Competition–dispersal tradeoff ecologically differentiates recently speciated marine bacterioplankton populations

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Although competition–dispersal tradeoffs are commonly invoked to explain species coexistence for animals and plants in spatially structured environments, such mechanisms for coexistence remain unknown for microorganisms. Here we show that two recently speciated marine bacterioplankton populations pursue different behavioral strategies to exploit nutrient particles in adaptation to the landscape of ephemeral nutrient patches characteristic of ocean water. These differences are mediated primarily by differential colonization of and dispersal among particles. Whereas one population is specialized to colonize particles by attaching and growing biofilms, the other is specialized to disperse among particles by rapidly detecting and swimming toward new particles, implying that it can better exploit short-lived patches. Because the two populations are very similar in their genomic composition, metabolic abilities, chemotactic sensitivity, and swimming speed, this fine-scale behavioral adaptation may have been responsible for the onset of the ecological differentiation between them. These results demonstrate that the principles of spatial ecology, traditionally applied at macroscales, can be extended to the ocean’s microscale to understand how the rich spatiotemporal structure of the resource landscape contributes to the fine-scale ecological differentiation and species coexistence among marine bacteria.

Significance

The resource landscape for marine microbes is composed of microscale resource patches, but whether this microheterogeneity can drive the ecological differentiation of natural microbial populations remains unclear. The tradeoff between two nascent populations of marine bacteria demonstrated here is significant for several reasons. First, it illustrates that principles of spatial ecology, so far only illustrated for animals and plants, apply to the ephemeral, microscale nutrient landscape of marine microbes. Second, the results suggest that differential behavior can ensure coexistence of otherwise very similar populations of organisms. Finally, because the demonstrated tradeoff induces microgeographic separation among the populations, it may be a crucial step in initiating gene flow barriers that ultimately allow the populations to embark on differential evolutionary trajectories.

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Significance

The resource landscape for marine microbes is composed of microscale resource patches, but whether this microheterogeneity can drive the ecological differentiation of natural microbial populations remains unclear. The tradeoff between two nascent populations of marine bacteria demonstrated here is significant for several reasons. First, it illustrates that principles of spatial ecology, so far only illustrated for animals and plants, apply to the ephemeral, microscale nutrient landscape of marine microbes. Second, the results suggest that differential behavior can ensure coexistence of otherwise very similar populations of organisms. Finally, because the demonstrated tradeoff induces microgeographic separation among the populations, it may be a crucial step in initiating gene flow barriers that ultimately allow the populations to embark on differential evolutionary trajectories.

Author contributions: Y.Y., O.X.C., M.F.P., and R.S. designed research; Y.Y. and F.M. performed research; Y.Y. and J.-H.H. contributed new reagent/analytical tools; Y.Y., O.X.C., and F.M. analyzed data; and Y.Y., O.X.C., M.F.P., and R.S. wrote the paper.

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underpinning their niche partitioning and the ecological tradeoff enabling their coexistence.

We demonstrate that the two *V. cyclitrophicus* populations have different ecological strategies to interact with microscale nutrient patches in the ocean. We show that the superior ability of the particle-attaching L population to colocalize with the highest nutrient concentrations is offset by the ability of the nonattaching S population to rapidly migrate to new nutrient sources upon a temporal change in the nutrient landscape. These results reveal that the ecological differentiation of the two sympatric populations is driven by a competition–dispersal tradeoff, whereby exploitation of individual nutrient patches comes at the cost of a reduced ability to migrate toward new patches. The ephemeral nature of marine microscale nutrient patches thus enables the coexistence of two foraging strategies, respectively favoring high access to resources at the individual patch level and at the microscale landscape level.

#### Results and Discussion

We began our systematic comparison of spatial behavioral adaptations by measuring the ability of the two populations to attach to and colonize surfaces, motivated by the predominant occurrence of the L and S populations in the large-particle fraction and the free-living fraction of seawater samples, respectively (16). Attachment to surfaces is a widespread phenotypic of marine bacteria (20), used to associate with particles and hosts (21–23), and a genomic comparison of the two populations suggested differential genetic potential for attachment (18).

We found strong and widespread differences in the attachment behavior of seven L and five S isolates, respectively. Imaging of attachment to polystyrene over 1 h (*SI Materials and Methods*) yielded a ratio, \( \alpha \), of 4.9 between the numbers of attached cells for L- (695 ± 161 cells per mm\(^2\)) and S-population isolates (143 ± 59 cells per mm\(^2\)) (Fig. 1A). The attachment ratio was even larger (\( \alpha = 42.4 \)) when the surface was more hydrophobic (Fig. S1A), and only mildly smaller (\( \alpha = 4.4 \)) when it was more hydrophilic. We measured similar differences in attachment to surfaces made of the polysaccharides found in the cell walls of algae and phytoplankton, specifically agarose (Fig. S1B), cellulose (\( \alpha = 4.5 \); Fig. S1B), and alginate (\( \alpha = 14.9 \); Fig. S1C), although not with chitin (Fig. S1C) and *SI Results and Discussion*). These results reveal a first, clear behavioral difference between the two populations, verifying the original hypothesis based on dissimilar distribution in the wild (18) that attachment to particles is an important mechanism in their ecological differentiation, and further demonstrating that differences in attachment strategies are largely independent of the precise chemical state of particles.

The different preference for surfaces was further manifest in the ability of the L population but not the S population to rapidly form biofilms and thus stably associate with surfaces (Fig. 1B and C and *Fig. S1D*). Biofilm formation, through the production of extracellular polymers that cement and protect cells, is a common bacterial behavior for the long-term colonization of surfaces (24, 25). Experiments under batch culture conditions showed that L-population isolates exhibited approximately ninefold higher values of area coverage than S-population isolates after 5 h, and this difference remained consistent throughout the early stages of biofilm formation (Fig. 1B). After 3 d, the S-population isolate 1F111 hardly formed any biofilm, whereas the L-population isolate ZF270 formed a dense biofilm (Fig. 1C).

Staining with an FITC-labeled lectin confirmed the biofilm nature of this cell assemblage by revealing the presence of a rich extracellular matrix (Fig. S1D). Even the precolonization of a surface by an isolate from the L population (ZF270) did not reduce the difference in surface colonization among isolates from the two populations (Fig. S1E).

These behavioral differentiations in surface colonization are supported by both genomic and phenotypic evidence. Genomic analysis has shown that only a small number of gene clusters are specific to either population (18). All isolates from the L population but none from the S population harbor the mannose-sensitive hemagglutinin (*msh*) gene cluster (18) (Fig. 1D), which encodes the mannose-sensitive hemagglutinin type IV pili and is involved in the surface attachment of *Vibrio cholerae* (26). The differential presence of the *msh* gene cluster exactly matches the difference in surface attachment between the two populations (Fig. L4 and *Fig. S1A–C* and *E*) and is further confirmed at the phenotypic level: By imaging 25 cells from two isolates per population via electron microscopy, we found that pili are present in 84–91% of cells from the L population and in 0% of cells from the S population (Fig. 1E). A second gene cluster...
harbored only by the L population is the symbiosis polysaccharide (syp) cluster (18) (Fig. 1D), which is involved in the production of extracellular matrix in *Vibrio fischeri* (27). Its differential presence matches the superior ability of the L population to form biofilms (Fig. 1B and C and Fig. S1D). Among all population-specific genes that have been reported for these two populations (18), the only genes potentially related to attachment are those in the msh and syp clusters. The specificity of the msh and syp gene clusters, together with their known functions in other *Vibrio* species, suggests that they play a fundamental role in regulating the different habitat preferences between the two *V. cyclitrophicus* populations.

In the ocean, particles are nutritional hotspots in an otherwise nutrient-poor water column, and considerably increase uptake rates of attached bacteria (28). Our observations of differential attachment then imply a competitive advantage for the L population, owing to its superior ability to colocalize with the high nutrient concentrations occurring on particles. We verified this hypothesis by quantifying growth on alginate as the sole carbon source: We provided cells the same total amount of alginate in either particulate or dissolved form, and compared their growth rate on the two forms of alginate to isolate the effect of the physical interaction with particles. Having resources concentrated on particles generally decreases the rate of nutrient exposure per cell compared with the case of uniformly dissolved nutrients. This spatial localization of resources had a much stronger negative impact on the S-population isolate 1F111, whose drop in growth rate on particulate alginate relative to dissolved alginate was more than twofold larger compared with the L-population isolate ZF270 (Fig. 1F), which was able to compensate by attaching to and colocalizing with the particles. This result confirms the hypothesis that attachment confers a growth advantage to cells from the L population and shows how the physical interaction between bacteria and particles has a considerable effect on growth.

What tradeoff, then, prevents the S population from being outcompeted? We hypothesized that the two populations differ in their ability to swim or respond to chemical gradients (chemotaxis), two widespread phenotypes in the ocean, where they allow bacteria to locate and exploit microscale nutrient patches (6, 7, 29). Specifically, we expected that the S population would have superior motility and chemotaxis to compensate for the disadvantage in attachment. However, detailed analysis of traditionally measured behavioral abilities—swimming speed and chemotactic preferences—yielded highly comparable responses for the two populations. Instead, a microfluidic system quantifying the response to temporal shifts in the nutrient landscape revealed a strong difference between L and S isolates, showing that the tradeoff between the two populations does not occur at the level of an individual patch but rather resides in the differentiation between a strategy that maximizes access to resources at the patch level (L population) and one that favors access to resources at the landscape level (S population). In the following, we demonstrate these results.

Cell tracking (SI Materials and Methods) showed that all 12 isolates exhibited comparable swimming speeds, averaging 59 ± 12 μm/s (S) and 54 ± 9 μm/s (L) (Fig. 2A). Additional measurements for S-isolate 1F53 and L-isolate ZF270 further showed that cell size and flagellation are comparable (Fig. 2A) and that the similarity in swimming speed is independent of the growth stage (Fig. 2A, Inset).

Chemotaxis toward steady resource gradients, generated by diffusion within microfluidic devices (design 1; Fig. S2), also revealed no significant difference between the two populations. This can be seen from the spatial distribution of cells for each of the 12 isolates along a gradient of the amino acid serine (Fig. 2B and C). A quantitative comparison of the chemotaxis magnitude, defined as the skewness of the cell distribution (Fig. 2D), yielded values of 1.1 ± 0.3 (S) and 1.0 ± 0.2 (L). An in-depth comparison between S-isolate 1F53 and L-isolate ZF28 showed comparable chemotaxis also toward other important marine chemotactants (Fig. 2E), including glucose, a product of photosynthesis by phytoplankton (30); N-acetyl glucosamine (GlcNAc), the monomer of the chitin exoskeleton of many marine organisms (31); and dimethylsulfiniopropionate (DMSP), a climatically important sulfur compound that serves a broad range of physiological and signaling functions in the ocean (32). Although we cannot rule out differences in the chemotactic preferences for other chemical compounds, these observations strongly suggest that the S population does not outperform the L population in terms of swimming ability and chemotactic responses to steady resource gradients.

Taken together, the results on attachment and motility imply that the tradeoff enabling the coexistence of the two populations cannot be understood at the single-patch level. Instead, the stable surface colonization by the L population together with the transient nature of resource hotspots in the ocean, where lysis (33), sloppy feeding (34), particle sinking (34), and turbulent stirring (29) all result in ephemeral patches of dissolved organic matter (35–37), led us to hypothesize that the S population gains its competitive advantage at the microscale landscape level by exploiting the temporal variability of the resource landscape and the continuous appearance of new patches. Specifically, we tested the hypothesis that S cells can better respond to a temporal change in the resource landscape. We first generated a steady resource gradient by releasing serine from the porous

![Fig. 2](image-url). Motility and chemotaxis are very similar for the two genetic populations of *V. cyclitrophicus*. (A) Swimming speed of the 12 isolates from the S (blue) and L (red) populations, measured at mid-exponential phase. Dashed lines and shadings denote averages and SDs for each population. (Inset) Growth-stage dependence of speed for one S (blue) and one L (red) isolate, whose cell shape is shown in the TEM images. (B) Swimming trajectories (white) in a steady, linear serine concentration profile for one S (blue) and one L (red) isolate. (C) Bacterial distribution along a serine gradient, quantified as the relative concentration of cells as a distance from the serine source (x = 0). (D) Magnitude of chemotaxis toward serine, quantified as the skewness of the bacterial distributions in C. Dashed lines and shadings denote averages and SDs for each population. (E) Bacterial distribution in gradients of o-glucose, GlcNAc, and DMSP for one S (blue) and one L (red) isolate. Numbers denote chemotaxis magnitude. In all panels (except B), data are averages over three experiments, and error bars denote SDs.
sidewall of a microfluidic device (design 2; Figs. S3 and S4), whereupon cells from both populations (isolates 1F111 and ZF270) rapidly (<3 min) migrated toward the serine-releasing surface (Fig. 3A). After 10 min, we switched the serine release to the opposite sidewall, thus inverting the direction of the gradient. Strikingly, only the S isolate (1F111) responded to the change, by migrating toward the new nutrient-rich surface within <10 min (Fig. 3B and C and Movie S1). In contrast, the L isolate (ZF270) remained attached to the original surface (for at least 30 min; Fig. 3C), despite the considerable nutritional impoverishment of that surface. Additional experiments with a device that allowed quantification of attachment in the presence of chemical gradients confirmed the differential behavior of the two populations (Figs. S5–S8 and SI Results and Discussion).

The steady attachment to particles thus creates a tradeoff between resource accessibility at the patch and landscape levels. Because of their stable attachment to particles, L cells have higher access to resources at the patch level. However, this comes to the detriment of their migration ability in response to temporal changes in nutrient availability, which limits their access to resources at the macroscale landscape level. In contrast, S cells do not commit to surfaces by attachment and biofilm formation—reducing their growth rate on particles compared with L cells (Fig. 1F)—but are continuously ready to migrate to new patches. Thus, the L and S populations differ in their ecological strategies for resource utilization, with the L population being better at accessing localized resources at the individual patch level and the S population being better at dispersing and discovering new patches. The result is a tradeoff between competition and dispersal (1, 4, 9–11, 14) that we expect favors either population depending on the spatiotemporal particle dynamics in the environment: Whereas the L population benefits from long-lived particles where steady attachment yields a competitive advantage (Fig. 1F), the S population benefits from high rates of particle turnover where frequent migration is advantageous (38, 39).

Our finding thus provides a new interpretation of the predominance of S and L populations in different size fractions of the marine water column (16, 18): Prevalent occurrence in small size fractions does not imply habitat specialization toward bulk water conditions but rather propensity to migration among hotspots over particle attachment. Furthermore, the ecological tradeoff we identified provides a simple explanation for coexistence even if populations fully overlap in their metabolic preferences, and indeed both the gene content and a Biolog assay suggest strong metabolic similarity between the two populations (Fig. S9 and SI Results and Discussion). Finally, a different experimental system capable of producing and renewing surfaces and gradients over several generations will be required to directly test coexistence, something that was not possible in our microfluidic devices. Such a system will further reveal the role of environmental conditions (concentration and composition of particles, rate of occurrence of new particles, particle size, duration of chemical release from a particle) in the outcome of the coexistence and relative prevalence of each population.

The systematic analysis of a broad spectrum of spatial behaviors suggests a model for the ecological and evolutionary differentiation between the two populations of *V. cyclitrophicus* (Fig. 4). Isolates from the L population find particles by chemotaxis, attach, and establish a steady association with them by building biofilms. This affords them access to the highest

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**Fig. 3.** Only the S population can rapidly respond to a temporal change in nutrient conditions. (A) Behavioral response of one S (blue) and one L (red) isolate in a temporally varying nutrient landscape. Large panels show swimming trajectories; small panels show the region close to the left sidewall, from which serine was released for the first 10 min. (B) Time course of the chemotaxis magnitude (as in Fig. 2D). Positive values of the chemotaxis magnitude indicate attraction towards the left side of the channel; negative values indicate attraction towards the right side of the channel. Because surface-attached cells are not included when quantifying chemotaxis, the magnitude of chemotaxis of the L population here is lower than in Fig. 2D, due to the strong attachment of the L population to the agarose sidewalls of the test channel in the design 2 device (Fig. 5B) used here, compared with the PDMS sidewalls in the design 1 device (Fig. S2) used to obtain Fig. 2D. (C) Time course of the number of cells swimming in the bulk (>5 μm from either sidewall). The decrease for the S population around 10 and 30 min was due to cells hovering adjacent to the (serine-releasing) sidewalls, where cell tracking is hindered by optical reflections (SI Results and Discussion). The dashed line indicates when the direction of the attractant gradient was switched. Data are averages from four experiments and error bars denote SDs.
nutrient concentrations: They are the first line of consumption of dissolved nutrients generated in the particle [e.g., by enzymatic processes (40)], making them strong competitors on these nutrient patches. In contrast, isolates from the S population find nutrient hotspots, including particles, by chemotaxis, but only associate with particles loosely by hovering in close proximity to their surface, without settling. Although this puts them in “second row” in terms of access to particle-originated nutrients, it buys them the flexibility to rapidly respond to new hotspots, for example when nutrients on the particle dwindle or a stronger hotspot appears nearby. We note that, beyond this fundamental differentiation in ecological strategies, coexistence can further be driven by other side effects of spatial partitioning, such as less-direct competition for resources between the S and L populations and different mortality rates due to higher preference of predators for particles. Additionally, a population-specific allele sweep in the rpoS2 sigma factor gene previously detected (18) may complement niche adaptation by mediating differential responses to stress.

These different ecological strategies could contribute to reductive reorganization between populations, promoting sympatric genetic differentiation and lineage diversion and explaining the gene flow boundary previously reported (16, 18). The origin of this gene flow boundary is thus considerably subtler than expected, because it does not require metabolic differentiation and operates only through differential propensity for attachment to particles and dissociation from nutrient hotspots. Given the ease with which these behavioral differences could evolve, for example by horizontal gene transfer of the msh or syp gene clusters, we propose that this bifurcation of spatial behaviors drove the early stages of population differentiation in V. cyclitrophicus. This work, then, provides a blueprint for connecting behavioral adaptations with microbial diversity and highlights how subtle a difference in spatial behavior can be sufficient to curtail gene flow among populations, and how important the spatial and temporal signatures of the marine microscale nutrient landscape are for microbial diversification in the ocean.

Materials and Methods

Microfluidic Devices. Two different versions of the microfluidic gradient generator, modified from ref. 41, were used in the chemotaxis experiments with steady linear concentration profiles (design 1; Fig. S2A; results in Fig. 2) and time-varying concentration profiles (design 2; Fig. S3A; results in Fig. 3). Design 1 corresponds to design 1 in ref. 41, whereas design 2 is a variant on the same design principle. Both gradient generators are made of three layers: a polydimethylsiloxane (PDMS) layer on top (impermeable to solutes), an agarose layer in the middle (permeable to solutes), and a glass slide at the bottom (for support). The design concept consists of fabricating microchannels within the layer of PDMS (Sylgard 184; Dow Corning) and/or the layer of agarose (Ultra Pure Agarose; Invitrogen) to establish chemotactant gradients by diffusion (Figs. S2 and S3). PDMS channels were designed using CAD software (Autodesk) and printed onto transparency film with a high-resolution image setter (Fineline Imaging). For details on fabrication and operation of microfluidic devices, see SI Materials and Methods.

Cell Tracking Within Concentration Gradients. Cells were imaged in phase contrast using a 10× (N.A. 0.30) or 20× (N.A. 0.45) objective by acquiring sequences of 200 frames (movies) at 50 frames per s with a CCD camera (PCO 1600; Cooke; 1,600 × 1,200 pixels). The field of view was 0.9 × 1.2 mm with the 10× objective and 0.45 × 0.6 mm with the 20× objective. Swimming trajectories were obtained from each movie by locating the maximum pixel intensity for each pixel over the duration of the movie. We performed image analysis by subtracting each frame from the following one, to focus only on motile cells, and by subsequently locating bacteria in each frame as peaks in a monochromic intensity field, using IPLab (Scionalytics). Bacterial positions were determined over all frames in a movie and binned to yield the relative bacterial concentration profile, as a function of the distance from the side-wall of the test chamber with the highest chemotactant concentration, using custom MATLAB (The MathWorks) routines.

Chemotaxis in Steady, Linear Chemoattractant Profiles. Design 1 (Fig. S2A) among the microfluidic gradient generators was used for the chemotaxis assays under steady linear profiles of chemoattractant concentration. In this design, the irrigation channels and the test channel are all located within the PDMS layer. By continuously flowing chemoattractant in one of the two irrigation channels and filtered autoclaved seawater in the other and relying on diffusion of the chemoattractant into the underlying agarose layer, this device produces a steady, linear concentration profile within the underlying agarose layer (41). By diffusion, this linear chemoattractant profile also extends into the cell suspension within the test channel (Fig. S2B). After >10 min, a time sufficient to ensure the establishment of the linear gradient (41), we injected mid-exponential-phase cells (OD600 0.5) into the test channel, thus exposing them to the linear chemoattractant profile. Cells were tracked as described above, yielding the distribution of cells along the width of the test channel (i.e., along the chemotactant gradient). The chemotaxis magnitude was measured by computing the skewness of the cell distribution. We used L-serine, D-glucose, N-acetyl D-glucosamine (GlcNAc), and dimethylsulfoniopropionate (DMSP) as chemoattractants, each at a concentration of 50 μM. Chemotaxis assays with L-serine were performed for all 12 isolates in Table S1, whereas those with D-glucose, GlcNAc, and DMSP were performed with 1 isolate from each population (1F53 and ZF28). Three independent experiments were performed in each case.

Bacterial Behavior Under Time-Varying Nutrient Conditions. Design 2 (Fig. S3A) among the microfluidic gradient generators was used to study bacterial behavior under time-varying nutrient conditions. In this design, only the irrigation channels were located in the PDMS layer, whereas the test channel was in the agarose layer. As in design 1, we created a linear concentration profile within the agarose layer by continuously flowing chemoattractant in one of the two irrigation channels and filtered autoclaved seawater in the other. This time, however, chemoattractant diffused into the test channel through the sidewalls (not the bottom surface) of the test channel (Fig. S3B). In this fashion, the agarose sidewall serves as a model for the surface of a nutrient particle, which releases chemicals and can be colonized by bacteria (Fig. 3 and Fig. S3B). Mid-exponential-phase cells (OD600 0.4) were diluted to
growth rates of isolates on the two forms of alginate. The growth rate (Fig. 1) of one S (1F111-pGFP) and one L (ZF270-pGFP) isolate was tested in this manner. A mid-exponential-phase culture (OD_{600} 0.5) was washed with fresh medium and diluted to ~ 3.0 \times 10^{7} CFU/mL in minimal medium containing 0.001% (v/v) of alginate in soluble or particulate form as the sole carbon source. A 300-μL aliquot was introduced into a test tube and incubated at 30 °C for 13 h. Ten-microliter aliquots were sampled at the start and end of experiments to determine CFU by plate counting, and the number of divisions per hour was then calculated. The minimal medium for these experiments was prepared as previously described (42), with omission of EDTA and addition of a vitamin mix (43). Experiments were performed in triplicate.

Other Methods. Methods describing bacterial isolates, cell-culture protocols, microfabrication, surface-attachment assays, biofilm experiments, swimming-speed measurements, genetic analysis, and substrate-utilization analysis are described in SI Materials and Methods.

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Growth-Rate Comparison. Growth on alginate as the sole carbon source was quantified by providing cells with the same total amount of alginate in either particulate (SI Materials and Methods) or dissolved form and comparing the growth rates of isolates on the two forms of alginate. The growth rate (Fig. 1F) of one S (1F111-pGFP) and one L (ZF270-pGFP) isolate was tested in this manner. A mid-exponential-phase culture (OD_{600} 0.5) was washed with fresh medium and diluted to ~ 3.0 \times 10^{7} CFU/mL in minimal medium containing 0.001% (v/v) of alginate in soluble or particulate form as the sole carbon source. A 300-μL aliquot was introduced into a test tube and incubated at 30 °C for 13 h. Ten-microliter aliquots were sampled at the start and end of experiments to determine CFU by plate counting, and the number of divisions per hour was then calculated. The minimal medium for these experiments was prepared as previously described (42), with omission of EDTA and addition of a vitamin mix (43). Experiments were performed in triplicate.
Supporting Information

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SI Results and Discussion

Attachment to Chitin Beads. Our results showed that the difference in attachment between bulk-fluid and small-particle isolates (S population) and large-particle isolates (L population) is largely independent of the chemistry (hydrophobic vs. hydrophilic), physical state (crystalline, hydrogel), and biological state (presence/absence of an existing biofilm) of the solid-phase material (Fig. 1A and Fig. S1.A–C and E). One exception we encountered is chitin. Chitin is an abundant polymer in the ocean, and the chitin-attachment protein GbpA (1) is common among *Vibrio* species. A genome comparison revealed the conservation of a *gbpA* homolog in both the S and L populations, implying that specific attachment to chitin is not involved in their ecological differentiation. Indeed, our attachment assay showed comparable attachment to chitin particles by one S and one L group isolate (Fig. S1C), in agreement with the absence of known genetic differences related to chitin attachment. This result is further corroborated by a recent report of the absence of *Vibrio cyclitrophicus* on specimens of copepod (2), which possess chitin-ous exoskeletons.

Interaction Between Cells and Chemoattractant-Releasing Surfaces. Our analysis of bacterial behavior under changing nutrient conditions showed that both S and L isolates accumulated near the chemoattractant-releasing agarose sidewall (Fig. 3). The number of swimming cells decreased to nearly zero for isolate ZF270 (L population) and did not increase again after the gradient direction was reversed (Fig. 3C), which we interpreted as cells having attached to the chemoattractant-releasing agarose surface. In contrast, although the number of swimming 1F111 cells (S population) also initially decreased, it increased again after gradient reversal (Fig. 3C), allowing cells to migrate to the new source (opposite sidewall) and suggesting that 1F111 cells did not attach to the chemoattractant-releasing agarose surface.

Here we investigate in more detail the surface-interaction dynamics associated with the gradient reversal by means of a gradient generator (design 3; Fig. S5) where the primary gradient (vertical) is perpendicular to the plane of observation (horizontal). In particular, we positioned the imaging plane directly below the sidewall (and Fig. S1A–C and E). One exception we encountered is chitin. Chitin is an abundant polymer in the ocean, and the chitin-attachment protein GbpA (1) is common among *Vibrio* species. A genome comparison revealed the conservation of a *gbpA* homolog in both the S and L populations, implying that specific attachment to chitin is not involved in their ecological differentiation. Indeed, our attachment assay showed comparable attachment to chitin particles by one S and one L group isolate (Fig. S1C), in agreement with the absence of known genetic differences related to chitin attachment. This result is further corroborated by a recent report of the absence of *Vibrio cyclitrophicus* on specimens of copepod (2), which possess chitin-ous exoskeletons.

Substrate Utilization. Our results showed that unspecific surface attachment and biofilm formation were consistent predictors of the preference for particles of the L population, suggesting that ecological differentiation between the two populations is mediated by differences in attachment rates to and residence times on particles. Here we consider whether another trait—metabolic preference—can be a key driver of ecological differentiation between the two populations. To this end, we analyzed substrate utilization using a Biolog assay. Results (Fig. S9) showed that both populations actively oxidize highly bioreactive carbon compounds, such as aldose sugars and protein-constituting amino acids, and neither population oxidized many compounds of lower bioreactivity (Fig. S9), supporting the idea that both populations exploit localized sources of highly bioreactive carbon compounds, such as marine particles (3–6). The lack of major differentiation in substrate utilization between the S and L populations, among the compounds tested, agrees with the finding that neither S- nor L-population isolates harbor any unique genes related to primary metabolism (7). Although identity in the metabolic preferences of S- and L-population isolates is exceedingly difficult to prove, both gene content and metabolic analyses suggest similarity between the two populations. Although metabolic activity could be differentially tuned in situ via differential gene-expression levels, these potential metabolic differences do not counter the proposed model, which, instead of metabolic differentiation, hinges on differentiation in the spatial behaviors of bacteria in response to the microscopic spatial structure of nutrients in their local environment.

SI Materials and Methods

Bacterial Isolates and Cell Culture. The isolates and plasmids used in this study are listed in Table S1. For routine culture, bacterial isolates were grown in 1/2 strength 2216 medium (8) or on 2216 agar plates at 30 °C. An orbital shaker (600 rpm) was used for liquid cultures. For culturing isolates harboring pGFP, spectinomycin (50 μg/mL) was added to maintain the plasmid. Unless otherwise stated, all assays were performed for each of the 12 strains listed in Table S1.

Fabrication and Operation of Microfluidic Devices. Polydimethylsiloxane (PDMS) channels were designed using CAD software (Autodesk) and printed onto transparency film with a high-resolution image setter (Fineline Imaging). Microchannels were fabricated by prototyping against a silicon master with positive-relief features using standard soft lithography techniques (9, 10). The PDMS layer was patterned with two or three parallel channels that were 20 mm-long, 100 μm-deep, and either 600 μm (design 1) or 1 mm (design 2)-wide. PDMS layers with embossed channels were obtained by molding against the silicon master, baking at 65 °C for 12 h, peeling off the hardened PDMS, cutting it to size, and punching inlets and outlets. Different configurations were used for the agarose layer. For design 1, we used a plain, 500 μm-thick agarose layer (Fig. S2A). For design 2, we used a 500 μm-thick agarose layer in which we carved a 20 mm-long, 1 mm-wide, 500 μm-deep trench spanning the entire thickness of the layer (Fig. S3A). In both cases, the agarose layer was made from a 3% (wt/vol) solution of agarose in filtered autoclaved seawater, heated in a microwave oven, injected into a mold with a plastic syringe, allowed to gel at room temperature, released from the mold, and used. For the mold, we used two stacked glass slides with a silicone gasket between them as an edge seal, and the thickness of the agarose slab was set by the thickness of the gasket. The trench (design 2) in the agarose layer was obtained by placing within the mold a 0.5 mm-thick, 1 mm-wide gasket strip before injecting the agarose.

The gradient generator was placed on an inverted microscope (Nikon; TE2000E). Flexible polyethylene tubing (Cole-Parmer;
inner/outer diameter 0.5/1.5 mm) was used to connect the inlets of the source and sink channels with two or three 3-mL plastic syringes (Becton Dickinson), driven by a syringe pump (Harvard Apparatus; PHD 2000) operated in “withdrawal” mode at 10 μL/min in all cases. After injection of the cell suspension, the inlet and outlet of the test channel were sealed using small PDMS blocks to prevent evaporation and residual fluid in the channel.

**Interaction Between Cells and Chemoattractant-Releasing Surfaces: Design 3.** In addition to the two gradient generators used to generate the data presented in the main text (design 1, Fig. S2; results in Fig. 2; design 2, Fig. S3, results in Fig. 3), a third microfluidic gradient generator (design 3, Fig. S5, results in Figs. S7 and S8) was used to support results on cell attachment under temporally varying gradients. Design 3, also modified from ref. 11, is made of three layers: a PDMS layer on top (impermeable to solutes), an agarose layer in the middle (permeable to solutes), and a glass slide at the bottom (for support). The PDMS layer and agarose layer were fabricated using the same methods as in designs 1 and 2. The PDMS layer was patterned with three parallel channels that were 20 mm-long, 100 μm-deep, and 1 mm-wide. We used a 1 mm-thick agarose layer in which we carved a 35 mm-long, 1 mm-wide, 500 μm-deep groove (Fig. S5A). The groove in the agarose layer was obtained by placing within the mold a 0.5 mm-thick, 1 mm-wide gasket strip before injecting the agarose.

Operation of the device also mirrored that of designs 1 and 2. Design 3 (Fig. S5A) among the microfluidic gradient generators was used to study the interaction of bacteria with the surface-releasing chemoattractant in greater detail compared with what was possible using design 2, because design 3 creates a gradient of attractant in the vertical direction, namely perpendicular to the imaging plane (Fig. S5B). As in design 2, the test channel was located within the agarose layer, but the PDMS layer now contained three irrigation channels. Flowing chemoattractant in the central irrigation channel and filtered autoclaved seawater in the two side irrigation channels resulted in a chemoattractant gradient with a strong vertical component across the agarose layer directly above the test channel. Consequently, a vertical chemoattractant gradient formed within the test channel by diffusion of the chemoattractant into the test channel from its top surface (Fig. S5B). After >10 min from the start of the irrigation, mid–exponential-phase cells (OD_600_0.4) were diluted to OD_600_0.04 with filtered autoclaved seawater and injected into the test channel. The vertical gradient attracted cells toward the agarose ceiling of the test channel, where cell concentration and cell behavior could be imaged at high resolution in a horizontal plane directly underneath the ceiling. Furthermore, cell attachment to the ceiling surface was visualized by epifluorescence microscopy (as described above). Epifluorescence images were analyzed using ImageJ (National Institutes of Health) to enumerate attached cells. To assay the response to a temporal change in nutrient conditions, 10 min after injecting cells into the test channel, we reversed the direction of the chemoattractant gradient by swapping the irrigation of chemoattractant and filtered autoclaved seawater. Fig. S6 shows the results of a numerical model (see below) of the time course of the chemoattractant concentration field in the agarose layer and the test channel. Experiments were performed with two isolates (1F111-pGFP and ZF270-pGFP), with three independent experiments per isolate (Figs. S7 and S8).

**Numerical Model.** The time evolution of the concentration field of chemoattractant, C(x,z), in a vertical cross-section (z) of each gradient generator device (Figs. S3 and S5) was obtained by solving the diffusion equation in a 2D domain comprising the agarose layer and the test channel with COMSOL Multiphysics (v. 4.1). We modeled the continuous flow in the source and sink channels as time-varying boundary conditions applied at the interface between flowing fluid and the agarose layer. We used a diffusion coefficient of the chemoattractant, D = 8.8 × 10^{-8} m²/s, both in agarose and in water (11). Interfaces other than agarose–fluid were modeled as no-flux boundary conditions.

To capture sudden changes in the chemoattractant concentration in the source and sink channels (e.g., at t = 20 min in Figs. S4 and S6), we used a smoothed step function. To model the injection of cells into the test channel associated with the complete replacement of the fluid in the test channel with new fluid at zero chemoattractant concentration (e.g., at t = 10 min in Fig. 3), we split each simulation into two parts: one running from t = 0 to 10 min and the second running from t = 10 to 40 min. For the first part, we used an initial condition C(x,z) = 0, whereas for the second part, we used as an initial condition the C(x,z) field obtained at t = 10 min from the first part for the agarose layer and C(x,z) = 0 for the test channel. The domain was discretized in a dense mesh of at least 15,000 triangular elements, and simulations were run with a time step of 1 s.

**Swimming-Speed Measurements.** Late–exponential-phase cells (OD_600_0.8) were harvested, washed with filtered autoclaved seawater, and introduced into circular microchambers (5 mm diameter, 100 μm height) made of PDMS. The microchambers were placed on an inverted microscope (Nikon; TE2000E), and video microscopy and image analysis were used to track cells and obtain their swimming speed as described previously (12, 13). Briefly, cells were imaged in phase contrast using a 20x objective (N.A. 0.45) by acquiring sequences of 200 frames (movies) at 50 frames per s with a CCD camera (Cooke; PCO 1600). A particle-tracking software developed in-house (BacTrack) was used to determine bacterial trajectories and calculate mean swimming speeds (14). The swimming speed was determined as the mean over all motile cells and represents a 2D projection of the 3D swimming speed. Time-course measurements of speed as a function of growth time were performed for isolates 1F53 and ZF270 by sampling small aliquots from liquid cultures. Triplet measurements were performed in all cases.

**Surface Attachment.** A mid–exponential-phase cell culture (OD_600_0.5) was washed with filtered autoclaved seawater, diluted to OD_600_0.005 (1.5 × 10⁶ CFU/mL), and then a 1-mL aliquot was inoculated in a 35 mm-diameter Petri dish and incubated at 30 °C for 1 h. After removing planktonic cells by gently washing twice with filtered autoclaved seawater, images of the bottom of the Petri dish at three locations were taken by phase–contrast microscopy using a CCD camera (Cooke; PCO 1600). Surface attachment was quantified by counting the enumerating attached cells using ImageJ. Three independent cultures were assayed for each isolate.

To test for attachment to surfaces of different hydrophobicity (Fig. S1A), we used nontreated (contact angle 79.1 ± 4.8°) and plasma-treated (contact angle 46.1 ± 3.7°) 35 mm-diameter polystyrene Petri dishes. To obtain polystyrene surfaces with an even lower contact angle (28.5 ± 3.6°), additional plasma treatment was performed (Electro-Technic Products; BD-20). Surface contact angles were measured by photography and image analysis.

To test the attachment to agarose (Fig. S1B), the bottom of a Petri dish was covered with agarose [3% (wt/vol)]. To test the attachment to cellulose (Fig. S1B), cellulose chromatography paper (Whatman) was placed at the bottom of a Petri dish. A mid–exponential-phase cell culture (OD_600_0.5) was washed with fresh medium, diluted to OD_600_0.01 (3.0 × 10⁶ CFU/mL), and then a 2-mL aliquot was introduced into the Petri dish. For the attachment assay with cellulose, GFP-expressing cells were used and visualized by epifluorescence microscopy (with the same illumination described above), because the opaque nature of cellulose hindered light microscopy. Two isolates expressing GFP...
(1F111-pGFP and ZF270-pGFP) were used for attachment assays with cellulose, and the two wild-type isolates (1F53 and ZF270) were used for attachment to all other surfaces. Triplicate measurements were performed in all cases.

To test for attachment to chitin and alginate (Fig. S1C), a mid-exponential-phase cell culture (OD$_{600}$ 0.5) was washed with fresh medium, diluted to OD$_{600}$ 0.01 (3.0 × 10$^6$ CFU/mL), and then a 1-mL aliquot was introduced into a Petri dish containing chitin beads (New England Biolabs) or alginate beads (prepared as described below) and incubated at 30 °C for 1 h. Planktonic cells were removed by gently washing twice with filtered autoclaved seawater for several seconds. Wild-type cells (1F53 and ZF270) were stained with a fluorescent dye (Syto9; Life Technologies). Cells attaching on a bead were imaged with confocal microscopy using an LSM510 laser scanning microscope (Carl Zeiss) equipped with a 40× objective (N.A. 0.6; Plan-Neofluar). Cells were illuminated by a 488-nm argon laser and detected using a 505-nm long-pass filter. Attached cells were then enumerated using ImageJ. Twenty-five (alginate) or 10 (chitin) particles were counted for each isolate.

**Preparation of Alginate Beads.** Unless otherwise indicated, all reagents were obtained from Sigma. For production of alginate microparticles, we used a protocol modified from Poncelet et al. (15). Briefly, a sodium alginate solution (Sigma; A2158) at 2% (wt/vol) was prepared with Milli-Q water (Millipore) by stirring until complete dissolution. NaOH (0.1 M) was added to adjust the pH of the alginate solution to 7.6. A calcium carbonate nanoparticle suspension in Milli-Q water was added to achieve a final concentration of 50 mM calcium carbonate. The suspension was degassed and stored at 4 °C until further use. A detergent oil solution was prepared by dissolving detergents in mineral oil at concentrations of 4.5% (vol/vol) Span, 0.4% Tween, and 0.05% Triton. One hundred milliliters of the detergent oil solution was stirred at 200 rpm, and 20 mL of sodium alginate, calcium carbonate suspension was slowly added. Stirring continued at 200 rpm for 15 min to create the emulsion of alginate, calcium carbonate droplets in oil. The stirring rate was increased to 350 rpm, followed by the slow addition of 20 mL of solution of mineral oil with 80 μL of glacial acetic acid at a rate of 0.5 mL/min. Stirring continued for 1 h at 350 rpm. The microparticles that formed were extracted into 150 mL of 50 mM calcium carbonate and separated by decanting the supernatant. The microparticles were washed with 50 mM calcium carbonate until the supernatant remained clear. The microparticles were extracted several times with water-saturated ether to remove residual oil. The microparticles were resuspended in Milli-Q water and filtered through 300- and 65-μm filters to obtain an intermediate size fraction. The microparticles were washed with Milli-Q water and stored in 20% ethanol at 4 °C until further use.

**Early-Stage Biofilm Formation.** An overnight cell culture was washed with 1/2 strength 2216 medium, diluted to OD$_{600}$ 0.005 (1.5 × 10$^6$ CFU/mL), and then a 1-mL aliquot was inoculated into a Petri dish containing chitin beads (New England Biolabs) or alginate beads (prepared as described below) and incubated at 30 °C for 1 h. Planktonic cells were removed by gently washing twice with filtered autoclaved seawater for several seconds. Wild-type cells (1F53 and ZF270) were stained with a fluorescent dye (Syto9; Life Technologies). Cells attaching on a bead were imaged with confocal microscopy using an LSM510 laser scanning microscope (Carl Zeiss) equipped with a 40× objective (N.A. 0.6; Plan-Neofluar). Cells were illuminated by a 488-nm argon laser and detected using a 505-nm long-pass filter. Attached cells were then enumerated using ImageJ. Twenty-five (alginate) or 10 (chitin) particles were counted for each isolate.

**Late-Stage Biofilm Formation and Confocal Microscopy.** To quantify late-stage biofilm formation, a mid–exponential-phase culture (OD$_{600}$ 0.5) was diluted to OD$_{600}$ 0.005 (1.5 × 10$^6$ CFU/mL) with 1/20 strength 2216 medium and injected to fill a 20-mm-long, 100-μm-high, 600-μm-wide microfluidic channel made of PDMS. After 30 min of no-flow conditions, which allowed initial cell attachment, a 2 μL/min flow of 1/20 strength 2216 medium was initiated and maintained for 72 h (3 d) using a syringe pump (PHD 2000; Harvard Apparatus) to supply attached cells with nutrients. Experiments were conducted at room temperature. Biofilms of cells expressing GFP and wild-type cells were imaged with epifluorescence confocal microscopy and confocal reflection microscopy, respectively. A Carl Zeiss LSM510 laser scanning microscope equipped with a 40× objective (N.A. 0.6; Plan-Neofluor) was used to acquire confocal microscopic images. For visualization of cells expressing GFP, cells were illuminated by a 488-nm argon laser and detected with a 505-nm long-pass filter. The biofilm thickness of GFP-expressing cells was quantified by analyzing images using COMSTAT (16) under MATLAB (The MathWorks). For visualization of the polysaccharide matrix (Fig. S1D), biofilms were stained with FITC-labeled lectin made from *Triticum vulgaris* (Sigma-Aldrich). FITC fluorescence was detected using the same light path as GFP. Confocal reflection microscopy (17) was used as an independent, non-fluorescence-based method to nondestructively acquire 3D images of biofilms. Biofilms were illuminated with a 633-nm helium-neon laser, and reflected light was collected through a 505-nm long-pass filter. Z-stack images were converted to a 3D projection (Fig. 1C and Fig. S1 D and E) by using LSM510 software (Carl Zeiss). Three independent cultures were assayed for each case.

**Cell Attachment to Biofilms.** To test for cell attachment to an established biofilm (Fig. S1E), a 3-d-old biofilm of wild-type isolate ZF270 (nonfluorescent) was prepared in a microchannel as described above. Then, GFP-expressing cells (1F111-pGFP and ZF270-pGFP), grown to mid-exponential phase, washed with filtered autoclaved seawater, and diluted to OD$_{600}$ 0.04 (1.2 × 10$^6$ CFU/mL), were introduced into the microchannel by flowing a cell suspension at 100 μL/min for 3 min and incubated for 1 h at room temperature. Channels were subsequently washed with filtered autoclaved seawater for 3 min at 100 μL/min to remove planktonic cells. Finally, biofilms were visualized by confocal reflection microscopy, and GFP-expressing cells attaching to the (nonfluorescent) biofilm were imaged by epifluorescence confocal microscopy, as described above. The number of GFP-expressing cells in each optical slice was quantified with ImageJ. Triplicate experiments were performed for each isolate.

**Comparison of Genetic and Phenotypic Information.** The unrooted phylogenetic tree of the 12 isolates was generated based on the *hsp60* gene sequence (7) using the neighbor-joining method. The color map, showing the strength of phenotypes of individual isolates relative to the strongest measurement for each phenotype (100%), was generated using MATLAB. Data of swimming speed (μm/s) (Fig. 2A), chemotaxis strength (Fig. 2D), number of cells attaching on the polystyrene surface (cells per mm$^2$) (Fig. 1A), and area surface coverage (%) (Fig. 1B) were used to compare swimming-speed, chemotaxis, surface-attachment, and early-stage biofilm-formation phenotypes, respectively.

**Substrate Utilization.** Biolog GN2 plates were used to determine patterns of sole carbon source utilization for six representative isolates (results in Fig. S9), as previously described (18). Briefly, Biolog GN2 plates are microtiter plates in which each well contains an individual carbon source. Ninety-five carbon sources were patterned for sole carbon source utilization for six representative isolates (results in Fig. S9), as previously described (18). Briefly, Biolog GN2 plates are microtiter plates in which each well contains an individual carbon source. Ninety-five carbon sources were patterned for each isolate.
plates were incubated for 4 d at 22 °C. Color development was evaluated by measuring the OD$_{588}$ of each well with a Varioskan Flash Spectral Scanning Multimode Reader (Thermo Scientific). An average of three wells was assayed for each substrate.

**Electron Microscopy.** A mid–exponential-phase cell culture (OD$_{600}$ 0.4) was gently washed twice with fresh filtered autoclaved seawater and placed on carbon-coated formvar grids (200 mesh), which were then negatively stained with 2% aqueous uranyl acetate for 1 min, rinsed, and imaged using a Tecnai Spirit transmission electron microscope (FEI) with an AMT CCD camera at 80 kV. The percentage of cells with pili and the average number of pili per cell were calculated from transmission electron microscopy images of 25 cells per isolate. Contrast enhancement, for the sole purpose of illustrating pili (Fig. 1E), was done with Photoshop CS5 (Adobe).

**Fig. S1.** Attachment of S and L isolates to various materials and biofilm formation. (A) Attachment to polystyrene surfaces with different contact angles, for one S (blue) and one L (red) isolate. Data are averages of three independent experiments, and error bars are SDs. (B) Attachment to agarose and cellulose surfaces after 1 h by one S (blue) and one L (red) isolate. Data are averages of three independent experiments, and error bars are SDs. (C) Attachment to alginate and chitin particles after 1 h for one S (blue) and one L (red) isolate. The y axis denotes attached cells per particle enumerated with confocal microscopy. Particles had a diameter of 65–300 μm. Data are averages of 25 (alginate) or 10 (chitin) particles, and error bars are SDs. (D) Extracellular polysaccharide matrix, stained with FITC-labeled lectin (yellow), in a 3-d-old L isolate (ZF270; purple) biofilm. (E) Attachment of GFP-tagged S and L cells (yellow) onto a previously established L-population biofilm (purple). Numbers in the top left of each panel denote attached cells per unit surface area (×10,000; mean ± SD; n = 3).
Fig. S2. Schematic of the design 1 gradient generator used to assay chemotaxis in steady, linear chemoattractant profiles. (A) Schematic of the cross-section of the assembled device (Right) and top view of each layer (Left). The top layer is made of PDMS, which is impermeable to solutes. The middle layer is made of agarose, which allows for the diffusion of chemoattractant used to establish the linear concentration profile. The bottom layer is a glass slide, used for support. Solid red arrows denote dimensions. (B) Schematic of the gradient-generation mechanism. The continuous flow of chemoattractant (yellow) and filtered autoclaved seawater (blue) within the two irrigation channels in the PDMS layer mediates the formation of a horizontal gradient (yellow-blue shading) in the underlying agarose layer and therefore in the test channel. Dashed red arrows in the cross-sectional view (Right) denote the different steps in the diffusion of the chemoattractant: Chemoattractant enters the agarose layer from the source irrigation channel (arrow 1), diffuses through the agarose layer (arrow 2), and is removed by diffusion into the sink irrigation channel (arrow 3), establishing a steady, linear, horizontal concentration profile across the agarose layer and therefore, by diffusion (arrow 4), also in the test channel. This design allows quantification of the bacterial swimming response to the steady chemoattractant gradient within the test channel (e.g., Fig. 2 B–E).
Fig. S3. Schematic of the design 2 gradient generator used to determine bacterial behavior under time-varying nutrient conditions. (A) Schematic of the cross-section of the assembled device (Right) and top view of each layer (Left). The top layer is made of PDMS, which is impermeable to solutes. The middle layer is made of agarose, which allows for the diffusion of chemoattractant used to establish the linear concentration profile. The test channel in this design is a “trench” spanning the entire thickness (500 μm) of the agarose layer. The bottom layer is a glass slide, used for support. Solid red arrows denote dimensions. (B) Schematic of the gradient-generation mechanism. The continuous flow of chemoattractant (yellow) and filtered autoclaved seawater (blue) within the two irrigation channels in the PDMS layer mediates the formation of a horizontal gradient (yellow-blue shading) in the underlying agarose layer and therefore in the test channel. Dashed red arrows in the cross-sectional view (Right) denote the different steps in the diffusion of the chemoattractant. Chemoattractant enters the agarose layer from the source irrigation channel (arrow 1), diffuses through the agarose layer and thus into the trench test channel (arrow 2), and is removed by diffusion into the sink irrigation channel (arrow 3). This design allows quantification of both the bacterial swimming response to the chemoattractant gradient and their attachment behavior at the agarose sidewall (e.g., Fig. 3).
Fig. S4. Temporal evolution of the chemoattractant gradient in the design 2 microdevice (Fig. S3), modeled numerically. Colors represent the chemoattractant concentration (color map) within the agarose layer. Black boxes identify the test channel within the agarose layer in all panels. Different rows correspond to different times, as indicated by the labels on the left. For the first 20 min, the left irrigation channel carried 50 μM chemoattractant and the right irrigation channel carried seawater (0 μM). At 10 min past the initiation of gradient generation, cells were introduced into the test channel. After 20 min, the conditions were swapped: The left irrigation channel carried seawater (0 μM) and the right irrigation channel carried 50 μM chemoattractant. This resulted in rapid reversal of the chemoattractant gradient. (Lower) Temporal evolution of the chemoattractant concentration profile along the horizontal midline of the test channel (shown as a white dotted line in one of the panels).
**Fig. S5.** Schematic of the design 3 gradient generator used to quantify the interaction between cells and chemoattractant-releasing surfaces. (A) Schematic of the cross-section of the assembled device (Right) and top view of each layer (Left). The top layer is made of PDMS, which is impermeable to solutes. The middle layer is made of agarose, which allows for the diffusion of chemoattractant used to establish the linear concentration profile. The test channel in this design is a “groove” with a depth (500 μm) equal to half the thickness of the agarose layer (1 mm). The bottom layer is a glass slide, used for support. Solid red arrows denote dimensions. (B) Schematic of the gradient-generation mechanism. The continuous flow of chemoattractant (yellow) in the central irrigation channel and of filtered autoclaved seawater (blue) in the two side irrigation channels within the PDMS layer mediates the formation of a gradient (yellow-blue shading) with a strong vertical component within the agarose layer and thus inside the test channel. Dashed red arrows in the cross-sectional view (Right) denote the different steps in the diffusion of the chemoattractant: Chemoattractant enters the agarose layer from the central irrigation channel (arrow 1), from where it enters the test channel through the test channel’s ceiling (arrow 2), finally leaving primarily from the test channel’s sidewalls and diffusing into the side irrigation channels (arrows 3). This design allows a detailed quantification of bacteria interacting with the horizontal ceiling surface of the test channel as a result of the vertical chemoattractant gradient (e.g., Figs. S7 and S8).
Fig. S6. Temporal evolution of the chemoattractant gradient in the design 3 microdevice (Fig. S5), modeled numerically. Colors represent the chemoattractant concentration (color map) within the agarose layer. Black boxes identify the test channel within the agarose layer in all panels. Different rows correspond to different times, as indicated by the labels on the left. For the first 20 min, the central irrigation channel carried 50 μM chemoattractant and the side irrigation channels carried seawater (0 μM). At 10 min past the initiation of gradient generation, cells were introduced into the test channel. After 20 min, the conditions were swapped: The central irrigation channel carried seawater (0 μM) and the side irrigation channel carried 50 μM chemoattractant. This resulted in rapid reversal of the chemoattractant gradient. (Lower) The temporal evolution of the chemoattractant concentration profile along the vertical midline of the test channel (shown as a white dotted line in one of the panels).
Fig. S7. Cell swimming trajectories directly underneath the agarose ceiling of the design 3 gradient generator device and attachment to that surface. White dots show attached cells imaged by epifluorescence microscopy with a 1-s exposure time. The blue and red overlays show swimming trajectories for the S- and L-population isolates, respectively. The gradient direction was initially upward across the test channel and toward the surface being imaged (Fig. S6), and was reversed 10 min after the inoculation of cells into the test channel (as shown by the schematics; Left). Images shown are representative results from three independent experiments.

Fig. S8. Time course of cell behavior at the agarose ceiling surface of the design 3 gradient generator device for the experiments shown in Fig. S7. (A) Number of cells swimming directly adjacent to the surface (hovering) per unit area. (B) Number of cells attached to the surface per unit area. Symbols are averages from three independent experiments, and error bars denote SDs.
Substrate utilization by six isolates of *V. cyclitrophicus* measured with a Biolog assay. Three isolates were from the S population (blue) and three were from the L population (red). Colors indicate the relative magnitude of the substrate utilization measured in terms of the OD_{590} color bar, with white indicating no utilization (OD_{590} < 0.5).

**Fig. S9.**

**Table S1.** Isolates of *V. cyclitrophicus* used in this study.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Population</th>
<th>Size fraction, μm</th>
<th>Notes</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1F111</td>
<td>S</td>
<td>1–5</td>
<td>All isolates are from the coast of New England, USA (1, 2)</td>
<td></td>
</tr>
<tr>
<td>1F273</td>
<td>S</td>
<td>1–5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1F175</td>
<td>S</td>
<td>1–5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1F53</td>
<td>S</td>
<td>1–5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1F97</td>
<td>S</td>
<td>1–5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZF14</td>
<td>L</td>
<td>&gt;63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZF205</td>
<td>L</td>
<td>&gt;63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZF270</td>
<td>L</td>
<td>&gt;63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZF28</td>
<td>L</td>
<td>&gt;63</td>
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<td>&gt;63</td>
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<td>ZF65</td>
<td>L</td>
<td>&gt;63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1F289</td>
<td>L</td>
<td>1–5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1F111-pGFP</td>
<td>S</td>
<td>1–5</td>
<td>Isolate 1F111 harboring pGFP</td>
<td>This study</td>
</tr>
<tr>
<td>ZF270-pGFP</td>
<td>L</td>
<td>&gt;63</td>
<td>Isolate ZF270 harboring pGFP</td>
<td>This study</td>
</tr>
</tbody>
</table>

The pGFP plasmid is a green fluorescent protein-expressing vector and is carrying a spectinomycin resistance gene as a marker (Clontech Laboratories).

Movie S1. Behavioral response of one S-population isolate (1F111) and one L-population isolate (ZF270) in a temporally varying nutrient landscape. The upper two panels show cell swimming trajectories of 1F111-pGFP (S) or ZF270-pGFP (L) isolates, as indicated. Shown is the vertical (xy in Fig. S3) cross-section of the test channel (width 1 mm). Serine release into the test channel occurred from the left sidewall for the first 10 min and was then switched to the right sidewall. The lower two panels show the temporal dynamics of the chemoattractant concentration field in this experiment (design 2; Fig. S3), modeled numerically. Each panel shows the concentration field within the vertical (xz) cross-section of the agarose layer and the test channel, as indicated. Colors represent the chemoattractant distribution (see color map in Fig. S4).