# AN EXPERIMENTAL TEST OF LOCAL ADAPTATION IN SOIL BACTERIA

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*Abstract.*—We extracted bacterial isolates of similar colony morphology from spatially located soil samples within 1 ha of old-growth forest. The same soil samples were used to prepare growth medium. Each isolate was then cultured in each medium and its growth recorded. There was no overall tendency for isolates to grow more successfully in their home site (i.e., the medium derived from the soil sample from which they had been extracted). Most isolates grew very poorly, however, and when the analysis was restricted to the minority of vigorous isolates there was clear evidence of local adaptation: isolates tended to grow better at their home site than did isolates from elsewhere and grew better at their home site than they did at other sites. The variation of growth within the 1-ha plot made up a complex fitness landscape of peaks, ridges, and valleys. Most of the vigorous isolates were found at or near a local fitness (growth) peak, although seldom at a global peak. In consequence, there was a tendency for growth to diminish away from the home site. The home isolate was about 50% more fit than average at its home site; fitness diminished exponentially away from the home site at a rate of 0.0577 per meter. These figures are similar to those previously reported for plants. This selection gradient has matched the bacterial assemblage to the edaphic structure of the environment, although the fit is far from perfect.

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The interpretation of biological diversity hinges on the relative strength of local dispersal and local selection. If all the genotypes or species of a given kind of organism have the same fitness, then the distribution of any particular type will be determined by dispersal alone. This will lead to patterns involving the abundance of species and the diversity of communities that can be described by neutral community models (Bell 2001; Hubbell 2001). Differences in diversity between regions will be attributed primarily to differences in dispersal rate. However, if the relative fitness of genotypes or species varies among sites, then different types will tend to predominate in different sites and divergent selection may preserve diversity within the region as a whole (Williams 1964; Soulé and Stewart 1970; Bell et al 2000). Differences in diversity between regions will be attributed primarily to differences in selection intensity. The balance between dispersal and selection changes with circumstances. In the first place, it changes with geographical scale: as the area of a site decreases, the proportion of the population recruited by immigration increases; consequently, selection will become less effective in maintaining local adaptation. Second, it changes with ecological scale: if sites differ more in productivity, then relative fitness is more likely to vary (Bell 1992) and selection will tend to maintain distinctive local adaptation. In extreme cases, either selection or dispersal is clearly dominant. Thus, if two sites are separated by 1000 km of latitude, or if one is a swamp and the other a chalk scarp, the plants that flourish in one are unlikely to be successful in the other. However, two adjacent 10-m sections of roadside may well differ in the species of herb they support, entirely because of the historical accidents of chance settlement and local proliferation. In the first case, the combined diversity of the two sites can be attributed largely to local adaptation caused by divergent selection; in the second case, distribution and diversity are the consequences of local dispersal alone. We are normally concerned, however, to inter-

pret patterns of distribution and diversity in less extreme cases.

Consider a single habitat that extends over a considerable area. "Single habitat" and "considerable area" are, of course, highly subjective terms based on human perception. We use them rather loosely to mean a region that is much larger than the characteristic dispersal distance of a set of organisms, any one of which may exist at any site within it. A few examples are: mosses in a 10-ha boreal peat bog, trees in a 1000-ha section of tropical dry forest, fish in a 100,000ha watershed. The imprecision of the concept is excused by the consideration that these are the circumstances in which we are most concerned to understand the ecological mechanisms that regulate distribution and diversity. Within such a tract, then, any two species are likely to be found at different sites, and any two sites are likely to contain different sets of species. The crucial question is, to what extent are these differences attributable to local adaptation?

The most powerful approach to this question is experimental and involves some system of moving individuals from one site to another. In most designs, the growth or reproduction of a genotype or a species is measured at its home site-the site where an individual of that type was found living-and at a number of other sites. The results can be used to investigate two aspects of local adaptation. In the first place, the success of a type at its home site or sites can be compared with the success of other types. This evaluates the efficacy of local selection, given that all types have at some point been dispersed to all sites. Most studies are directed toward this issue. There is, however, a second issue: the success of a type at its home site or sites can be compared with its success elsewhere. This evaluates the efficacy of dispersal, given the operation of local selection. Taken together, these two comparisons provide a description of how closely the composition of the assemblage has become fitted to the structure of the landscape.

Transplant experiments of various designs have sometimes provided dramatic evidence of local adaptation, even at very small distances. Such cases, however, usually involve clear environmental transitions, such as mine boundaries (Antonovics and Bradshaw 1970) or forest edges (Lovett Doust 1981), or clear ecotypic differentiation, as in Diodea (Jordan 1992) or Polemonium (Galen et al 1991). In other cases, where neither environmental nor genetic discontinuities were evident, little evidence of home-site advantage has been found (Schemske 1984; Cheplick 1988; Rapson and Wilson 1988; Rice and Mack 1991; van Tienderen and van der Toorn 1991: Helenurm 1998); the topic has been reviewed by Linhart and Grant (1996). The experimental evidence for local adaptation of similar types within a single habitat is thus rather weak and equivocal. Against this may be set the consideration that the labor of transplanting individuals and measuring their reproductive success in the field is often very great, so that experiments tend to be limited in scope and might not be powerful enough to detect modest but biologically significant levels of selection.

We have taken a somewhat unconventional approach to investigate the spatial structure of fitness in detail. Two aspects of its design are especially important. First, we studied the properties of bacterial isolates isolated from forest soil. Using bacteria greatly simplifies the measurement of growth, reduces the labor of cultivation, and avoids the complexities of sexual genetics. Second, we conducted an explant experiment, growing the isolates in the laboratory in aqueous extracts of soil samples, rather than studying them in situ. This makes it possible to measure growth semi-automatically and thereby to conduct a moderately large experiment involving thousands of cultures. The question that we set out to answer was whether natural communities of soil microbes are specifically adapted to conditions of growth at a scale of tens of meters.

## MATERIALS AND METHODS

## Study Site

Soil samples were taken from 1 ha of old-growth forest at the Gault Nature Reserve, Mont St-Hilaire, Quebec. This is a permanent Smithsonian Institution Man and Biosphere (SIMAB) site, described in more detail by Richard et al. (2000). The site was divided up by ropes into  $10\text{-m} \times 10\text{-m}$ cells. After surface obstructions such as twigs and fallen leaves had been removed, a single sample of about 100 g of surface soil was taken with a trowel from each  $10\text{-m} \times 10\text{-m}$ m cell (specifically, from the center of the northwest quadrant of each cell). These samples were stored at 4°C for two weeks and then sieved and sorted to remove stones and roots. Each was then used for two purposes: to provide bacterial isolates and to prepare culture medium.

#### Culture Medium

From each sample, 10 g of air-dried soil was sealed into a pouch made of tea-bag material and infused with 150 ml distilled water at room temperature for 24 h. The aqueous extract was then passed through a sterilizing filter (0.22 micron) and made up with 10 g/L of glycerol to provide a carbon source. Glycerol was chosen because it diffuses freely into bacterial cells without requiring specific membrane transporters; the concentration is the same as the King's B medium used in selecting and expanding the isolates. Filtration was preferred to autoclaving because it does not modify the chemical composition of the extract, but passage of virus and naked DNA, and subsequent infection or transformation, cannot be excluded. For convenience, this preparation is henceforth called a "site," reflecting the 10-m  $\times$  10-m cell within the study area from which it was derived.

#### **Bacterial Isolates**

A pinch of soil from each sample was suspended in 15 ml distilled water and left for 10 min, after which 20  $\mu$ l of liquid was pipetted onto each of two culture plates containing standard King's B agar medium and incubated for 24 h. From the diversity of bacteria that grew on the plates, we selected a type with a distinctive colony morphology, white in color and with wrinkled edges, that appeared on all plates. One colony of typical appearance was chosen from each plate, then restreaked and reisolated before being expanded in liquid King's B medium in one well of a 96-well microwell plate and stored in 50% glycerol at  $-80^{\circ}$ C. Sterile procedures were used throughout the experiment once the isolates had been selected. This procedure supplied two replicate isolates of similar colony morphology from each of 96 soil samples. Four of the original 100 sites were discarded at random.

All isolates were Gram-positive rods. Ten isolates (six vigorous and four nonvigorous, see below), including the four shown in Figure 4, were sent for identification by fatty acid composition to Microbial ID, Inc. (Newark, DE). All were determined to be *Bacillus mycoides* GC subgroup A.

## Growth Assay

The purpose of the growth assay was to estimate the growth of each isolate at each site. We prepared two sets of 96 assay plates, each plate bearing 96 samples of the soil extract prepared from a given site. The two replicate isolates from each site were thawed and reconditioned on plates bearing King's B liquid medium. They were then transferred to the assay plates, so that each assay plate contained all the 96 isolates from one of the two replicate sets growing in the soil extract prepared from a single site. The plates were then incubated unshaken for 48 h at 28°C. The absorbance of each well at 660 nm was measured with an automated plate reader immediately after inoculation and then again after incubation; the timing of inoculation and measurement being staggered so that the duration of growth was the same, within 10 min, for each well. Growth is defined as the difference between initial and final absorbance. The total number of cultures scored was  $2 \times 96 \times 96 = 18,432$ , with no missing data.

We decided to score the greatest possible number of isolates rather than to use replicate cultures of each isolate and, consequently, have no direct estimate of the error variance involved in the assay procedure. To evaluate this variance, we used the mutator strain SBW25  $\Delta mut$ S of *Pseudomonas fluorescens* (R. C. McLean, unpublished) to construct 69 closely related but nonidentical lines expressing wide variation in growth. These were treated in a manner similar to

0.30

0.25

the experimental isolates. They were grown up in King's B medium overnight in tubes at 28°C, then diluted 100-fold into fresh King's B medium and replicated 14-fold into 96-well microwell plates, which were incubated for 24 h at 28°C without shaking before being scored on the same plate reader used in the experiment.

#### RESULTS

## Growth of Explants

Growth was positive for all isolates and all sites, but varied considerably. Variation among isolates (CVs for two replicate sets 0.544, 0.520) was greater than variation among sites (CVs 0.328, 0.344). Many isolates grew poorly at all sites, whereas a minority of about 15–20% of vigorous isolates grew well in most sites.

The growth of the *Pseudomonas* mutator lines used to evaluate the error variance was similar to that of the most vigorous isolates. The average variance of replicate cultures of the same genotype was 0.000566 (average CV 0.072) and was only weakly related to the mean ( $r^2 = 0.15$ , not improved by log transformation). The average variance of the vigorous isolates over all sites was 0.003375 (average CV 0.74); thus, the error variance associated with replicate culture and measurement seems to be only a small fraction (0.000566/ 0.003375 = 0.17) of the overall variance among sites.

## Performance of Home Isolates

There was no general tendency for the growth of an isolate to exceed that of others in its home site (Fig. 1). Indeed, the majority of isolates grew somewhat more poorly than average at their home site. Absorbance readings of less than 0.1 have low repeatability from plate to plate and may indicate poor growth or no consistent growth at all, whereas readings in excess of 0.1 nearly always indicate consistent and repeatable growth (unpubl. obs.). Restricting the analysis to isolates with absorbance greater than 0.1 showed that the minority of vigorous isolates that grew well almost everywhere grew better than the average of isolates at their home site. This might mean that all isolates that are readily capable of growth in the soil extracts are locally well adapted. A simpler explanation, however, is that an isolate that grows well almost everywhere will usually grow well at its home site, whereas most isolates grow poorly at all sites, including this one. The wide variance among isolates would thereby lead to the apparent overyielding of generally superior isolates at their home site.

There was likewise no general tendency for an isolate to grow better at its home site than at other sites (Fig. 2). Again, however, generally superior isolates were distinctive: they grew better than average at their home site than elsewhere. This cannot be attributed to the variation of growth over sites, because there is no reason, apart from local adaptation, that the home site of a given isolate should differ consistently from other sites.

Taken together, these two observations provide support for a limited degree of local adaptation. The majority of isolates grow poorly everywhere and show no tendency to perform exceptionally well at their home site. A minority grows well



FIG. 1. Local adaptation: effect of selection. The two parts of the figure show the two replicate series. The line represents equality: above the line, the home isolate is superior to the mean performance of all isolates at the home site.

at most sites, and these isolates seem to grow particularly well at their home site.

### The Fitness Landscape

The fitness landscape is the spatial structure of the fitness of an isolate. The design of the experiment makes it possible to reconstruct the spatial structure of growth in the 1-ha plot. Fitness itself may involve many other factors, of course, including complex social interactions among bacteria; we assume that the amount of growth achieved in pure culture in the laboratory is an important component of fitness. The spatial structure of mean growth has a complex topography (Fig. 3). There is a steep ridge of high growth extending roughly west–east through the middle of the plot, with peaks at each end. It is separated by a deep valley from a subsidiary ridge to the southeast, with a broad and deep basin in the southwest. Both northern and southern boundaries have abrupt peaks and depressions. A similar landscape can be drawn for each isolate, showing how the growth of a single isolate varies



FIG. 2. Local adaptation: effect of dispersal. The two parts of the figure show the two replicate series. The line represents equality: above the line, the performance of an isolate at its home site is superior to its mean performance over all sites.

over the 1 ha that was sampled. These landscapes are interesting, because they provide a direct visual test for local adaptation: a locally adapted isolate will show a peak in the fitness surface at or near its home site. For the minority of generally superior isolates, this was usually the case. Two examples are shown in Figure 4, one involving a major homesite peak at a marginal site (Fig. 4A) and the other a minor home-site peak at an interior site (Fig. 4B). In both cases the topography of growth is complex. A moderate displacement of 10-20 m from the home site is usually associated with a decrease, often a considerable decrease, of growth. At greater distances, however, there are other peaks, some of which may be higher than the home-site peak. Thus, isolates that are able to grow well in the soil extracts typically occupy local peaks that are surrounded by areas of lower growth but that are not necessarily global maxima.

A very few isolates grew very well overall without having markedly superior growth at their home site; these are the outliers to the upper right in Figure 1, falling near the line of equality. The fitness landscape of one of these generalist



FIG. 3. The fitness landscape. Mean growth (absorbance of cultures after 48 h incubation) over all isolates at 10-m intervals in 1 ha of forest floor.

types is shown in Figure 4C. It is generally elevated, but with very steep relief. The home site occupies an undistinguished position on a ridge of high growth that runs west–east along the southern edge of the survey area. This is separated by a very deep valley from a more complex region of high growth to the north. In this case, then, the home site occurs within a region of generally high growth but is not itself on or near a local peak.

The majority of isolates that grow poorly everywhere shows no distinctive spatial patterns. An example is shown in Figure 4D. The landscape has a generally very low elevation, with a few modest peaks here and there. The home site is situated on a low plain between a marginal peak and an interior basin. There is no sign of local adaptation.

#### Distance Decay of Growth

We have chosen two ways of summarizing the spatial patterns displayed by generally superior isolates. The first is the average growth at all sites within a given distance of the home site, which is expected to decrease with increasing distance. The second is the frequency of sites at a fixed distance from the home site where growth is higher. This should also decrease with distance if the home site is near a local peak, although the complex topography of the fitness landscapes suggests that the probability of finding at least one site where growth exceeds the home site may actually increase as more and more distant sites are examined.

Mean growth collapses to about 60% of the home site value at sites 10 m distant. Thereafter, it declines steadily to about 45% of the home site value at very distant sites (Fig. 5A). This pattern can be compared with that of randomized data. An artificial dataset was created by re-assigning west–east and south–north coordinates at random to the set of mea-



FIG. 4. The fitness landscape for single isolates. The arrow marks the home site of the isolate. (A) a specialist isolate whose home site is a major fitness peak; (B) a specialist isolate whose home site is a minor fitness peak; (C) a generalist isolate; and (D) an inferior isolate.

surements at each site. Thus, the pattern of genetic response at each site remained the same, but any spatial correlation between sites was destroyed. In a minority of cases, growth at the redesignated home site will have been allocated, by chance, a high value, corresponding to that of the generally superior isolates in the dataset. The analysis is restricted to these cases. At a distance of 10 m from the home site, growth will necessarily be much less in almost all cases. Thereafter, however, there is no consistent tendency for it to decrease further (Fig. 5B). Thus, the observed pattern can be interpreted as follows. The collapse of growth in sites next to the home site may be in part an artifact of analyzing only highgrowth isolates. The most conservative comparison is between the zero-intercepts of the randomized data (0.662) and the real data (0.605), suggesting that a small displacement from the home site is associated with a loss of growth of about 10% (i.e., [0.662 - 0.605]/0.662 = 0.086). Further displacement causes a sustained reduction in growth that is not shown by the randomized data and can only be attributed to loss of local adaptation with distance.

0.4



FIG. 5. The decay of growth away from the home site: mean growth of isolate at all sites within a given radius of the home site. Solid and hollow circles are means ( $\pm 2$  SE) for the two replicate series. (A) Data from survey. Combined data has linear regression slope of -0.002161 per meter,  $r^2 = 0.86$  (for means), P < 0.001. (B) Randomly relabeled sites. Combined data has linear regression slope of -0.000085 per meter,  $r^2 = 0.004$  (for means), P > 0.1.

A similar analysis can be conducted for the probability that growth at a random site located a given fixed distance from the home site exceeds growth at the home site. In practice, we computed this probability for sites falling on the rim of a square whose side is displaced d units from the home site. For adjacent sites with d = 1, 10 m from the home site, the probability that a random site is more productive is only p= 0.210. Because the home site tends to lie at or near a local fitness peak, this probability declines steadily with distance to a value of about 0.1 at distances approaching 100 m (Fig. 6A). Note, however, that the probability that at least one site exceeding the home site exists at a given fixed distance is 1  $-(1-p)^n$ , where n is the number of sites at that distance. Because *n* increases with d (n = 8d) the probability that at least one higher peak exists is roughly  $1 - \exp(-8dp)$ , which rapidly approaches unity even for modest values of p. This is consistent with the complex topography of the fitness land-



FIG. 6. The decay of growth away from the home site: the probability that a site at a fixed distance supports higher growth of the home isolate. Solid and hollow circles are means ( $\pm 2$  SE) for the two replicate series. (A) Data from survey. Combined data has linear regression slope of -0.001603 per meter,  $r^2 = 0.70$  (for means), P < 0.001.  $r^2$  for exponential decay is 0.74 (adjusted, 0.70). (B) Randomly relabeled sites. Combined data has linear regression slope of -0.00050 per meter,  $r^2 = 0.21$  (for means), P > 0.1

distance from home site

scapes, in which the home site usually sits near a local but not a global maximum of growth. For randomized data the estimate of p was 0.246, so that again most of its initial drop might be attributable to restricting the analysis to generally superior isolates. Thereafter, however, it shows no consistent pattern of change (Fig. 6B), showing that the decline observed in the real data is a second manifestation of local adaptation.

The distance decay of growth causes a decline in relative fitness away from the home site, where relative fitness is defined as the growth of the home isolate at a given site, relative to the mean of all isolates at that site. We have restricted the analysis to the 36 superior isolates (from both replicate series) alone. The fitness of the home isolate exceeds the mean fitness at the home site by 48% (Fig. 7). Because



FIG. 7. Distance decay of fitness. Fitness is estimated as the growth of the home isolate relative to the mean growth of all isolates for any site at a given distance from the home site. Plotted points are means ( $\pm 2$  SE) for the vigorous isolates in both replicate series combined. Fitness for all sites except the home site was fitted to the three-parameter exponential function  $w_d = w_{\infty} + \alpha \exp(-\beta d)$ , where *d* is distance from the home site in meters, with  $r^2 = 0.99$  (for means), P < 0.001, to obtain the estimates of  $w_{\infty} = 0.735$ ,  $\alpha = 0.377$ ,  $\beta = 0.0577$ .

only superior isolates are included in the calculation of the mean, this estimate is unlikely to be an artifact of genetic variance in growth rate. The decrease of relative fitness away from the home site, excluding the value at the home site itself, is extremely well fitted by an exponential decay function:

$$w_d = w_\infty + \alpha \exp(-\beta d), \tag{1}$$

where  $w_d$  is the relative fitness at distance d from the home site,  $w_{\infty}$  is the relative fitness approached at a great distance from the home site, and  $\beta$  is the decay coefficient. The estimate of asymptotic fitness away from the home site is  $w_{\infty}$ = 0.735. The added fitness representing the excess fitness at the home site relative to the mean fitness of the home isolate at distant sites is  $\alpha = 0.377$ . Thus, the increase in fitness at the home site, relative to a random distant site, is 0.377/0.735 = 0.51. This is close to the estimate of 48% obtained from a direct comparison of the fitness of the home isolate at the home site with that of all other isolates, most of which will come from distant sites. Away from the home site, it may be useful to think in terms of a standardized home-site advantage,  $w_{\text{home}} = (w_d - w_{\infty})/\alpha$ , which has value unity at the home site and decreases asymptotically toward zero with distance. It expresses the excess fitness of an isolate attributable to its proximity to the home site, relative to the mean fitness of

#### DISCUSSION

The techniques that we used in this experiment enabled us to obtain reliable and precise estimates of the spatial structure of growth. At the same time, they introduced difficulties in applying these results to the problem of how diversity is maintained in natural communities. We shall discuss these difficulties before attempting to draw any broad conclusions from the experiment.

First, nondescript bacterial isolates are not clearly comparable either with species or with genotypes of multicellular sexual organisms. It is undeniably good practice to attempt to identify the organisms being studied, to the extent that this is practicable, as we have done. It will be very difficult or very expensive, unfortunately, to identify the hundreds of microbial isolates that might be used in studies of this sort. The problem that we addressed, however, does not necessarily require the material to be identified (however interesting it might be for other reasons to do so). It requires merely that we are able to compare the fitness of ecologically similar organisms at the site where they were found with their fitness elsewhere. Whether the organisms should have the same or different names or should belong in a Linnean category of given rank is not specified by the problem and is not relevant to its resolution. We note, moreover, that the basic theory of selection in heterogeneous environments descending from the original model of Levene (1953) does not require sex or diploidy or multicellularity. It applies in exactly the same way to species of sexual organisms, alleles in sexual populations, and nondescript bacterial isolates.

Second, all the isolates whose species were determined turned out to be Bacillus mycoides, a common aerobic bacterium in the Bacillus cereus group. It forms endospores, and it is possible that some or many of our isolates germinated from spores that had been dispersed from some distant site or had lain for many years in the soil. If so, they might be less likely to show local adaptation, because they would not have been selected in the site where they were assayed. The hypothesis under test is that selection will lead to local specialization, and measuring fitness in the propagule pool will always yield a lower estimate of the degree of specialization than measuring it in the adult pool; in the extreme case of a Levene-type model with completely random dispersal in every generation, measuring fitness in the propagule pool would fail to reveal any local adaptation at all. This objection, or reservation, applies to all transplant, explant, and implant experiments. It is minimized when seeds or ramets are collected from adult plants growing in situ and replanted at the same or different sites, although even in this case the sample might consist mainly of recent immigrants that, although viable, are not particularly well adapted to the site where they were found.

Finally, the explant design necessarily excludes much (perhaps most) of the environmental heterogeneity that it is intended to evaluate. This is a just and severe criticism that cannot be refuted, although it can be palliated. The bacteria

that we studied may well have been living in intimate association with particular kinds of surface, experiencing distinctive physicochemical conditions varying at scales of microns, and interacting with a great variety of other organisms. Our procedure involved removing them from this context, exposing them to novel conditions of growth in the laboratory, and then restoring only the bulk chemical environment represented by water-soluble components of the soil samples. This is again a reservation that extends to all kinds of transplant experiments, albeit more strongly to some than to others. If we were to work with an organism such as Pseudomonas fluorescens, for example, we would have greater confidence that our isolates had grown in the sites from which they were collected; on the other hand, because this bacterium is usually associated with plant surfaces, we would have less confidence that our assay environments were appropriate.

These two objections, that either the material or the assay conditions are inappropriate, can be made to any transplant experiment. Negative results-that the home isolate has no special relationship with the home site-are correspondingly difficult to interpret, because all the isolates might be either recent immigrants or residents adapted to environmental factors that the assay procedure had eliminated. Negative results from transplant experiments are in fact seldom published, perhaps for this reason. Where positive results have been obtained, however, the case for local adaptation is very strong: because the material is always in some degree inappropriate and the conditions of growth always in some degree unrealistic, any striking and consistent relationship between fitness and location is correspondingly convincing. Thus, while the two objections that we have described are always made, and always well taken, they imply that transplant experiments have high stringency and that their interpretation is necessarily conservative.

Setting these criticisms to one side, our experiment produced two principal results. The first was that most isolates grow poorly at all sites. This might mean only that most isolates grow poorly on microwell plates in the laboratory, however. Bacillus is normally a facultative aerobe in the soil; it may be poorly adapted to growing in liquid medium on unshaken plates that may have become anaerobic by the end of the assay. These potentially inappropriate environments are necessary for the experiment to be practicable, but the poor growth of most isolates shows how they weaken its interpretation. The second result was that the minority of isolates that grew well overall grew particularly well at their home site relative to other isolates. The variance of growth among isolates, however, prevents this fact from demonstrating local adaptation, because a generally superior isolate is very likely to exceed the average at its home site. We also found, however, that any given superior isolate tended to grow better at its home site than at a random site. Taken together, these two observations provide convincing evidence that the most vigorous isolates, at least, were adapted to the general chemical conditions of growth at their home sites.

The degree of local adaptation was considerable, with the growth of the home isolate exceeding the mean growth at the home site by about 50%. This degree of adaptation is, of course, maintained by selection. The steepness of the selection gradient responsible is expressed by the parameter of

the exponential decay function relating relative growth to distance from the home site, which was estimated to be 0.0577 per meter. The spatial extent of local adaptation can be expressed as the distance from the home site at which the growth of the home isolate becomes equal to the mean growth of all isolates. This is estimated to be  $-(1/\beta)\ln[(1-y_0)/\alpha] =$ 6.1 m. Because this is somewhat less than the 10-m grain of the survey, we may take 10 m to be roughly the spatial scale at which distinctive local adaptation can be detected. It should be emphasized again that this local adaptation involves the general chemical conditions of growth alone. Bacterial growth is no doubt influenced by many factors that act at much smaller spatial scales and that will not be detected by our procedures. The spatial scale that is addressed by our experiment is not the scale at which bacteria grow and interact, but rather the scale at which diffusible substances alter the nutrient characteristics of soils.

There are few comparable studies of the spatial structure of fitness at scales of less than 1 km. Galloway and Fenster (2000) found no relationship between fitness and distance from the home site in the annual plant Chamaecrista, except at distances of 1000 km or more; the smallest distance in their experiment, however, was 100 m. Schmitt and Gamble (1990, table 5) found that the overall fitness of inbred progeny of another annual herb, Impatiens, fell to 0.52 relative to home-site fitness at a distance of 12 m. The exponential rate of fitness decline was 0.031 per meter (albeit calculated from only three points). Waser and Price (1989, table 3) obtained a mean fitnesses of 0.52 and 0.62 for outcrossed seeds planted at 10 m relative to those planted at 1 m from the home site in the perennial herb Ipomopsis. These values are strikingly similar to those that we report, given that bacteria and plants might be expected to show quite different spatial patterns. Their similarity suggests that bacteria and plants alike are adapting to environmental heterogeneity at a scale of a few meters. In the case of the bacteria, the agents responsible for this heterogeneity are certainly water soluble and therefore diffusible substances. Both the source of selection and the spatial scale of adaptation are reasonable, because we have already demonstrated that forest soils are patchy at scales of 1-10 m with respect to nutrients such as phosphate and nitrate (Lechowicz and Bell 1991) and that plant growth responds to this patchiness in explant experiments (Bell and Lechowicz 1991). The work on Impatiens was conducted in a forest environment (oak-hickory woodland in New England) similar to ours, whereas the Ipomopsis study site was dry montane grassland.

The spatial structure of fitness has been used to calculate the intensity of local selection  $\Delta w/w$  if dispersal rate is known. For the *Impatiens* experiment by Schmitt and Gamble (1990), this gives a value of about  $2 \times 10^{-3}$  per generation (Burt 1995). No corresponding estimate can be made for our bacterial isolates, whose dispersal rate is unknown. An indirect estimate can be obtained, however, by noting that the relative increase in mean fitness will be equal to the standardized genetic variance  $V_w^2$  (Burt 1995). This variance can be estimated from the mean of the 96 standardized squared differences between the replicate isolates in our survey, which yields an estimate of  $V_w^2 = 0.180$  (SE = 0.026). This is no doubt inflated by genotype-environment interaction and lies close to the upper bound of 0.3 suggested by Burt (1995). It is not very different, however, from comparable indirect estimates ranging up to 0.20 reported for *Impatiens* by Mitchell-Olds (1986). Thus, our data are broadly consistent with the proposition that plants and bacteria are both adapted to edaphic heterogeneity at a scale of a few meters.

Underlying these average responses is the detailed spatial structure of growth. Our experiment allows us to visualize how the distribution of bacterial isolates is related to their capacity for growth at different sites throughout the sampling area. In most cases, isolates that were generally capable of vigorous growth showed a complex landscape of ridges, peaks, and valleys of growth. This spatially structured environmental variance will give rise to selection that will tend to drive any particular isolate from the valleys up the slopes toward the nearby peaks. Fine-scale variation in selection has been observed before in both natural (Stewart and Schoen 1987) and experimental (Stratton and Bennington 1998) populations of plants. Furthermore, we have shown previously that the microbial community of soil contains a great deal of selectable genetic variation that enables it to adapt rapidly to different conditions of growth (Koelewijn et al. 2001). The existence of environmental heterogeneity, the presence of selectable genetic variation, and the occurrence of divergent selection are all prerequisites for the emergence of local adaptation, but none of them separately, nor all of them together, guarantee that local adaptation will evolve. In fact, most vigorous isolates appear to be located close to a local growth maximum. That is, isolates tend to be found within a small area where their growth is substantially greater than it would be in the surrounding region. The topography of growth was markedly idiosyncratic, however, with different isolates showing peaks and troughs in quite different locations within the sampling area. The environmental heterogeneity of edaphic factors that we have previously demonstrated in these forest soils is thus accompanied by genotypeenvironment interaction that provides an opportunity for local selection.

Local selection seems to have sculpted the distribution of the most vigorous members of this bacterial assemblage so that it roughly matches the underlying environmental structure. The match of assemblage to environment is by no means perfect, however. Isolates rarely occupy a global peak of growth rate. Any random displacement from the home site is expected to result in a loss of fitness, but there are almost certain to be some sites at any given distance where growth would be greater. The imprecision of the match between assemblage and environment is likely to be attributable to the failure of immigration or mutation to introduce well-adapted types before environmental factors change. Because selection will continually tend to re-establish a match as fast as it is degraded by environmental change, the selection gradients reported here may be a permanent feature of the soil environment.

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