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Rapid evolution of adaptive niche construction in experimental microbial populations

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Many species engage in adaptive niche construction: modification of the local environment that increases the modifying organism's competitive fitness. Adaptive niche construction provides an alternative pathway to higher fitness, shaping the environment rather than conforming to it. Yet, experimental evidence for the evolutionary emergence of adaptive niche construction is lacking, leaving its role in evolution uncertain. Here we report a direct observation of the de novo evolution of adaptive niche construction in populations of the bacteria *Pseudomonas fluorescens*. In a laboratory experiment, we allowed several bacterial populations to adapt to a novel environment and assessed whether niche construction evolved over time. We found that adaptive niche construction emerged rapidly, within approximately 100 generations, and became ubiquitous after approximately 400 generations. The large fitness effect of this niche construction was dominated by the low fitness of evolved strains in the ancestrally modified environment: evolved niche constructors were highly dependent on their specific environmental modifications. Populations were subjected to frequent resetting of environmental conditions and severe reduction of spatial habitat structure, both of which are thought to make adaptive niche construction difficult to evolve. Our finding that adaptive niche construction nevertheless evolved repeatably suggests that it may play a more important role in evolution than generally thought.

KEY WORDS: **Adaptation, mutations, population biology, selection—experimental.**

New lives inherit from their ancestors more than just genes: they also inherit environmental modifications made by their ancestors. Environmental modification that affects the fitness of the modifying organism is known as "niche construction." Niche construction surely participates in evolution, but the importance of its role and the time scales on which it acts are less certain (Odling-Smee et al. 1996, 2003).

The evolution of niche construction is complicated by the feedbacks that are introduced when evolving organisms influence the selective pressures driving their evolution (Lewontin 2001). Shifts in the fitness landscape caused by the evolution of niche construction can drive evolutionary dynamics that qualitatively differ from evolution in a fixed landscape (Laland et al. 1996, 1999). The most well-known form of niche construction is *negative*: organisms deplete resources, thereby limiting their own growth more than that of competitors who use different resources. The evolution of such niche construction, particularly its role in niche partitioning resulting in coexistence, has been extensively studied (MacArthur and Levins 1964; Schoener 1974; Geritz et al. 1997; Chow et al. 2004).

The evolutionary importance of *adaptive* niche construction—environmental modification that improves the modifier's competitiveness—is more controversial (Odling-Smee et al. 2003; Laland and Sterelny, 2006; Kylafis and Loreau, 2011; Scott-Phillips et al. 2014). Adaptive niche construction has two requirements: causing an environmental change and responding to that change better than competitors. For example, a population might make the environment more conducive to its own growth by producing a "public good," such as a resource-unlocking siderophore, and the transporter to use it (Driscoll 2010; Zhang

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and Rainey 2013). Alternatively, a population might retard growth in a way it can overcome, for example bacteria excreting an antibiotic while also expressing a resistance gene (Chao and Levin 1981; Riley and Wertz 2002; Nahum et al. 2011). The many examples of adaptive niche construction in nature have led some authors to argue that it has been improperly neglected by "standard evolutionary theory" that assumes that the selection pressures imposed by the environment are unaffected by the evolutionary response to that selection (Odling-Smee et al. 2003; Laland and Sterelny 2006).

However, there is substantial disagreement as to whether the evolutionary role of adaptive niche construction is of sufficient importance to justify the technical and conceptual complications it introduces to the study of evolution (Dawkins 2004; Laland and Sterelny 2006; Scott-Phillips et al. 2014). In many situations, niche construction that is personalized—not shared between individuals—can be considered analogous to a standard organismic phenotype in its evolutionary and ecological effects, in which case standard evolutionary theory is sufficient provided we interpret the evolving phenotype more broadly (the "extended phenotype") (Dawkins 1999; Bailey 2012). At the other extreme, mutants whose niche construction would collectively have an adaptive effect may be unable to invade from initial rarity when their modifications would seemingly be diluted to insignificance and shared with the incumbent population. More generally, if evolutionary changes are small, then evolved niche construction might be a doubly small product of small changes in environmental modification and small changes in the fitness response to such modifications.

We know that adaptive niche construction can evolve because it is found throughout the tree of life (Odling-Smee et al. 2003). The conditions under which adaptive niche construction can evolve have been explored theoretically (Laland et al. 1996, 1999; Odling-Smee et al. 2003), with spatial structure implicated as an enabling factor (Chao and Levin 1981; Silver and Di Paolo 2006; Lehmann 2008; Mitri et al. 2011), and some specific forms studied in more detail (e.g., Mousseau and Fox 1998; Driscoll 2010; Van Der Putten et al. 2013). The frequency of adaptive niche constructing traits have been shown to respond to selection in experimental systems (Chao and Levin 1981; Saltz and Foley 2011; Zhang and Rainey 2013). But there has been little experimental study of the evolutionary emergence of adaptive niche construction from de novo variation, and thus major questions remain. Does the evolution of new adaptive niche construction require rare large changes and the long times on which major evolution occurs? Or can it evolve quickly? Does the evolutionary emergence of adaptive niche construction depend on specific details of organisms and environments? Or do the complexities of environments and organisms cause it to commonly evolve—albeit in unpredictable ways? Because niche construction (or "ecosystem engineering") has been shown to affect the generation and maintenance of biodiversity, these questions have significant implications for our broader understanding of life on earth (Jones et al. 1996; Erwin 2008).

Microbial populations are ideal for studying the evolution of niche construction, because large populations can be followed in controlled laboratory conditions for many generations. We evolved multiple lineages of the bacterium *Pseudomonas fluorescens* for hundreds of generations in a novel environment—serial growth and dilution cycles in a shaken complex media. The absence of spatial structure and the impermanence of environmental modifications make this a conservative model system for exploring the evolution of niche construction. Nevertheless, we found that adaptive niche construction evolved rapidly and reproducibly from de novo mutations. We characterized several phenotypic changes associated with these evolved niche constructors, and used the frozen fossil record from our experiments to investigate their evolutionary history.

Materials and Methods

EVOLUTIONARY PROTOCOL

Evolution experiments were initialized with the SBW25 strain of *P. fluorescens* (the ancestor, **A**; Rainey and Bailey 1996; Rainey and Travisano 1998). Experimental populations were cultured in 30 mL universal glass vials containing 6 mL of King's B (microcosms), and incubated at 28ºC, inclined at 45ºC, in an orbital shaking incubator at 150 rpm (shaken conditions), thereby eliminating spatial structure and preventing cellular aggregation. Vial caps were loose during culture to allow oxygen flow. Serial transfers were performed every 48 h at 1:100 dilution (60 μL, $N_{bottleneck} \approx 3 \times 10^8$), thus populations double 6.66 times each transfer. In these conditions cultures exceed half their carrying capacity within 24 h, so evolving populations spent substantial evolutionary time out of exponential growth in a heavily modified environment (Fig. S1, S2). Eight (s)hort evolution experiments **S1**–**S8** were evolved for 30 days (15 transfers, 100 generations) at Stanford University with 1 mL samples from each transfer stored in glycerol solution at –80ºC. Eight (l)ong evolution experiments **L1**–**L8** were evolved for 120 days (60 transfers, 400 generations) at the Institute of Natural and Mathematical Sciences in New Zealand, with samples frozen for 40, 80, and 120 days. **L1**–**L8** correspond to the US1–US8 evolution experiments of Zhang and Rainey (2013).

CHOICE OF CLONAL STRAINS

The colony morphology of *P. fluorescens* is evolutionary labile, and has previously been associated with ecological function (Rainey and Travisano 1998). The end-point populations of

our evolution experiments all exhibited visually distinguishable colony morphotypes, which we used as a crude proxy for the diversity in those populations (Tables S1, S2). The two most common morphotypes in each population always accounted for >90% of the colonies, and there was little to no visible intramorphotype variation within populations. Therefore we chose representative colonies for the most-frequent (**maj**, "majority") and second-most-frequent (**min**, "minority") distinct morphotypes as the basis for our niche construction study. Clonal isolates were picked, re-plated to verify morphotype heritability and clonality, and then stored as frozen stocks. By focusing on clonal isolates instead of population samples we robustly separate niche construction—environmental modification that affects the organism's *own* fitness—from environmentally mediated interactions *between* types such as cross-feeding (Pfeiffer and Bonhoeffer 2004).

ASSAY FOR EVOLVED NICHE CONSTRUCTION

The evolutionary change in the niche construction of an evolved strain X (its "evolved niche construction") was measured by constructing two environments, one modified by the evolved strain (E_X) and the other modified by the ancestor (E_A), competing the evolved strain X against the ancestor in both environments, and comparing the outcomes of those competitions (Fig. 1).

1. Construct: Two overnight microcosms were inoculated from frozen stocks of the strain of interest X and the ancestor A, cultured in shaken conditions for $20 \pm 1/2$ h, transferred at 1:1500 dilution (4 μL) into fresh microcosms, and cultured again for $20 \pm 1/2$ h at which point the cultures had reached approximately half their carrying capacity. Bacteria were then removed by filtration at 0.2 μm, and the remaining bacteria-free media transferred into empty, sterile microcosms, forming E_X and E_A. Media in the constructed environments was not amended in any way. Due to loss during filtration, constructed environments contained 4 mL, rather than 6 mL, of media.

2. Compete: Overnight cultures at half carrying capacity of X and the fluorescently labeled ancestor A_g were combined in a 50:50 mixture. This mixture was homogenized, then used to inoculate both E_X and E_A at 1:1000 dilution. The paired competitions, X versus A_g in both E_X and E_A, were cultured for 24 h in shaken conditions.

3. Compare: The competitive fitnesses $F_X(E_X)$ of X in E_X, and $F_X(E_A)$ in E_A, were measured from the change in ratio of X to A_g over the course of the paired competitions (see below). Evolved niche construction was then quantified as the *difference* between the competitive fitness of X in E_X and in E_A: $\Delta F_X(\Delta E_X) \equiv F_X(E_X) - F_X(E_A)$. A positive value of $\Delta F_X(\Delta E_X)$ indicates an adaptive *change* in niche construction from that of the ancestor. For this to occur, X must modify the environment differently than the ancestor ($E_X \neq E_A$), *and* its fitness must respond differently to the changed environmental modification: $F_X(E_X) \neq F_X(E_A)$.

The final density attained in constructed environments was a lower, but still substantial, fraction of the carrying capacity in fresh medium (one-fifth is a typical value) corresponding to approximately eight to nine population doublings over the course of competitions in the constructed environments. At each stage of this assay all cultures were performed side by side, minimizing cryptic differences in conditions. When replicating this assay (as in Fig. 2) we performed total replicates, that is independent experiments performed in different weeks.

The key choice in our assay was the density to which bacteria were cultured when making the constructed environments. The magnitude of environmental modification increases with population growth, but near carrying capacity there is less future growth and hence fewer generations for selection to act. We measured niche construction at the point where cultures reach half their carrying capacity (13–15 h in the 48-h cycle of our evolutionary protocol) when the impact of niche construction on the full-cycle frequency dynamics is likely to be largest. However, this means that our assay only probed part of the competition over the 48-h cycle (the *full-cycle fitness*) that drove the evolution, and was insensitive to components of fitness that contribute when population sizes are small and environmental modification relatively minor.

MEASURING COMPETITIVE FITNESS

The competitive fitness $F_X(E)$ of strain X in environment E was obtained from the change in ratio of X to the standard competitor A_g—a GFP-tagged variant of the ancestral SBW25 strain (Fig. S3, Supplementary Methods)—during competition from low density in shaken conditions. Ratios of nonfluorescent to fluorescent cells at the start (R_i) and finish (R_f) of competitions were measured in a BD FACSCalibur flow cytometer, with 50,000 events recorded. Fitness was quantified as $F_X(E) = log_2(R_f/R_i)$, the number of population doublings of X relative to A_g: for example, $F_X(E) = +2$ means that X doubled twice more than A_g. Competitions performed in constructed environments were inoculated at a 1:1000 dilution; competitions performed in unmodified King's B were inoculated at the 1:100 dilution of our evolutionary protocol. All competitions were inoculated with roughly equal mixtures of the competing strains unless otherwise noted.

Results

EVOLUTION LED TO ADAPTIVE NICHE CONSTRUCTION

We assayed the evolved niche construction of representative strains isolated from the end-point populations of 30-day evolutions **S1–S8** and 120-day evolutions **L1–L8** (Fig. 2). Significant adaptive niche construction evolved in almost all of these. The

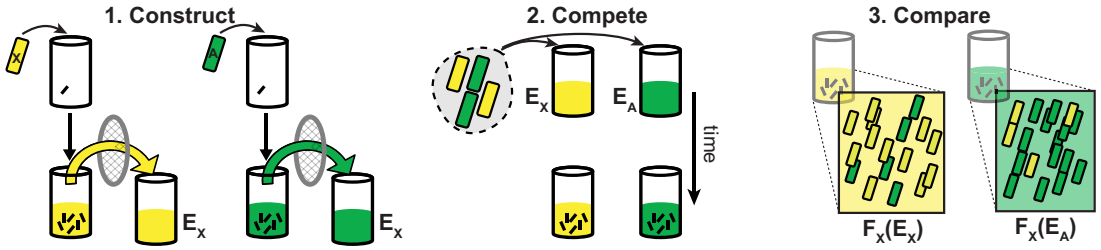

Figure 1. Schematic of assay for the evolved niche construction of strain X. First the modified environments E_X and E_A are constructed by culturing X and A (the ancestor), respectively, to half their carrying capacity, and then removing the bacteria by filtration. X and A are then competed in both environments, and the competitive fitness F_X of X measured in each. The evolved niche construction of X is defined as the *difference* in its fitness between E_X and E_A: $\Delta F_X(\Delta E_X) \equiv F_X(E_X) - F_X(E_A)$.

evolution of niche construction that lowered the modifier's competitive fitness was never observed. The 30-day evolved populations were polymorphic: representatives of the minority morphotypes (**S#min**) were adaptive niche constructors ($\Delta F_X(\Delta E_X) > 0$, $P < 10^{-6}$ pooled sign test), but representatives of the majority types (**S#maj**) were not. This polymorphism was resolved by 120 days: **L** strains in general were adaptive niche constructors ($P < 10^{-6}$ pooled sign test).

The magnitude of the evolved niche construction of the 30-day minority types (**S#min**) averaged $\Delta F_X(\Delta E_X) \approx +4$. That is, relative to the ancestor **S#min** strains did better by four doublings more in the environment they constructed than in the environment the ancestor constructed. The 120-day strains evolved even more niche construction than the **S#min** ($P = 4.6 \times 10^{-6}$, Wilcoxon rank-sum test), with average $\Delta F_X(\Delta E_X) \approx +6$. With typically eight to nine doublings during competition in the constructed environments, niche construction was responsible for $\approx$45% (**S#min**) and $\approx$70% (**L#maj/L#min**) of their competitive fitness in E_X.

LOW FITNESS IN THE ANCESTRAL ENVIRONMENT

There are multiple ways—involving positive or negative effects of either the ancestral or the evolved strains on either themselves or the other—that evolved strains could realize higher competitive fitnesses in self-modified environments (i.e., evolve adaptive niche construction). To discriminate between these, we plot the fitness of the evolved strains in their own environment $F_X(E_X)$ and in the ancestral environment $F_X(E_A)$ in Figure 3 A.

The evolved niche constructors are characterized by low fitness in the ancestral environment ($F_X(E_A) \ll 0$). The evolved niche constructors do typically outcompete the ancestor in the self-constructed environment ($P = 2.7 \times 10^{-12}$, pooled sign test), but only by a narrow margin: $F_X(E_X) \approx +1/2$ doublings on average. In comparison, the evolved niche constructors lose miserably in the ancestral environment ($P = 2.7 \times 10^{-20}$, pooled sign test), typically managing $F_X(E_A) \approx -3.5$ (**S#min**) and $F_X(E_A) \approx -5.5$ (**L#**) fewer doublings than the ancestor.

The low fitness in the ancestral environment is accompanied by changes in cell phenotype. Figure 3 B–C shows an example for **S2min**: At the start of competition in E_A, **S2min** and $\mathbf{A_g}$ had essentially identical optical profiles, but at the end of competition the **S2min** population had shifted dramatically toward higher forward- and side-scattering, which correspond roughly to cell size and shape. This change did *not* occur in E_{S2min}. Microscopy corroborated the large cellular changes in E_A: A culture of **S2min**

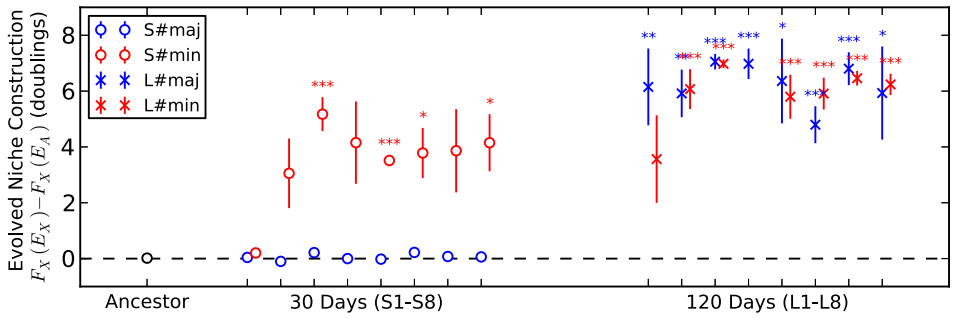

Figure 2. The evolved niche construction, $F_X(E_X) - F_X(E_A)$, of 30-day (S1–S8) and 120-day (L1–L8) evolved strains. At 30 days most minority strains (red) have evolved adaptive niche construction, that is $F_X(E_X) > F_X(E_A)$. At 120 days adaptive niche construction is ubiquitous. Points are the mean of three to five measurements and bars the standard error of the mean. Error bars for most values near zero fall within the points. The per-strain significance of the difference of the mean from zero is indicated. $^{*}P < 0.05$, $^{**}P < 0.025$, $^{***}P < 0.01$ (Student's t-test).

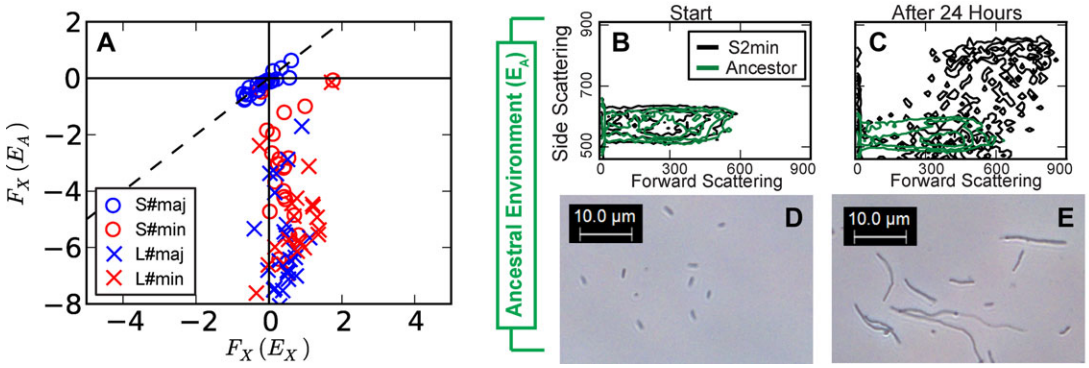

Figure 3. Evolved niche constructors are unfit in the ancestor-constructed environment E_A. (A) The fitness of evolved strains X in the self-constructed environment, $F_X(E_X)$, versus their fitness in the ancestor-constructed environment, $F_X(E_A)$. The dashed line indicates the null expectation of no niche construction, $F_X(E_X) = F_X(E_A)$. Niche constructing strains (S2min–S8min and all L strains) are universally unfit in E_A, an effect that is much stronger than their fitness advantage in E_X. Flow cytometry profiles obtained at the (B) start and (C) finish of a 24-h competition between the niche constructing strain S2min and the GFP-tagged ancestor in E_A. Forty times phase-contrast microscopy of cells sampled at the (D) start and (E) finish of a 24-h monoculture growth of S2min in E_A. After 24 h in E_A a substantial proportion of S2min cells develop an elongated filamentous morphology, apparent both by their optical profile (C) and by microscopy (E). This effect is absent when S2min cells are grown in E_{S2min}.

cells in E_A began with size and shape typical of *P. fluorescens*, but after 24 h (Fig. 3 D, E) a substantial fraction of the **S2min** population became long filamentous cells typically five to 10 times and as much as 50× the length of normal cells. Similar phenotypic responses to E_A occurred in all niche constructing strains, but not in non-niche-constructing evolved strains (Fig. S4).

Filamentation is often associated with "sick" cells, but in certain conditions it can be an adaptive response to stressful conditions (Justice et al. 2008). That is, filamentous cells can have a different reproductive potential than normal cells. One simple possibility is that the reproductive potential of a cell scales with biomass. If that is the case here, then we might be incorrectly inferring low fitness from the low cell counts of niche-constructing strains after competition in E_A, because those counts fail to include the higher growth potential of the systematically larger filamentous cells the niche constructors develop in that environment. We directly tested this possibility by transferring samples from the end of competitions in E_A and E_X into next-cycle cultures: fresh microcosms inoculated at 1:100 dilution and cultured for 48 h as per the evolutionary protocol (Fig. S5). Because at the end of the next cycle there was no longer a systematic difference in cell morphologies, cell counts should capture all fitness effects. This experiment revealed that long filamentous cells did have a higher reproductive potential than normal cells, but that this higher potential did not come close to making up for the far lower numbers of such cells. Measured by the change in log-ratio, niche constructors recovered on average of 22% of their fitness deficit in E_A due to the higher reproductive potential of the long cells they develop in that environment (i.e., roughly one doubling out of the four to six doubling deficit was recovered). This was less than would have occurred if the filamentous cells had reproduced in direct proportion to their biomass.

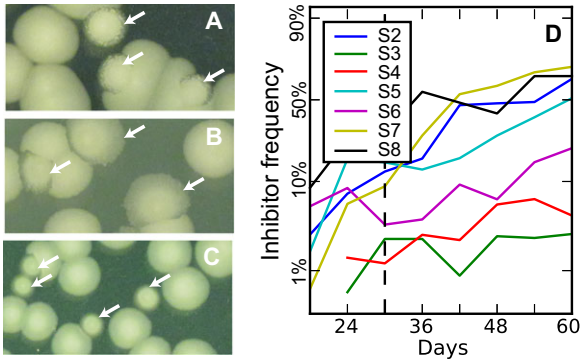

Figure 4. The characteristic colony morphotypes and evolutionary trajectories of the early niche constructors S2min–S8min. The colonies of (A) S2min, (B) S3min, and (C) S6min (indicated by arrows) exhibit the low-density outer rings and asymmetric contact boundaries when abutting a non-niche-constructing S#maj colony that characterize the "inhibitor" morphotype shared by all the early niche constructors. (D) The frequency of the inhibitor morphotype increased in every case when evolutions S2–S8 were extended to 60 days, showing the evolutionary success of the early niche constructors.

CHARACTERISTICS OF EARLY NICHE CONSTRUCTORS

On plates all 30-day adaptive niche constructors **S2min–S8min** (**S1min** not a niche-constructor) exhibited an "inhibitor" colony morphotype (Fig. 4 A–C). These colonies share three key features: they asymmetrically bulge into the wild-type "smooth morph"

(SM) colonies that are still in the majority (**S#maj**), they develop a ring of lower density on their boundaries, and they resist overgrowth by neighboring colonies. These features are not present in **S1maj–S8maj** or **S1min**. Time on the plate and the presence of noninhibitor colonies noticeably increases the intensity of the inhibitor morphotype. Inhibitor features are similar for colonies from the *same* evolved population, but vary considerably *between* evolutionary replicates.

We quantified two other measures of the fitness of the 30-day strains from growth curves (Supplementary Methods, Figs. S1, S2). No consistent difference was found between the growth rates of early niche constructors **S2min–S8min** and the majority types (Fig. S6), but the carrying capacities of early niche constructors were generally significantly lower than those of the co-occuring majority type (Fig. S7).

EVOLUTIONARY COURSE OF NICHE CONSTRUCTION

Niche construction is a two-part phenotype, a niche-constructing organism must both modify the environment (ΔE) and its fitness must respond to that modification ($\Delta F(\Delta E)$). The evolution of adaptive niche construction then presents a chicken-and-egg problem: which came first the environmental modification or the adaptation to it? And given that they appear to incur a significant fitness penalty in the ancestral environment, how did the niche constructors invade?

To investigate these questions, we measured the fitness of early niche constructors **S2min–S5min** in environments modified by their whole community, and environments modified by several different isolates of their co-occuring and non-niche-constructing majority type (Fig. 5). **S2min–S5min** were less fit in the environments modified by their community than in self-modified environments, although fitter in the former than the ancestor-modified environment. There was considerable fitness variability in environments modified by individual co-occurring strains, but little significant systematic difference between the fitness in these environments and in the ancestor-modified environment.

The effect of niche construction on invasion (and asymmetric competitions generally) depends on how the fitness response varies with the level of environmental modification. We thus studied environments constructed by varying mixtures of the competitors, for example, $E = 1/3\ E_X + 2/3\ E_A$. We found that the fitness of niche constructors increased super-linearly with the portion of the environment they modified (Fig. S8); they received most of the fitness benefit of their niche construction when modifying just one-third of the environment. This suggests that environmental modification by a small fraction of the preexisting community could decrease the barrier to invasion.

To probe long-term behavior, we isolated strains from the majority morphotype at the 40-, 80-, and 120-day time points of evolutions **L1–L4**, and measured their competitive fitnesses in

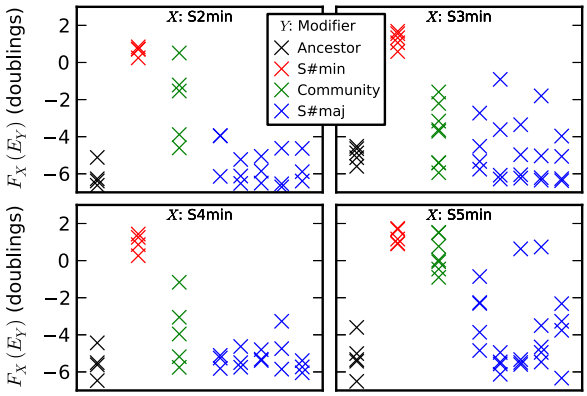

Figure 5. The fitness of early niche constructors in various modified environments. The competitive fitnesses of S2min, S3min, S4min, and S5min were measured in environments modified by several different strains: the ancestor (black), themselves (red), their entire community (green), and several isolates from their co-occuring and non-niche-constructing majority morphotype (blue). Early niche constructors were most fit in their own environment, least fit in the ancestral environment, and intermediate in the environment modified by their community. In environments modified by majority isolates fitness varied, but with little systematic increase over the ancestral environment.

the evolutionarily relevant environments modified by the *communities* present at each of the 0-, 40-, 80-, and 120-day time points of the same evolution experiments (Fig. S9). All evolved strains tested had their fitnesses rescued equally by the environmental modifications made by the 40-, 80-, or 120-day communities. This indicates that the crucial modification, ΔE_X, arose by 40 days and changed little thereafter. But the fitness response, $\Delta F_X(\Delta E_X)$, likely continued to evolve, as strains from different time points had different fitnesses in the same environment. The fitness in evolutionary conditions of the early-niche-constructer inhibitor morphotypes was directly observed: inhibitor frequency increased systematically in **S** evolutions extended to 60 days (Fig. 4 D).

NICHE CONSTRUCTION AND OTHER COMPONENTS OF FITNESS

Over the full 48-h cycle of growth and saturation of our evolution experiments, evolved niche constructors had a 5–10% per-generation fitness advantage over the ancestor (Fig. S10). If the differential modifications in the environment caused by the evolved strains were comparable, and the differences in the ancestor's and evolved strains' response to such modifications were also in the 10% range, one might guess that the magnitude of the niche construction would be roughly the product of these: $\sim 1\%$. So the magnitude of the niche construction we observe in our assay is surprising. However, the relatively modest net fitness difference between the ancestral and evolved strains

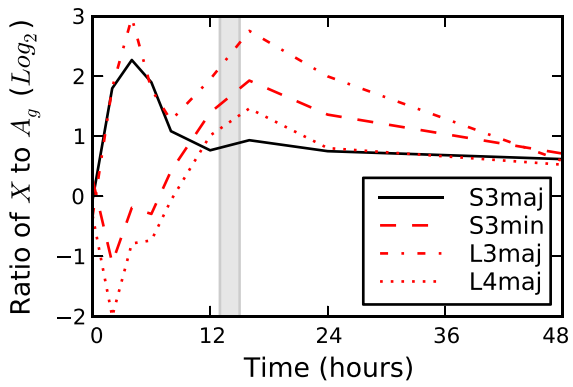

Figure 6. The competitive dynamics between four evolved strains and the ancestor during the evolutionary batch-culture cycle. Forty-eight-hour competitions between the GFP-tagged ancestor A_g and the niche-constructing strains S3min, L3maj, L4maj (red), and non-niche-constructing S3maj (black), were sampled at the 2-/4-/6-/8-/12-/16-/24-/48-h time points. Competitions were inoculated at 1:100 dilution as per our evolutionary protocol, and at ≈1:1 ratio. Frequency changes during the 48 h of batch culture are much larger than the net change over the full cycle, suggesting strong trade-offs between different components of fitness. Gray shading indicates the approximate time at which cultures reached half carrying capacity, corresponding to the level of environmental modification in the niche-construction assay. These dynamics were retained when varying the starting ratios from 1:1 to 1:8 (Fig. S11).

masks much larger effects. The competitive dynamics between evolved strains and the ancestor during the 48-h cycle are complex (Figs. 6, S11) with ratios of their frequencies changing greatly: for some reaching more than four and for others as low as a quarter during the cycle (i.e., gaining or losing two doublings). Thus the net changes in frequency ratio over the full cycle of 30–70% represent the sums of large negative and positive contributions, suggesting strong trade-offs between multiple components of fitness (including lag time, maximum growth rate, late-stage growth, etc.). Niche construction is expected to primarily contribute when densities are high enough to drive significant environmental modification. To test this, we carried out a niche construction assay in which constructed environments were modified by growth up to just one-tenth of carrying capacity instead of half in our standard assay: no significant niche construction effects were observed (Fig. S12).

Discussion

In the simple conditions of our evolution experiments, conventional adaptation was accompanied by significant evolutionary change in niche construction. While the ancestor already niche constructs in various ways, most of the strains evolved additional adaptive niche construction. This was rapid and repeatable, with such evolved niche constructors eventually taking over the population. Ubiquitous features of the evolved niche constructors, especially very low fitness in an environment modified by the ancestor (E_A), were found in strains many of whose other properties—dynamics during the lag, rapid growth, and saturation phases of the cycle, aspects of their colony morphology, and their population frequency from 24 to 60 days—differed considerably.

HOW DID ADAPTIVE NICHE CONSTRUCTORS EVOLVE?

At first glance, the low fitness of the evolved niche constructors in the ancestor-modified environment would appear to prevent such mutants from invading from initial rarity. And that fitness deficit is only partially ameliorated by the modifications made by the rest of their community, which could have evolved first. But a fitness deficit in E_A of $\sim 50\%$ would correspond to just half a doubling per-cycle if it only acts subsequent to the half-carrying-capacity point from which we measured niche construction: this is equivalent to a 6% per-generation cost over the full cycle. While still a substantial fitness effect by most evolutionary standards, the very strong performance early in the cycle of the niche constructing strains that we measured in detail (Fig. 6) suggests that such benefits could have enabled niche constructors to overcome this penalty and invade without the fitness benefits of their environmental modifications.

To understand the evolutionary possibilities, one must consider both qualitative and quantitative aspects of the evolved niche constructors. There are several salient observations. First, colony morphology suggests that early niche constructors were highly uniform within populations, but morphology and growth curves reveal substantial variation among replicated evolutions. Second, niche constructors had a 5–10% per-generation advantage over the ancestor averaged over the cycle. Third, niche constructors constituted 2–30% of the population at 100 generations, and all (or nearly all) of the population at 400 generations. Finally, evolution occurred in large microbial populations: the effective population size was $\sim 2 \cdot 10^9$, large enough that most point mutations occur every generation and many beneficial mutations arise, compete, and some acquire further beneficial mutations before any fix (Gerrish and Lenski 1998; Desai and Fisher 2007).

One simple scenario is that all 30-day strains were the product of single-driver mutations. If the rate at which such mutations arise is $\sim 10^{-6}$ then 12% advantage mutants would *collectively* take over the population by 100 generations, but there would be much diversity from competing mutants. This is a likely scenario for the majority smooth-morphs in the 30-day populations, but our observations suggest a different scenario for the niche constructors. If early niche constructors had a slightly smaller selective advantage than the smooth-morph mutants, or a substantially smaller target size for mutations to create them, they could still *collectively* rise

to the 2–30% frequencies observed at 30 days. But this scenario conflicts with the diversity *between* but not *within* populations of the early niche constructors: 100 generations is not enough time for diversity to be purged within them unless one phenotype strongly dominated—and then the between-population diversity would not exist.

Alternative scenarios can explain low within-population diversity of early niche constructors, but these require significantly higher selective advantages to reach the observed 100-generation frequencies. One possibility is 17% niche-constructing mutations with a very small target size: for example, a small number of sites in one gene with some phenotypic variability. Another is that niche constructors arose via a second mutation from a rapidly growing population of smooth-morph mutants, in which case the combined mutations require a total benefit in the 25% range. The "first past the post" nature of both these processes—the first mutant that establishes dominates numerically—yields a dominant strain within each population, but between populations the dominant strain can differ.

The main problem with all of the above scenarios, which ignore niche construction, is that even after 120 days, the measured fitness advantage of the niche constructors in full-cycle competition with the ancestor is only 5–10%: much less than required for them to have reached the observed frequencies at 30 days and to be outcompeting the already-majority smooth-morphs.

Even simple evolutionary scenarios that explicitly include niche construction are not easy to square quantitatively with all of our results. A natural hypothesis is that the 1–3% per-generation increase in frequency of the early niche constructors from 24 to 60 days is due to the advantage over the non-niche-constructing majority that they gain from their niche construction (inclusive of all trade-offs). But if niche constructors' fitness is only affected by environmental modifications that they themselves make, then they must have been sufficiently fitter than the ancestor at low frequencies to reach appreciable numbers well before 100 generations (30 days), at which point niche construction began contributing. This still runs afoul of the relatively small advantage of the niche constructors over the ancestor when the two are competed from equal frequencies.

A quantitatively consistent evolutionary scenario seems impossible unless fitness differences are nontransitive: that is, the competitive results of X versus A and Y versus A do not fully determine the outcome of X versus Y (Kerr et al. 2002). Such nontransitive fitnesses have been observed in some previous evolution experiments (Paquin and Adams 1983; Rainey and Travisano 1998). In the mixed, unstructured conditions of our experiment it is only via environmental modifications that competitors can interact nontransitively. Thus some of the evolution of the niche constructors *must* be driven by changes in the environment and the fitness response to those changes. More experiments are needed to fully disentangle the evolutionary possibilities. Yet the importance of feedback from environmental modifications caused by earlier evolution on the competitive fitness of evolved strains is clear from our data. And the feedback effects were very different than simple resource partitioning among diverged strains.

WHAT IS THE MECHANISM OF THE EVOLVED ADAPTIVE NICHE CONSTRUCTION?

At this point we do not know the mechanistic basis of the adaptive niche construction that evolved in our experiments. The presence or absence of niche construction in evolved strains was not correlated with any of the simple potentially causative environmental modifications, such as pH and pyoverdin production, that we observed. And while clear similarities across evolutionary replicates suggest a common mechanism, the differences seen on closer inspection (e.g., Fig. 6) allow for the possibility of multiple mechanisms as well. Nevertheless, our results do offer some clues.

There is certainly a loss-of-function aspect to the evolved niche construction: these strains lost much of their ability to grow and divide in the ancestrally modified environment. This may not reflect a loss of function at the molecular level (e.g., a nonsense mutation in an expressed gene), but given the prevalence of such adaptations in other experimental evolution studies, that would not be surprising (Behe 2010). In any case, there is a major dysfunction in how niche constructing strains are growing and dividing in *some*—but not all—depleted, late-stage media (e.g., Fig. 5). And changes in the medium from earlier stages of growth have little effect: evolved niche construction was only significant when measured in environments conditioned by previous growth to high-enough densities.

One broad mechanism that would comport with all the results of our experiments is that evolved niche constructors either re-regulated or re-engineered their cell cycle in the depleted media. Poor performance in the ancestor-modified environment could then be a deleterious side-effect of adaptive improvements in the late stages of growth if those improvements depended on environmental signals or metabolic products not produced by the ancestor. Re-regulation linking the cell cycle to specific aspects of the depleted environment is a particularly attractive explanation for the appearance of filamentous cells in E_A as products of misregulated division: the development of aberrant filamentous cells has often been associated with breakdowns in control of cell growth and division (Justice et al. 2008).

A mechanism associated with changes in the regulation of late-stage growth suggests a plausible evolutionary pathway. First, there was a metabolic adaptation that caused a cumulative change of the chemical composition of the environment as a side effect. Then a second adaptation coupled the regulation of late-stage growth to those chemical changes. For now this is only speculation, but one of the great advantages of experimental

evolution is that it enables retrospective testing of such hypotheses, for example, by resequencing and experimental manipulation. A cautionary note is, however, in order. As *P. fluorescens* is a metabolically and responsively complex organism—as reflected in its wide range of colony morphologies—it surely has many sensory inputs to its cell-cycle regulation, and potentially many variants of the cycle and switch to stationary phase. Thus going from a set of genetic changes to a mechanistic explanation of the evolutionary history and phenotypic phenomena we have observed may be challenging. But such complications are a crucial part of the richness that enables adaptive evolution.

ECOLOGICAL IMPLICATIONS OF EVOLVED ADAPTIVE NICHE CONSTRUCTION

Even if adaptive niche construction accounts for only a small part of evolved fitness—as may be the case in our system—it can have outsize impacts on diversity by influencing the way evolved strains interact in local habitats. If one strain arrives before the other, or diverged strains mix unequally, the strain that is present earlier or in larger numbers may modify the environment to their competitive benefit. Thus adaptive niche construction can cause priority effects, in which early-arriving strains inhibit the establishment of late-arriving ones (Drake 1991; De Meester et al. 2002; Fukami et al. 2007). Migration between previously separated populations is a particularly clean example: if the competing strains are adaptive niche constructors, and invading migrants are not accompanied by their environmental modifications, then established populations will be systematically advantaged, thereby preventing invaders from reaching a viable frequency. In this way the evolution of adaptive niche construction might amplify the process of adaptive radiation by preserving the distinctness of nascent populations and by lessening the separation between niches required to prevent the collapse of niche-specialists into a single ecotype (Habets et al. 2006). More generally, the evolution of priority effects would tend to lower the diversity of strains within local populations (alpha-diversity) by promoting competitive exclusion rather than coexistence, while increasing the diversity between local populations (beta-diversity) as long as local habitats vary in the arrival history of different strains.

CONCLUSION

Our results were obtained in a model system in the laboratory, and therefore are not directly applicable to natural populations. But our system was conservative in ways that would seem to make the evolution of adaptive niche construction less likely: environmental conditions were reset every two days and the shaken microcosm eliminated spatial structure. Moreover, the complex media we used and the growth–saturation–dilution cycle of batch culture could enable evolution of many forms of negative niche construction that could have dominated. Thus the fact that, nevertheless, adaptive niche construction evolved so repeatably and was so ubiquitous after just a few hundred generations suggests that it might also evolve in many other conditions, and on time scales that are short by evolutionary standards, with potentially substantial implications for the generation and maintenance of biodiversity across spatial scales.

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DATA ARCHIVING

The flow cytometry data used in this manuscript are deposited at Dryad (doi:10.5061/dryad.nf757).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1: The colony morphotype censuses from the terminal populations of the 8 replicate 30-day evolutions S1–S8.
Table S2: The colony morphotype censuses from the terminal populations of the 8 replicate 120-day evolutions L1–L8.
Figure S1: 48-hour growth curves of 30-day evolved strains.
Figure S2: The first 12 hours of the logged growth curves of 30-day evolved strains.
Figure S3: Competitions between the GFP-tagged ancestor *Ag* and the untagged ancestor *A*.
Figure S4: Flow cytometry profiles at the finish of 24 hour competitions between 30-day evolved strains and the ancestor in constructed environments.
Figure S5: The fitness effect of the long, filamentous cells that niche constructors develop in the ancestor-modified environment.
Figure S6: Maximum growth rates of 30-day evolved strains as estimated from optical density measurements taken from 48 hour growths.
Figure S7: Carrying capacities of 30-day evolved strains as estimated from optical density measurements taken from 48 hour growths.
Figure S8: The fitness of evolved niche-constructing strains in environments partially modified by their actions, and partially modified by the ancestor.
Figure S9: The fitness of strains isolated from different time points, indicated by color, of 120-day evolutions L1–L4 were tested in the environments constructed by the evolved communities from different time-points, indicated along the x-axis.
Figure S10: Calculation of the selective advantage in evolutionary conditions for selected evolved niche-constructing strains.
Figure S11: The competitive dynamics between evolved strains and the ancestor over the evolutionary batch culture cycle, starting at different initial ratios.
Figure S12: The evolved niche construction of 120-day evolved strains as a function of the density to which strains were grown when making the constructed environments E_X and E_A.