a modest loss of fitness in an "away" environment (Hereford 2009; Kassen 2014). Notable by their absence are studies that assess the spatial and temporal scale of local adaptation in nonparasite microbial species or genotypes isolated from natural habitats (but see studies by Belotte et al. [2003] and Keymer et al. [2009]).

Thus, while the Baas-Becking hypothesis has played an important role in guiding our thinking about microbial biogeography, we actually know comparatively little about one of its key assumptions, that the microbial genotypes isolated from natural communities are locally adapted to the prevailing conditions of growth. To fill this gap, we have undertaken a field-based study directly measuring the scale of local adaptation in space and in time for the soil microbe *Pseudomonas*, a cosmopolitan genus commonly found in soils. The soil matrix is characterized by both a large amount of microbial biomass (estimated to be 1–2 t/ha; Killham 1994) and a high bacterial biodiversity (between 26,000 and 52,000 different 16S ribosomal RNA [rRNA] sequences per gram; Roesch et al. 2007). Moreover, soil bacteria are functionally important for soil resistance against invading pathogens (Weller et al. 2002) and play a key role in nutrient cycling (Altieri 1999; Kennedy 1999; Grundmann 2000), erosion control, and water retention (Kennedy and Papendick 1995). The high degree of soil structure provides, from a bacterium's point of view, ample ecological opportunity on several scales. For example, different soil types and environment have been shown to select for significantly different bacterial communities (Smalla et al. 2007; Uroz et al. 2010). On an even finer scale, the soil matrix itself, with its water-coated pores, provides multiple spatially separated niches that can be characterized, for example, by differential availability of oxygen (Stotzky 1997). In the temporal dimension, this ecosystem is governed by seasonality both within different kingdoms of microorganisms (e.g., a shift from fungal to bacterial dominance between winter and summer; Lipson et al. 2002) and within the bacterial community (Lipson and Schmidt 2004). Seasonal differences in water and nutrient ion availability as well as differences in temperature will shrink or extend the breadth of certain niches, whereas other niches will vanish or appear de novo, which in turn is expected to influence the composition of the microbial community (Murray et al. 1998; Höfle et al. 1999; Lipson and Schmidt 2004; Thompson et al. 2004).

Here, we use a reciprocal transplant experiment to investigate the spatial and temporal scale of local adaptation of *Pseudomonas* and related genera in the soils from beech-maple forests in western Quebec, Canada. Our study allows us to directly investigate the nature of adaptation in this ubiquitous taxonomic group to their local environment in both space and time. We find evidence consistent with the idea that selection is strongest when environments are most distant from each other in both space and time, although the strength of selection increases more rapidly in space than in time at the scales we sampled. Interestingly, divergent selection never became strong enough to generate local adaptation capable of maintaining diversity, at least for the genotypes in our study. Taken together, these findings indicate that selection can be a significant factor in structuring natural microbial communities, and our study offers further insight into how microbial biogeography is connected to fitness.

Material and Methods

Soil Sampling

Soil samples were collected monthly from undisturbed beech-maple woodland sites at the Gatineau Park Reserve (Gatineau, Quebec, Canada) by carefully brushing away leaf litter and scooping soil into two sterile 40-mL Falcon tubes per sampling point. We sampled a total of three locations (600–1,150 m distant from each other), designated C, N, and R, respectively. At each location, we collected soil from every meter along a 12-m transect. All sites at each location were sampled once a month for 8 months from April to November 2012 (designated as month 1 to month 8). Soil samples were transported immediately to the laboratory and stored for no more than

24 h at 4°C before processing. Just before clonal isolation, samples were passed through a sterile sieve with a 3 × 3-mm mesh to remove remaining leaf litter, sticks, and stones. Freshly sieved soil was used for clonal isolation, whereas the remainder of the sample was stored at −20°C for further use.

Clonal Isolation

Approximately 0.5 g of soil was used immediately after sieving to isolate clones by adding 15 mL of sterile ddH_20 to the soil, vortexing the sample, and then letting it stand for 10–20 min following the protocol of Belotte et al. (2003). We then diluted the soil suspension with ddH_20 onto *Pseudomonas* isolation agar plates (Thermo Fisher Scientific, Lenexa, KS) and incubated these plates for 48 h at 28°C. After incubation, all plates were very briefly screened under UV light, and a visibly fluorescent colony was picked at random. We ensured clonality of all isolates by streaking them thrice onto Kirby-Bauer (KB) agar plates and incubating at 28°C before inoculating the clones into liquid KB medium; growing them until turbid at 28°C, 150 rpm; and then mixing them with glycerol to a final concentration of 20% for frozen storage at −80°C. A clone thus represents a bacterial genotype isolated from a given site. Below, we describe in more detail the haplotype, or genetic identity, of these clones.

Soil Tea Preparation

We followed the protocol developed by Belotte et al. (2003) for soil extract "tea" preparation. In brief, sampled soil was dried at 70°C for 48 h before measuring 3.33 g of each dried soil sample into an empty tea bag and sealing the bag with metal clips. We then added 50 mL of ddH_20 to each soil tea bag for 24 h at room temperature before filter-sterilizing the resulting "tea" suspension using a 0.2-μm filter. Sterile soil tea was brought to 50 mL of volume with ddH_20 before adding 10 g/L of glycerol to facilitate growth. Glycerol is a simple sugar that diffuses readily across bacterial cell membranes in an energy-independent manner (Dills et al. 1980) and so should enhance growth of all clones indiscriminately.

Growth Assays

We chose a subset of clones and environments sampled from meter-scale positions 1, 6, and 12 of each transect at each month and assayed their growth in a fully factorial design. The growth of each clone was thus measured in 3 meter-scale positions for 3 transects for 8 months, giving 72 distinct sites. Assays were performed in two blocks of 36 sites in triplicate, with each replicate being run at a separate time. Within each temporal replicate, we assigned sites to each block randomly. To initiate growth assays, we inoculated a 96-well microplate with all test clones plus the laboratory strain SBW25 *lacZ* directly from frozen stocks into 300 μL of KB liquid medium. SBW25 *lacZ* acts as a control that allows us to ensure that the medium supports growth. Clones were allowed to grow without shaking for 24 h at 28°C. The position of clones on a plate was randomized for each temporal replicate. We inoculated 5 μL from these overnight cultures into a second microwell plate containing 145 μL of soil tea from the different sites. We measured optical density (OD) at a wavelength of 660 nm as a proxy for growth just after inoculation (time = 0) and then again after 48 h of incubation at 28°C. To avoid biases due to biofilm formation, OD readings are taken as the mean of three repeated measurements after vigorous shaking for 20 s each time. Growth rates are an appropriate and widely used measure of fitness in the absence of competitor genotypes or species, because they provide a direct measure of performance that is sufficient to describe fitness in the absence of nontransitive interactions among genotypes. Fitness can also be measured via competition experiments against a focal clone; however, the diversity of clones in our assay precludes the choice of a single, focal competitor clone against which measures of fitness would be meaningful and unbiased.

Clone Identification

Clones were grown from frozen stocks overnight at 28°C, 150 rpm. On the next day, we spun down 0.5 mL of turbid culture at 10,000 *g* for 3 min and discarded the supernatant. We isolated genomic DNA from the cell pellet using either the Qiagen Blood and Tissue kit (Qiagen, Valencia, CA) or the DNAWizard kit (Promega, Madison, WI). We amplified a region of the 16S rRNA gene spanning the loops V2–V9 with the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). Polymerase chain reaction (PCR) conditions were as follows: 3 min at 94°C, 35 cycles of 45 s at 94°C, 1 min at 50°C, and 1 min at 72°C, followed by a final elongation at 72°C for 10 min, then cooling to 4°C. All PCR products were visually confirmed on a 1% agarose gel before Sanger sequencing with primer 27F or 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). We aligned all sequences with CodonCode Aligner (CodonCode, Centerville, MA) and based clonal identification on a fragment of the gene ranging from position 142 to 701 of the 16S rRNA gene. We identified all clones using the Classifier algorithm of the Ribosomal Database Project (Wang et al. 2007; table S1, deposited, along with all supplemental tables, in the Dryad Digital Repository,

http://dx.doi.org/10.5061/dryad.76670 [Kraemer and Kassen 2015]). Unique haplotypes (clones sharing an identical 16S rRNA sequence) were identified by visually inspecting single-nucleotide polymorphisms and InDels in CodonCodeAligner. All sequences are deposited in GenBank under accession numbers KM819176–KM819461. Sequence data were used to calculate the pairwise genetic distance between clones belonging to the *Pseudomonas* genus with the MEGA 5 software (Tamura et al. 2011) using a Kimura-2 parameter model with gamma-distributed rates.

Data Collection

We calculated the average initial and final OD from the three initial and final measurements for each well. Next, we calculated ΔOD by subtracting the initial OD from the final OD. To control for background growth, we additionally assessed the ΔOD of six control wells per plate, which had been inoculated with sterile KB medium from the same microplates used for the initial overnight growth step. We observed occasional growth in this sterile medium, so we account for this in the following way: We first calculated the average ΔOD for the control wells and subtracted this value from all ΔODs on the same plate to obtain a contamination-"corrected" ΔOD (ΔOD_c). All negative ΔOD_c values were set to zero. We then assessed the likelihood of contamination (p_c) for each soil tea plate by recording the fraction of control wells that had ΔODs larger than 0.1 (this ΔOD was chosen as a cutoff value, taking into account measurement errors due to slight condensation on the lids of plates during measurements). We then calculated a contamination-"corrected" growth rate (W) for each clone-site combination as

$$W = p_c \times \Delta OD_c + (1 - p_c) \times \Delta OD. \quad (1)$$

We take W to be a measure of absolute fitness of a clone at a given site. Importantly, this measure of corrected fitness by necessity assumes that there is an additive relationship between clones and potential contaminants in each well. Because the identity of the contaminants is unknown, we are unable to account for possible competitive interactions between clones and potential contaminants. We did not detect a correlation between the number of contaminated control wells per soil medium with either site (on either meter or kilometer scale) or month of sampling (generalized linear model with binomial error distribution, all $P > .05$). Counts of contaminated control wells and average ΔOD for all control wells per plate can be found in table S2.

Environmental Parameters

We describe the overall quality of a given environment as the average growth of all identified clones in a given site. We calculate genetic variation supported within each environment as the standard deviation of growth for all clones measured at a given site.

Model Fitting

Model fitting was performed using the gls function of the nlme package and the lmer function of the lme4 package in R (R Development Core Team 2009). Our estimates of fitness were modeled using the following basic equation:

$$\text{fitness} = \text{genotype} + \text{environment} + (\text{genotype} \times \text{environment}). \quad (2)$$

We found that enforcing equal variances for each clone significantly improved model fit. We additionally investigated several residual autocorrelation structures that also improved model fit over the basic model. However, no single autocorrelation structure improved the model fit as much as enforcing equal variances, and models containing both equal variances and autocorrelation structures failed to converge. We therefore used a model with equal variances for each clone but no autocorrelation structure in all analyses that follow. The best-fit models for each analysis are shown in table 1. Additional details can be found in table S3.

The models can be analyzed at different nested genetic (clone, haplotype, or genus) and environmental scales (sites or locations, corresponding to the meter or kilometer scale, respectively). To determine the appropriate level for analysis, we calculated and compared variance components of nested terms. We found that the haplotype level explained the highest percentage of variance (59.68%), followed by clone (6.27%), whereas genus did not explain any of the variation observed (0%). Therefore, all of the following models were performed with haplotype as the genotype term in equation (2). For the environmental variable, both scales explained a similarly small percentage of variance observed (0.2% at the meter scale and 0.85% at the kilometer scale), so we performed all analyses with environment modeled on the meter scale.

Components of Genotype-by-Environment Interaction

The genotype-by-environment interaction (GE) term in equation (2) quantifies the extent of nonadditive variation in fitness in our collection of genotypes and environments. As a general rule, GE variation is expected to increase as environments become more different in the conditions of

Table 1: Best-fitting models for each analysis with fitting parameters

Covariates	Weights	AIC	df	*N*	AICc
Full model:					
Haplotype + environment + (haplotype × environment)[a]	1|Clone	−11,390.75	2,804	14,552	−10,051.65
Reduced model:					
Haplotype + site + month + (site × month) + (haplotype × site)	1|Clone	−24,635.11	473	14,552	−24,603.26
Spatial adaptation model:					
Haplotype + site + month + (site × month) + (haplotype × distance)[a]	1|Clone	−25,759.1	215	14,552	−25,752.62
Full-model *Pseudomonas*:					
Haplotype + environment + (haplotype × environment)[a]	1|Clone	−3,596.128	1,180	5,992	−3,016.8
Reduced-model *Pseudomonas*:					
Haplotype + site + month + (site × month) + (haplotype × site)	1|Clone	−8,787.73	235	5,992	−8,768.46

Note: All best-fitting models reported here had an AICc of >10 below the next best model. Details of all compared models are available in table S3. AIC = Akaike information criterion; AICc = corrected AIC; df = degree of freedom.

[a] Meters.

growth being offered. The reason for this relationship can be understood by examining Robertson's (1959) decomposition of GE into two components, termed consistency and responsiveness. For two environments, GE variance represents the sum of inconsistency and responsiveness as follows:

$$\sigma^2\text{GE} = \sigma_{G1} \times \sigma_{G2} \times (1 - \rho_{G1G2}) + \frac{1}{2} \times (\sigma_{G1} - \sigma_{G2})^2, \quad (3)$$

where σ_{G1} and σ_{G2} are the genetic standard deviations of fitness of all genotypes growing in two different environments, and ρ_{G1G2} is the genetic correlation of growth between them. The first term on the right-hand side is inconsistency, whose relative value is governed by the genetic correlation of growth across environments, ρ_{G1G2}. A positive genetic correlation means that the same genotype is fittest across both environments, whereas a negative genetic correlation implies that different genotypes are favored in different environments. Inconsistency thus measures the extent to which the relative fitness rank of genotypes changes across a pair of environments and is expected to increase as environments become more different, because the genetic correlation should decrease and eventually become negative as environments become more highly contrasted in the conditions of growth they offer. The second term on the right-hand side is responsiveness, a measure of the difference in genetic variation expressed by the same set of genotypes across environments. A collection of genotypes whose fitness is measured in very similar environments should display very similar amounts of genetic variation in each, so responsiveness will be small. As environments become more distinct and conditions of growth between them become more different, the amount of genetic variation in fitness expressed in each is likely to become more different as well, and thus responsiveness increases. These expectations are summarized in the schematics shown in figure 1.

Equivalently, GE can be interpreted in terms of genetic divergence rather than environmental divergence (Kassen and Bell 2000; Barrett et al. 2005) as follows:

$$\sigma^2\text{GE} = \sigma_{E1} \times \sigma_{E2} \times (1 - \rho_{E1E2}) + \frac{1}{2} \times (\sigma_{E1} - \sigma_{E2})^2, \quad (4)$$

where σ_{E1} and σ_{E2} are the environmental standard deviations of fitness of two clones in all environments and ρ_{E1E2} is the environmental correlation of growth or, in other words, a measure of how similar a pair of genotypes perceives a series of environments. As before, total GE variance should increase as a function of the genetic dissimilarity among genotypes. Two closely related genotypes—for example, distinct isolates of the same genotype—will rank environments in near-identical ways, so the environmental correlation in growth between them is close to 1. On the other hand, two very distinct genotypes—for example, species from different genera—are unlikely to have similar growth profiles across a collection of environments, and so the environmental correlation between them will be close to zero or even negative. The formula $\frac{1}{2} \times (\sigma_{E1} - \sigma_{E2})^2$ denotes genotype responsiveness. This term is more intuitive than the equivalent term in equation (3), because it simply expresses differences in plasticity between the two genotypes, which again should increase as relatedness among genotypes decreases. Figure 1 summarizes these expectations schematically.

To examine the relationship between GE and its components and both environmental and genetic divergence of clones belonging to the *Pseudomonas* genus, we used a full model of the form outlined in equation (2) with clone as the genotype term and SoilID, a term describing the meter-scale environment at each month, as the environ-

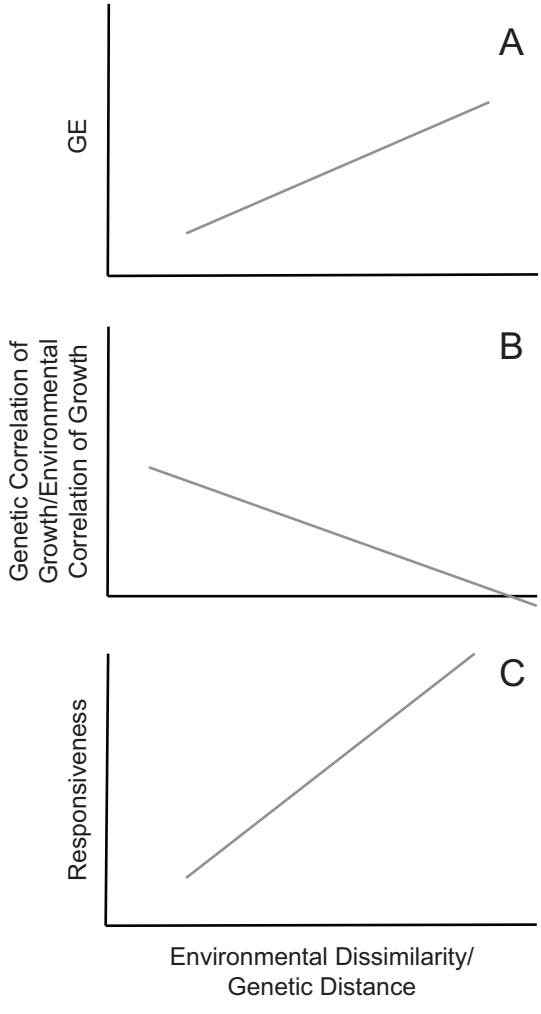

Figure 1: Schematic representation of genotype-by-environment interaction (GE) and its components, plotted against increasing environmental dissimilarity or increasing genetic distance, behaving in a pattern consistent with adaptation to local conditions. *A*, GE of a collection of clones increases with increasing dissimilarity between a pair of environments or genotypes. *B*, The genetic or environmental correlation of growth is negatively correlated with environmental or genotype dissimilarity. The intersection between the correlation of growth and the *X*-axis indicates local adaptation. *C*, Responsiveness is positively correlated with environmental dissimilarity or genetic distance.

ment term. We base all of our calculations on the interaction coefficients between each clone and each environment obtained from this model. Following previous work (Bell 1992; Kassen and Bell 1998; Bataillon et al. 2011), we measure environmental dissimilarity as the standard deviation of the difference of average fitness of all isolates between two environments. Genetic dissimilarity is measured as the pair-wise genetic distance of all isolates. We regressed the genetic parameters obtained (total GE, ρ_{G1G2}, genetic responsiveness) against environmental dissimilarity and the environmental parameters (total GE, ρ_{E1E2}, environmental responsiveness) against genetic dissimilarity to evaluate the interpretation of GE outlined above. We tested the significance of these regressions using permutation tests with 10,000 bootstraps because of the nonindependence of the individual data points.

Lastly, we calculated the genetic correlation of growth between all pair-wise combinations of environments and genotypes to draw a map of genetic correlation across temporal and spatial distances between environments using the Tps function of the fields package (R Development Core Team 2009) under default settings.

Results

Natural History

The 16S rRNA sequences we obtained allowed us to identify 286 of the 288 isolated clones belonging to eleven genera. *Pseudomonas* made up the majority of the clones (121), followed by, in descending abundance, *Stenotrophomonas* (38 clones), *Serratia* (35 clones), *Yersinia* (30 clones), *Erwinia* (28 clones), *Burkholderia* (17 clones), *Aeromonas* (10 clones), and *Janthinobacteria* (4 clones). *Bacillus, Luteibacter*, and *Achromobacter* were present as a single clone only and so were ignored in all subsequent analyses (table S1). The preponderance of *Pseudomonas* isolates is not surprising, given that we actively selected for them in our isolation procedure by using *Pseudomonas* isolation agar.

Rather than assign each clone to the species level, which can be challenging when based on a single gene, we assign a unique haplotype identifier to each clone with a unique sequence. Figure A1 (figs. A1–A4 available online) shows the frequency distribution of all haplotypes sampled. As expected in a nonexhaustive sampling design, a number of haplotypes were only represented by a single clone. However, the majority of haplotypes were sampled at least twice, with one haplotype (*Erwinia* haplotype 4) being represented by 22 individual clones in our data set.

Patterns of diversity for each transect over time are illustrated in figure A2, expressed both as the relative proportion of clones comprising each genus and as the reciprocal of Simpson's diversity index (Magurran 1988) at the genus level. We did not detect any pronounced differences in average diversity across the three transects and the growth season.

Environmental Quality

For all following experiments, we limit our collection of clones and environments to those obtained from meter-

scale sites 1, 6, and 12 at each of the three transects for each of the 8 months of sampling. The taxonomic distribution of clones used in this experiment is as follows (clones/haplotypes): *Pseudomonas*: 27/16; *Stenotrophomonas*: 11/4; *Yersinia*: 9/3; *Serratia*: 9/3; *Aeromonas*: 4/3; *Janthinobacteria*: 3/2; *Burkholderia*: 2/2; *Erwinia*: 6/2. We were not able to assign two *Serratia* and two *Stenotrophomonas* clones unambiguously to a single haplotype, and we excluded these four clones from all further analyses. The final experimental design thus included a total of 68 clones assayed across 72 environments and replicated three times.

Following standard agronomic practice (Simmonds 1981), we measured the overall quality of the environment as the average growth rate for all clones at a given space-time combination. We found both meter-scale location and month of sampling as well as their interaction to be significant predictors of growth (linear mixed model: $P < .001$, $P < .05$, and $P < .05$, respectively), thus indicating that environments vary significantly in both space and time. An example for average growth of all clones measured in three replicates for all three meter-scale sites at a single transect (transect C) is shown in figure 2. Data for all other transects are available upon request.

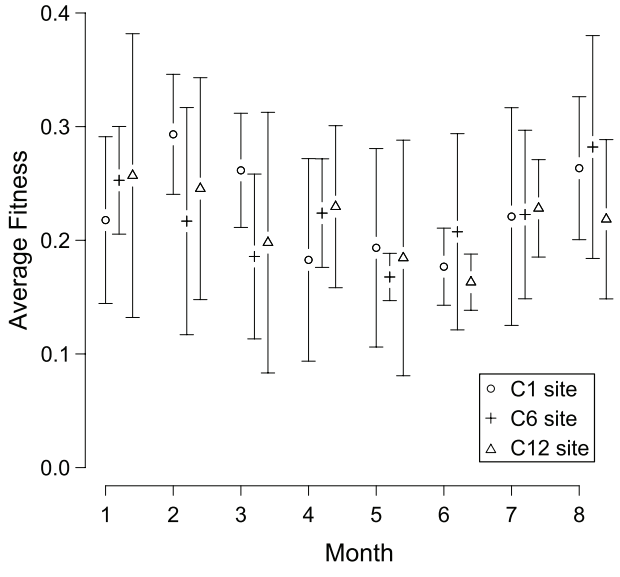

Figure 2: Average environmental quality of 68 isolates at each meter-scale site of the C transect across all months sampled. Error bars indicate 95% confidence intervals across the three replicate blocks.

Genetic Variation in Fitness within a Site

The genetic variation in fitness within a given site was calculated as the standard deviation of fitness of all 68 clones growing in a given space-time combination. Plotting this value against the average environmental quality (fig. 3*A*) gives a strongly positive relationship ($R^2 = 0.18$, $P < .001$), suggesting that high-quality environments support more genetic variation in fitness. Plotting the actual fitness values against the rank of environmental quality (where 1 is the poorest quality environment and 72 is the best; fig. 3*B*) confirms that this relationship does not derive from the fact that low-quality environments are so poor that most clones do not grow, nor is it due to a subset of a few high-fitness clones that grow especially well in high-quality environments.

Local Adaptation

The standard approach to detecting local adaptation is to decompose the total variance in a collection of genotypes whose fitness is assayed across a series of environments into components due to genotype, environment, and their interaction. A significant interaction term is often taken to indicate local adaptation. For our data set, such a full model reveals that genotype (which we take here to be the haplotype) explains the majority of the variation observed (62.27%), and environment (at the meter scale) explains just 3.46% of the variation, although both are statistically significant ($P < .05$). Notably, the GE interaction component does not contribute to the variation observed. Limiting the analysis to isolates belonging to the *Pseudomonas* genus does not change the results qualitatively (variance explained: haplotype = 70.28%, environment = 2.25%, haplotype-by-environment interaction = 0%). As in the full model, haplotype and environment are both statistically significant, whereas the interaction term is not.

Time shift analysis is typically performed to detect patterns of temporal adaptation (Blanquart and Gandon 2012). However, it relies on a significant GE term to estimate time shift coefficients. Because this is not the case here, time shift analysis was not appropriate for our data set. However, even though overall GE was not found to be a significant predictor of fitness compared with the Genotype and Environment terms used in the model, it is nonetheless possible that subcomponents of the GE term—for example, expressed as temporal or spatial adaptation—might be. Therefore, we extended the analysis using a model that distinguishes between temporal and spatial GE effects by distributing the variation between genotype-by-site interactions (potential spatial adaptation) and genotype-by-month interactions (potential temporal adaptation) as follows:

$$\begin{aligned}\text{fitness} = {} & \text{haplotype} + \text{site} + \text{month} \\ & + (\text{haplotype} \times \text{site}) + (\text{haplotype} \times \text{month}) \\ & + (\text{haplotype} \times \text{site} \times \text{month}). \end{aligned} \quad (5)$$

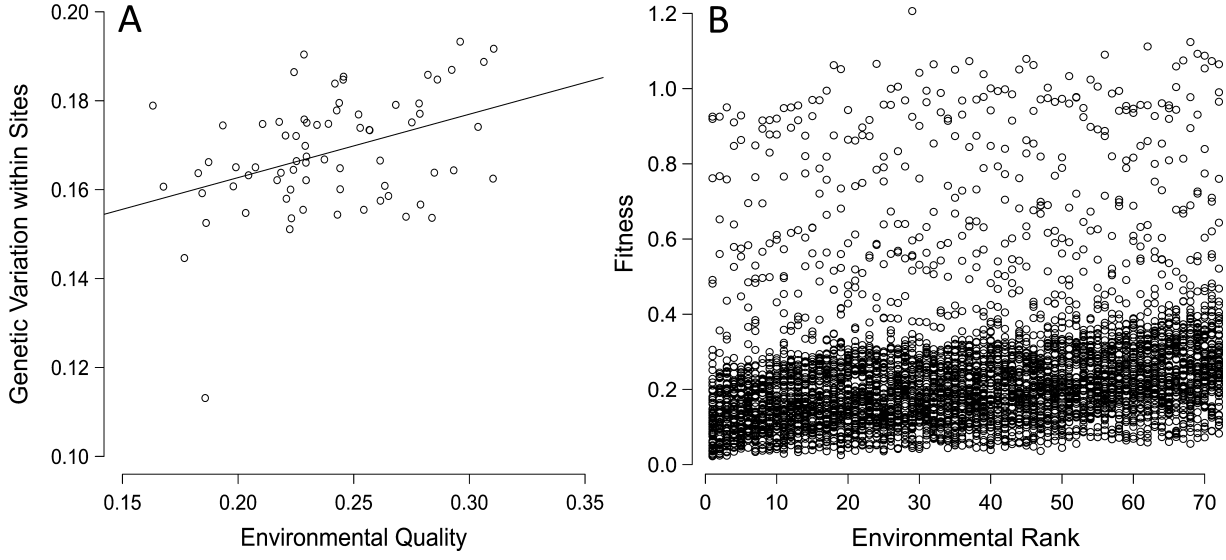

Figure 3: *A*, Genetic variation within sites plotted against the environmental quality of each meter-scale environment. *B*, Fitness of all 68 isolates plotted against the environmental rank of each environment.

Again, haplotype explains the most variation observed (62.07%), whereas both site and month alone explain 0.56% and 0.11%, respectively, of variation observed. Interestingly, although we found no indication of a significant haplotype-by-month interaction (0%), we were able to detect a weak but significant interaction between haplotype and site (1.06% of observed variation), which might be indicative of adaptation in space.

We investigated this effect further by refitting all significant model terms and replacing the haplotype-by-site interaction with an interaction of haplotype and spatial distance between the point in space the haplotype was isolated from and the test environment as follows:

$$\begin{aligned}\text{fitness} = {} & \text{haplotype} + \text{month} + \text{site} \\ & + (\text{month} \times \text{site}) \\ & + (\text{haplotype} \times \text{spatial distance}).\end{aligned} \tag{6}$$

Local adaptation in this model would be consistent with the observation that haplotypes perform significantly worse at distant sites compared with their "home" site, as revealed by a negative haplotype-by-spatial distance interaction coefficient for a haplotype. Our results are partially consistent with this hypothesis: 16 of the 38 coefficients of individual haplotype-by-spatial distance interactions are significantly or nearly significantly different from 0 ($P < .1$). Of those, 10 coefficients are negative, indicating that over half of the tested haplotypes with significant coefficients show evidence consistent with local adaptation in space (table S4). The six haplotypes displaying significantly positive interactions between fitness and spatial distance seem to be maladapted in space and may represent recent migrants to the sites they were sampled from.

Inconsistency, Responsiveness, and Overall GE across Gradients of Genotypic and Environmental Dissimilarity

Another approach to study local adaptation is to dissect the overall GE term into its two components, inconsistency and responsiveness, and investigate how these components covary with either genetic or environmental dissimilarity. First, we calculated overall GE, the environmental correlation of growth (the value governing inconsistency) and the variation of the environmental standard deviation (responsiveness) from the model interaction coefficients of pairs of clones belonging to the *Pseudomonas* genus across all environments. We found a significant positive correlation between overall GE and genetic distance (fig. 4*A*, slope: 0.09, permutation test: $P < .001$). Thus, GE is, on average, higher if two less similar clones are compared (for example, two clones belonging to different species). The correlation between the environmental correlation of growth and genetic distance is significantly and strongly negative (fig. 4*B*, slope: -5.05, permutation test: $P < .001$). In other words, pairs of less similar clones have less consistent responses across environments, and the GE term in the model is at least partly due to the crossing of reaction norms. Lastly, plotting responsiveness against ge-

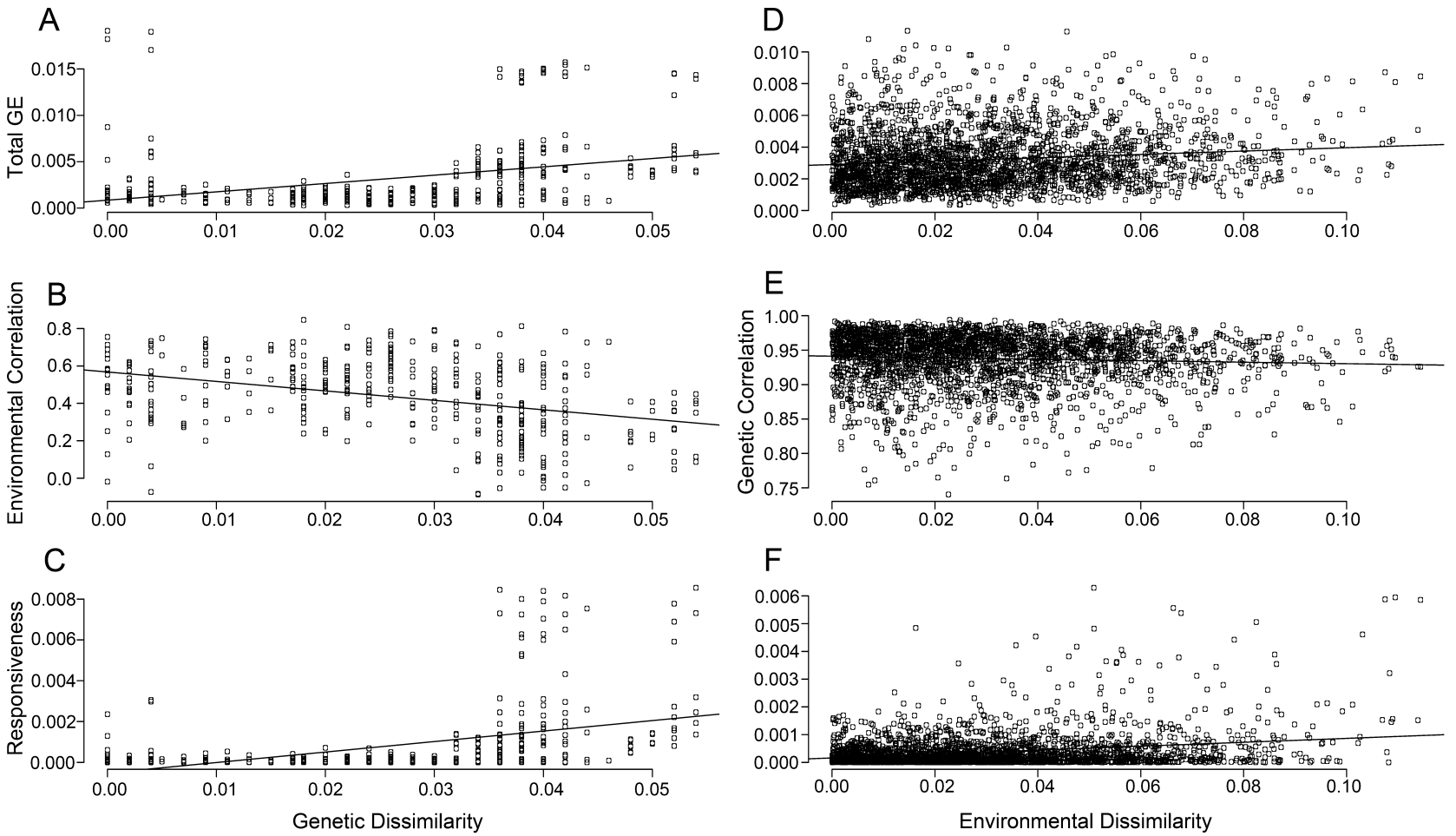

Figure 4: Total environmental genotype-by-environment interaction (GE; *A*), environmental correlation of growth (*B*), and responsiveness (*C*), plotted against genetic dissimilarity (genetic distance) and total GE (*D*), genetic correlation of growth (*E*), and responsiveness (*F*) plotted against environmental dissimilarity.

netic distance revealed a significantly positive relationship (fig. 4*C*, slope: 0.05, $P < .001$), indicating that dissimilar clones are likely to be more variable in plasticity than closely related isolates.

A linear relationship may not be an appropriate descriptor for the correlation between the GE components and genetic distance. It rather seems that a genetic distance of approximately 0.3 represents a threshold value above which values change markedly. Intriguingly, this is roughly consistent with the traditional species definition of bacteria that indicates that clones with 97% sequence identity of the 16S rRNA gene belong to the same species (Stackebrandt and Goebel 1994). However, it is important to note that we based calculation of genetic distance on only a partial 16S rRNA sequence, rather than on the whole gene.

Similarly, we calculated the genetic components of GE according to Kassen and Bell (2000) and regressed them against environmental dissimilarity (the standard deviation in fitness between the two environments compared). We found a weak but significant positive correlation between overall GE and environmental dissimilarity (fig. 4*D*, slope: 0.01, permutation test: $P < .001$). The genetic correlation of growth of a range of genotypes across a pair of environments is significantly negatively correlated with environmental dissimilarity (fig. 4*E*, slope: -0.11, permutation test: $P < .001$) but does not become negative within the range of environments sampled. Thus, genotypes perceive environments differently, but not so differently as to change their rank order of fitness. Finally, the relationship between environmental dissimilarity and genetic responsiveness is weakly but significantly positive (fig. 4*F*, slope: 0.007, permutation test: $P < .001$), indicating that environment-specific gene expression is in part responsible for the GE interaction observed.

The nature of our data set allows us to investigate the temporal and spatial components of adaptation in more detail by focusing on, for example, the relative fitness of all genotypes isolated at a particular spatial location at different times (temporal adaptation) or, conversely, all genotypes isolated at the same time but from different spatial locations (spatial adaptation). It is notable that the GE variance and its components respond in a similar way for these more restricted data sets, which suggests that patterns of GE variation result from the combined effects of local adaptation in space and time (figs. A3, A4).

A more direct way to interrogate our data set for evidence of local adaptation is to ask how the average genetic correlation of growth between pairs of clones changes as a function of the distance between two sites separated in space or in time, respectively. The results, plotted in terms of the temporal distance between a pair of sites in months and a spatial distance in meters, are shown in figure 5 in the form of a quasi-topographical map with distance between sites in space and time along the axes and the genetic correlation of growth as the third dimension. The general pattern that emerges is quite clear: the genetic correlation of growth decreases as sites become more distant from each other both in space and in time. As a result, the genetic correlation decreases along the diagonal from the bottom left (sites close in space and time) to the top right (the most distinct sites in both dimensions). Although sampling points are evenly spaced in time (once every month), they are not evenly distributed in space (data points are at distances 0, 6, 12, 600, 990, and 1,100 m). Thus, interpretations of the fine-scale topography of the landscape, such as the "ridge" at 400 m distance, are difficult and might be due to either autocorrelation of environments in space or time or other statistical artifacts. Finally, it is important to note that, for this collection of genotypes at least, the genetic correlation remains positive across the entire set of environments studied. Thus, although we detect the characteristic signal of local adaptation being more likely in environments that are more distant in space and time, there is no indication that divergent selection becomes sufficiently strong to cause different genotypes to be favored in different environments, even among the most spatially and temporally distinct sites.

Discussion

A guiding principle of microbial biogeography is captured by the idea, often referred to as the Baas-Becking hypothesis, that "everything is everywhere but the environment selects." In other words, microbial species are readily dispersed, and the types that are recovered from a given location reflect the action of strong selection favoring local adaptation to the prevailing conditions of growth. The extent to which this hypothesis represents an accurate description of the structure of natural communities of microbes remains a matter of some debate, however, with the available evidence pointing toward both environment-driven selection and dispersal limitation playing a role.

Under the Baas-Becking hypothesis, we expect that microbial genotypes will be locally adapted, in the sense that they have high fitness in their "home" environment and lower fitness in all other "away" environments. We have evaluated this interpretation directly by investigating the extent of local adaptation among sites in both space and time. We used different genotypes of the ubiquitous gram-negative bacterium *Pseudomonas* and its relatives, isolated from soil samples collected in a temperate deciduous forest across a growing season, and estimated the extent of GE interaction for fitness from a reciprocal transplant experiment. The strength of this approach is that it is the only direct way of estimating the degree of local adaptation;

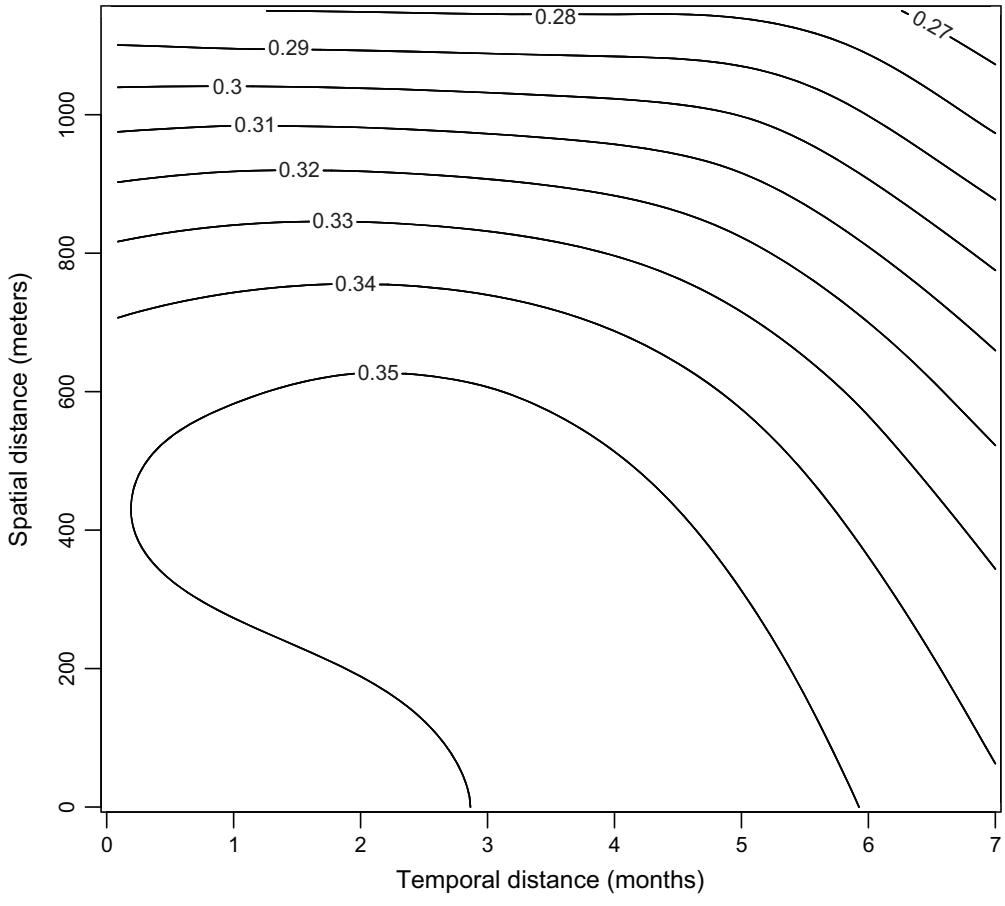

Figure 5: Genetic correlation of growth of all pairs of genotypes and environments plotted against spatial and temporal distance between the environmental pairs.

inference about process based on patterns coming from genetic markers, which themselves have little to do with fitness, can be avoided. Its weakness is that, for the experiments to be manageable, a certain degree of abstraction from the natural environment is necessary.

The level of abstraction that we have chosen here is to focus on one genus of bacteria, *Pseudomonas*, and its close relatives, growing in liquid extracts derived from natural soil environments. The choice to focus on *Pseudomonas* and its close relatives is justified on two counts. First, the strictest interpretation of the Baas-Becking hypothesis is that all genotypes isolated from a given habitat will be locally adapted regardless of taxonomic group. It thus matters little, from the perspective of theory, whether we choose to study *Pseudomonas, Bacillus*, or any other ubiquitous, cosmopolitan group. We chose *Pseudomonas* because these are readily cultured in the laboratory, and our laboratory is well equipped to study this group. Future work will examine the biogeography of both culturable and nonculturable soil microorganisms. Second, previous studies of this group have observed varying degrees of population structure (Haubold and Rainey 1996; Cho and Tiedje 2000; Morris et al 2010), suggesting the existence of some degree of clustering in natural populations, perhaps due to local adaptation.

The use of soil "teas" as an abstraction of the soil environment is likely to capture the prevailing abiotic features of the soil, such as major macro- and micronutrients, pH, and one aspect of the biotic environment, free-living phage. Previous work justifies this approach: soil "teas" have been used to demonstrate strong patterns of local adaptation in space in *Bacillus* (Belotte 2003) and time in phage (Koskella 2013), and additional work has demonstrated the importance of soil tea components such as pH (Fierer and Jackson 2006), inorganic components (Högberg et al. 2007), and phages (Vos et al. 2009) in structuring soil microbial communities. We note that our experiments are not intended to represent the true spatial structure of the soil matrix nor the range of other microbial species with which our isolates might normally be competing. Our

results should thus be interpreted in the appropriate light: as a test of the degree to which *Pseudomonas* are locally adapted to the prevailing macroenvironmental properties of different soil sites.

Briefly, our results can be summarized as follows: First, there is substantial variance in average performance of our clone collection across a growing season and across different locations within a forest, consistent with the idea that temporal and spatial variation can generate strong divergent selection. Second, the behavior of GE variance and its components, inconsistency and responsiveness, reflect patterns that are consistent with a signal of local adaptation: GE increases as environments become more distant in space and time, and it also increases as the genotypes being compared become more genetically divergent, in line with what has been observed for other microbes (Kassen and Bell 2000). Third, the strength of divergent selection, measured as the genetic correlation of growth across pairs of environments, increases with distance between sites in space and time. This result is in line with our intuition that environments become more different the farther apart they are from each other in either space or time. However, it is important to note that the genetic correlation never became negative, even among the sites most divergent in space and time, indicating that divergent selection was not strong enough to generate local adaptation capable of maintaining diversity in our collection of isolates.

Taken together, our results suggest that, consistent with the view of environmental structure under the Baas-Becking hypothesis, there is substantial variation in the strength of selection among environments in these temperate forest soils. Moreover, the strength of divergent selection among sites increases as those sites become more distant from each other in both space and time. Additionally, our results suggest that, over the course of a single growing season at least, spatial variation contributes more to the variance in fitness among soil microbes than temporal variation does, at least on the scales measured here. This result is in accordance with a number of evolutionary studies finding spatial variation to be more effective at supporting genetic diversity than temporal variation, because the latter tends to select for more generalized types (e.g., Kassen 2002; Coffey et al. 2008; Perron et al. 2008). Intriguingly, however, our results are in stark contrast to several studies of microbial biogeography that found strong and predictable temporal structuring of communities (Yannarell et al. 2003; Caporaso et al. 2012; Gilbert et al. 2012). At this point, it is unclear whether this discrepancy is due to differences in the methodology used (growth rate vs. taxonomic abundance and diversity), to the different spatial and temporal scales investigated, to our focus on metabolically active cells versus the presence of both active and dormant cells in biogeographic studies, or to the fact that we focused our efforts on a relatively narrow taxonomic group. Future work clearly needs to be aimed at correlating biogeographic methods based on 16S rRNA sequence diversity with direct fitness measures in a bacterial community.

Our results also did not show strong evidence that the genotypes in our collection were highly specialized for the sites from which they were collected. Rather, the genetic correlation in fitness across sites, which must be negative for selection to maintain specialized genotypes in different environments, was positive across the entire range of space and time studied here. This result likely reflects the ecologically versatile nature of *Pseudomonas* and its relatives, which are found in a wide variety of habitats including soil, water, plants, animals, and humans. Alternatively, it may reflect the fact that we have not adequately measured the degree of local adaptation because our protocols have missed important aspects of the environment, such as the composition of the biotic community. Although we cannot exclude this possibility directly with the data presented here, it seems unlikely that our results are strongly biased against detecting local adaptation to the environmental conditions we have measured. We picked genotypes at random among all sizeable, fluorescent clones present on a plate, meaning that, for the community of *Pseudomonas* at least, we have obtained a representative sample of this taxonomic group. We note further that a similar protocol used by Belotte et al. (2003) was capable of detecting strong patterns of spatial adaptation, lending further support to the idea that our results reflect real patterns of adaptation for soil-dwelling *Pseudomonas*.

There are two other possible explanations for the absence of strong local adaptation in our study. First, we may not have sampled a wide enough range of environments in space or time. Our soil samples were collected from the same general habitat type—a temperate beech-maple forest—and so may not have included habitats that were different enough to support strongly niche-specialized types. Second, dispersal rates may be so high as to overwhelm any signature of selection to a local habitat. We note that high levels of dispersal could explain why several of the haplotypes included in our study were significantly maladapted in space.

Two other features of our results deserve mention. The first is the positive relationship between average environmental quality and variance in fitness within sites. The precise nature of this relationship has been debated for many years by agronomists and animal breeders in a very different context, deciding in which environment to conduct artificial selection to produce the greatest response to selection on a trait of interest. Recently, Martin and Lenormand (2006) used a multivariate extension of

Fisher's geometric model of adaptation to show that the expected relationship between environmental quality and genetic variation within sites is negative: when genetic variance derives from mutation alone, more genetic variance is supported in poorer environments. This relationship emerges in Fisher's model because of the assumption of a single-peaked fitness landscape: the fitness function is steeper for more maladapted types than it is for types closer to the fitness peak. It is tempting, therefore, to interpret our contrasting results as being due a more complex fitness landscape composed of multiple fitness peaks in our environments. However a more detailed appraisal of this hypothesis is not possible in the absence of both a theoretical extension of Fisher's model to multiple peaks and a detailed characterization of the fitness landscapes in our study.

The second observation of note is the behavior of environmental consistency and responsiveness as a function of genetic distance. In both cases, there seems to be a threshold value at a genetic dissimilarity roughly corresponding to traditional bacterial species definitions of 97% 16S rRNA sequence similarity (Stackebrandt and Goebel 1994; Stackebrandt et al. 2002). The 97% similarity cutoff has been criticized as being arbitrary (Cohan 2002; Staley 2006); however, our results suggest that it may reflect genuine differences in ecological responses to environmental variation. This hypothesis deserves further investigation.

Although we have found evidence that the strength of divergent selection increases in a predictable way in space, consistent with that seen in a similar study by Belotte et al. (2003), our work differs from theirs in two important respects. First, we expand on their analysis to include the response of fitness among genotypes in both space and time. As might be expected, selection becomes stronger between sites the further they are from each other in time. Second, we limited our isolate collection to a specific subgroup of soil microbes, the *Pseudomonas* and closely related genera. Belotte et al. (2003), by contrast, isolated the most abundant and vigorously growing colonies that possessed a predetermined colony morphology on a less restrictive growth medium. The Belotte study has often been interpreted as evidence supporting strong local adaptation, whereas in our study, the evidence for local adaptation was weaker. The reasons for this discrepancy may stem from the different taxonomic groups being studied, but the absence of information on strain identity in the Belotte study makes it difficult to evaluate this proposition.

Collectively, our results shed light on the scale and nature of divergent selection in natural communities of soil microbes. Although the biogeographic structure of bacterial communities has been investigated for a century (Beijerinck 1913; Fierer and Jackson 2006; Hanson et al. 2012), little is known about how effective selection is at generating local adaptation in space or time. Under the Baas-Becking hypothesis, it is often assumed that divergent selection is strong, yet few attempts have been made to measure it in natural populations. Our work is an attempt to remedy this situation by studying the scale at which divergent selection operates in space and time. Because we have not studied dispersal directly, however, we cannot evaluate the validity of the Baas-Becking hypothesis as a general explanation for the distribution of *Pseudomonas* in temperate forest soils. Nevertheless, we see our results as a first step toward gaining insight into evaluating the role of divergent selection in structuring natural communities of microbes. The fact that we observe a positive relationship between the strength of divergent selection and distance in both space and time suggests that some degree of dispersal limitation exists for the microbial communities, similar to that seen in other studies of microbial biogeography (Fenchel 2003; Whitaker et al. 2003; Vos and Velicer 2008) and is reminiscent of patterns seen in macroorganisms (Hereford 2009; Fraser et al. 2011). Thus, a similar set of rules may govern the biogeography and community structure of natural communities of both microbes and macrobes.

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