

Population size shapes trade-off dilution and adaptation to a marginal niche unconstrained by sympatric habitual conditions

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Abstract

How does niche expansion occur when the habitual (high-productivity) and marginal (low-productivity) niches are simultaneously available? Without spatial structuring, such conditions should impose fitness maintenance in the former while adapting to the latter. Hence, adaptation to a given marginal niche should be influenced by the identity of the simultaneously available habitual niche. This hypothesis remains untested. Similarly, it is unknown if larger populations, which can access greater variation and undergo more efficient selection, are generally better at niche expansion. We tested these hypotheses using a large-scale evolution experiment with *Escherichia coli*. While we observed widespread niche expansion, larger populations consistently adapted to a greater extent to both marginal and habitual niches. Owing to diverse selection pressures in different habitual niches (constant vs. fluctuating environments; environmental fluctuations varying in both predictability and speed), fitness in habitual niches was significantly shaped by their identities. Surprisingly, despite this diversity in habitual selection pressures, adaptation to the marginal niche was unconstrained by the habitual niche's identity. We show that in terms of fitness, two negatively correlated habitual niches can still have positive correlations with the marginal niche. This allows the marginal niche to dilute fitness trade-offs across habitual niches, thereby allowing costless niche expansion. Our results provide fundamental insights into the sympatric niche expansion.

Keywords: niche expansion, marginal environment, trade-off dilution, fluctuating environments, fluctuation predictability, fluctuation speed

Introduction

Marginal niches, where most individuals of the species under consideration have initially poor survival and reproduction, are critical sources of ecological opportunities for evolutionary changes (Kawecki, 2008). Adaptation to such niches can lead to significant changes in the species' ecological capabilities, ultimately reshaping the set of conditions that support its growth and reproduction, also known as the niche width (Holt & Gaines, 1992; Hutchinson, 1961). Adaptation to marginal niches has been conventionally studied in macroscopic organisms near the physical boundaries of species ranges (Hoffmann & Blows, 1994), making dispersal dynamics a vital determinant of this phenomenon (Kawecki, 2000; Lenormand, 2002). However, marginal niche adaptation remains understudied in sympatric ecological scenarios where spatial constraints are absent and the habitual niche is simultaneously available. Addressing this gap, here we investigate how various population genetic and ecological factors interact to shape bacterial adaptation to a new marginal niche in the presence of various habitual niches.

Imagine that a marginal niche becomes available to an asexual bacterial population in a habitat that simultaneously offers access to the habitual niche. For example, this can occur if a bacteriophage simultaneously encounters two

bacterial hosts in its environment: one, habitual and the other, novel (Bono et al., 2013, 2015; Duffy et al., 2006). Moreover, the mammalian gut can be a representative of such an environment (Scanlan, 2019). The nutrient niche theory states that ecological niches in the gut are defined and delimited by the available nutrients (Freter et al., 1983; Pereira & Berry, 2017). A change in the host's diet can make a novel low-productivity carbon source (the marginal niche) available to the bacteria in addition to the habitual niche comprising high-productivity carbon source(s) (Payne et al., 2012; Phillips, 2009). Moreover, this notion of nutrient niches has also been used in evolution experiments with bacteria (Jasmin & Kassen, 2007). Owing to the marginal niche's low-productivity, bacterial growth will be largely dependent on the high-productivity habitual niche *until* there is sufficient adaptation to the marginal carbon source. The first step toward such an adaptation will be the appearance of a mutation that increases the usability of the marginal carbon source. Such a mutation will then need to survive genetic drift before it comes under the purview of selection (Sniegowski & Gerrish, 2010). The per generation rate at which mutations with a beneficial effect size s survive drift is $NU_b s$, where N is the population size and U_b is the rate of beneficial mutations (Desai & Fisher, 2007; Desai et al., 2007). Moreover, apart from having better access

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to rare large-effect beneficial mutations, larger populations also show greater efficiency of natural selection (Chavhan et al., 2019; Desai & Fisher, 2007; Neher, 2013). This leads to our first hypothesis that the larger populations should show greater adaptation to the marginal niche.

Under sympatric conditions, the selection coefficient s in the expression $NU_b s$ is influenced by the simultaneous presence of the habitual (high-productivity) and the marginal (low-productivity) niches. Given the differences in productivities across the habitual and marginal niches, mutations that increase fitness in the marginal niche would have a greater chance of surviving drift if they were not too deleterious in the habitual niche. In other words, mutations that are beneficial in the marginal niche are more likely to succeed if they do not show antagonistic pleiotropic effects in the habitual niche. Thus, maintaining fitness in the sympatric habitual niche should be a strong constraint while adapting to the marginal niche. An extension of Fisher's geometric model (FGM) that includes multiple fitness optima (Martin & Lenormand, 2015) can act as a useful theoretical framework for understanding marginal niche adaptation in the presence of a sympatric habitual niche. In this model, the distance of the ancestral genotype from the fitness optimum in a given condition scales inversely with the ancestral fitness in that condition. Moreover, adaptation to a given condition brings the evolved genotype closer to the fitness optimum in that condition. By definition, the distance between the ancestral genotype and the marginal niche optimum would be greater than that between the ancestral genotype and any given habitual niche optimum (Figure 1). At one extreme, if the ancestral genotype falls between the two optima, marginal niche adaptation is expected to lead to a concomitant habitual niche maladaptation (Figure 1A). In this case, fitness maintenance in the habitual niche should strongly constrain marginal niche adaptation. Alternatively, if the ancestral genotype is not present between the marginal and habitual niche optima, adaptation to the marginal niche can be achieved without concomitant loss of fitness in the habitual niche (Figure 1C). At the other extreme, the habitual niche optimum can be situated between the ancestral genotype and the marginal niche optimum; in this case, adaptation to the marginal niche is expected to show a high positive correlation with habitual niche adaptation (Figure 1D). Thus, how the ancestral genotype will adapt to a marginal niche under consideration will be determined by the location of the habitual niche optimum, and the latter would depend on the identity of the habitual niche. This leads to our second hypothesis that adaptation to the marginal niche should be influenced by the habitual niche's identity.

A comprehensive test of hypothesis 2 should include a large variety of habitual niches that can present diverse selection pressures. This is particularly important because the demand to maintain fitness can be considerably different in cases where the habitual niche applies a single constant selection pressure vs. cases where it imposes multiple dynamically fluctuating selection pressures. Whereas fluctuations in selection pressures make fitness trade-offs instrumental in shaping evolution, the constant application of a single selection pressure makes evolution oblivious to such trade-offs (Bono et al., 2017; Kassen, 2002). Thus, the constraint to maintain fitness in the habitual niche should be very different if the habitual niche presented constant vs. fluctuating selection pressure(s). On the one hand, an extension of FGM to understand marginal niche adaptation will require two fitness optima (one marginal and the other habitual) if the habitual niche presents

a single unchanging selection pressure. On the other hand, several more fitness optima (and the trade-offs between them) will need to be incorporated into the extended FGM if the habitual niche fluctuates over multiple selection pressures.

Previous experiments have shown that the speed of selection pressure fluctuation can significantly influence both fitness correlations across different component selection pressures and the underlying genetic architecture (Boyer et al., 2021; Salignon et al., 2018). Moreover, both theoretical (Tagkopoulos et al., 2008) and empirical studies (Boyer et al., 2021; Dhar et al., 2013; Hughes et al., 2007; Karve et al., 2018; Mitchell et al., 2009) suggest that the predictability of environmental fluctuations can be important determinants of evolutionary outcomes. Adding a further layer of nuance, adaptation to the marginal niche can also be influenced by the interactions of the above ecological factors with each other and with the population size. Indeed, a recent study shows that the relative significance of fitness trade-offs under constant vs. fluctuating selection pressures depends on the population size (Chavhan et al., 2021). To the best of our knowledge, the effects of the various ecological factors described earlier (in combination with the population size) and their interactions on adaptation to marginal niches remain uninvestigated experimentally.

We conducted experimental evolution with *Escherichia coli* at two population sizes in several constant and fluctuating environments to determine the population genetic and ecological determinants of the adaptation to a marginal niche under sympatric availability of habitual niches. We also investigated if and how population size interacted with the habitual niche's identity to shape fitness in high-productivity habitual niches. We further determined if fitness in fluctuating habitual niches is shaped by the interactions of population size and the speed and predictability of environmental fluctuations. To our knowledge, such three-way interactions have not been put to experimental tests yet. We found that larger populations gained higher fitness in marginal niche regardless of the habitual niche's identity. Surprisingly, despite the differences in the constant and fluctuating selection pressures applied by the various habitual niches across our treatments, adaptation to the marginal niche was not influenced by the identity and stability of the sympatric habitual niche(s). Moreover, larger populations consistently gained greater fitness in both constant and fluctuating habitual niches.

In contrast to marginal niche adaptation, fitness in constant habitual niches was significantly influenced by the habitual niches' identities. Moreover, neither the speed nor the predictability of environmental fluctuations significantly influenced adaptation to the fluctuating habitual niches. We found that two negatively correlated habitual niches can still exhibit positive fitness correlations with the marginal niche. Incorporating this within an extension of FGM accounted for our observations across both the habitual and marginal niches in both constant and fluctuating environments at multiple population sizes. Our results elucidate the substantial evolutionary potential for rapid and costless expansion of bacterial niche width in the face of a new sympatric ecological opportunity, even at low mutational supply rates.

Materials and methods

Experimental evolution

We founded *E. coli* MG1655 populations from a single common ancestral clone and cultured them in eight different

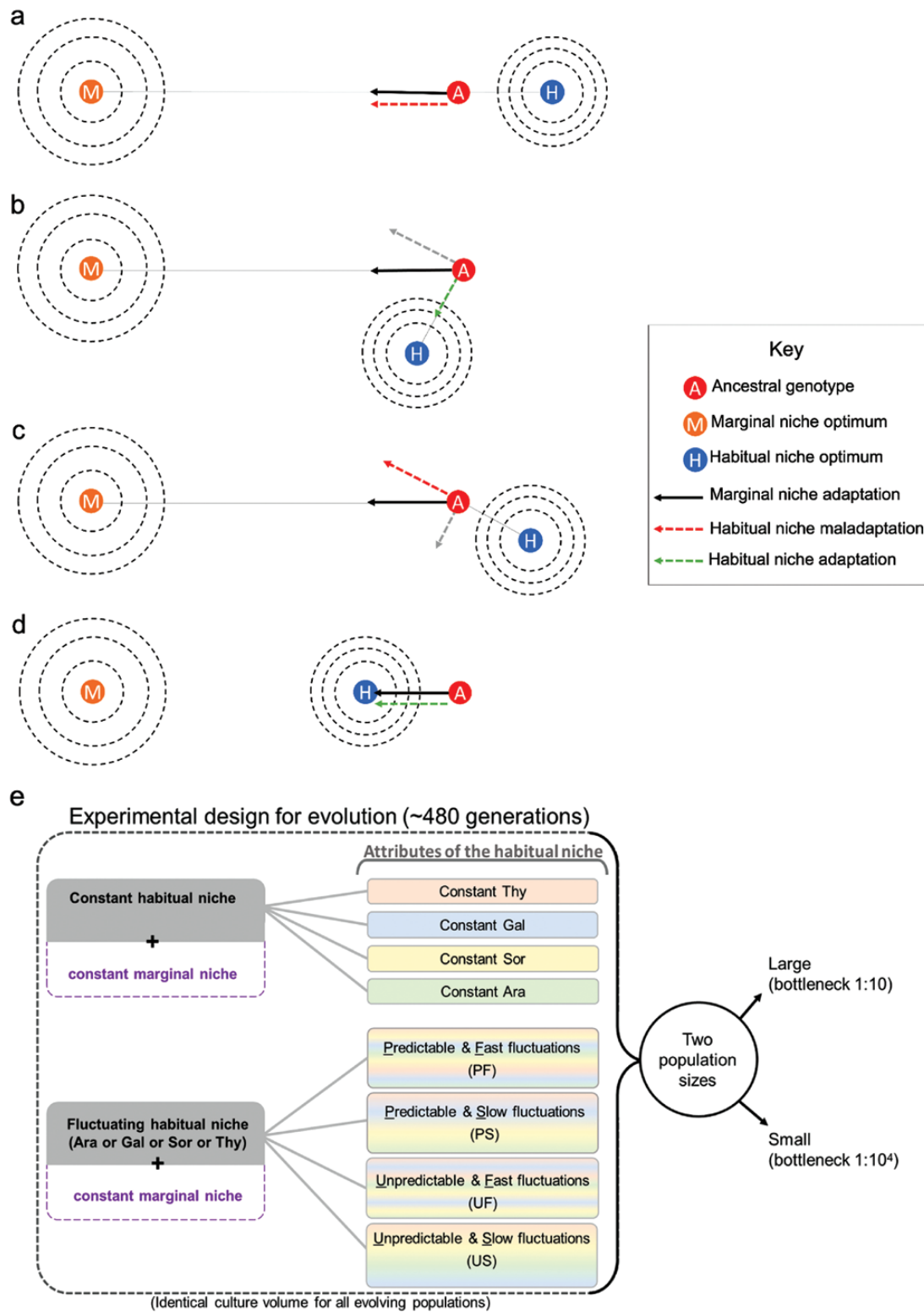


Figure 1. The rationale behind the design of our evolution experiment. (A) When the ancestral genotype is situated between the marginal and habitual fitness peaks, adaptation to the former will likely lead to a concomitant maladaptation in the latter. (B) If both the marginal niche peak and the habitual niche peak are on the same side of the ancestral genotype but the three are not colinear, adaptation to the marginal niche should lead to a relatively weaker adaptation to the habitual niche (*the small green arrow*). The *gray arrow* depicts an adaptation to the marginal niche that is neutral in the habitual niche. (C) If both the marginal niche peak and the habitual niche peak are on different sides of the ancestral genotype but the three are not colinear, adaptation to the marginal niche should lead to maladaptation to the habitual niche, albeit such maladaptation should be weaker than in case of case “a” (*red arrow*). (D) When the habitual niche is situated between the marginal fitness peak and the ancestral genotype, adaptation to the marginal niche should lead to a concomitant adaptation to the habitual niche. (E) A schematic representation of our experimental design: There were 16 distinct environmental regimens (populations derived from a common ancestor evolved in eight different environmental conditions at two different population sizes). In all the eight environmental conditions, the marginal niche (Acetate) was always present in addition to the habitual niche, which had distinct attributes in different environments. See the text for details.

environmental conditions at two distinct population sizes (Figure 1; see Supplementary Appendix S1 for detailed information about the ancestral strain and culture media). All eight environments offered Acetate as the low-productivity carbon source that constituted the marginal niche. Out of the eight environments, four offered a single distinct high-productivity carbon source (constant habitual niche) for bacterial growth (one of thymidine [Thy], galactose [Gal], sorbitol [Sor], or arabinose [Ara]). In the other four environments, the habitual niche (high-productivity carbon source) fluctuated over time in all combinations of predictable vs. unpredictable and fast (switching every ~13.3 generations) vs. slow (switching every ~40 generations) fluctuations. We designated these fluctuations in the habitual niche as predictable and fast (PF), predictable and slow (PS), unpredictable and fast (UF), and unpredictable and slow (US). Taken together, a combination of two different population sizes and eight different environments gave rise to 16 separate evolutionary regimens (Thy-L, Thy-S, Gal-L, Gal-S, Sor-L, Sor-S, Ara-L, Ara-S, PFL, PFS, PSL, PSS, UFL, UFS, USL, and USS; here L and S refer to large and small population sizes, respectively; Figure 1). We chose Acetate as the marginal carbon source because it could only support severely restricted growth as compared to the substantial growth supported by Ara, Gal, Sor, or Thy (the habitual carbon sources; see Supplementary Figure S1). We included Gal and Thy as the habitual carbon sources because *E. coli* showed strong reciprocal fitness trade-offs across Gal and Thy in an earlier study (Chavhan et al., 2020), which suggests that maintaining fitness in Gal vs. Thy as the habitual carbon sources should impose contrasting selection pressures.

We note that the categorization of the five carbon sources used here into marginal and habitual niches is based on the differences in the absolute ancestral fitness in them (Supplementary Figures S1 and S3). We also add that fast evolving microbes like *E. coli* can show significant and rapid fitness gains in the habitual carbon sources. For example, *E. coli* showed sustained fitness gains over >50,000 generations on its most preferred carbon source (glucose, a sugar whose uptake involves the phosphotransferase system [PTS]) in Richard Lenski's long-term evolution experiment (Wiser et al., 2013). Our experiment only concerns non-PTS carbon sources (four habitual and one marginal). These carbon sources are less preferred by *E. coli* as compared to glucose and can thus also allow rapid fitness gains/losses. At the beginning of each growth phase, the environment of each fitness regimen contained equal quantities (in terms of weight per unit volume) of both the habitual and the marginal carbon source.

We propagated six independently evolving biological replicates of each regimen, making a total of 96 independently evolving experimental populations. All the populations were allowed to evolve for ~480 generations in 96-well plates at a culture volume of 300 μ l incubated at 37 °C while being shaken continuously at 150 rpm. We followed the standard methodology for growing microbial populations of different sizes at identical culture volumes (Desai et al., 2007; Raynes et al., 2014; Vogwill et al., 2016). The large (L) populations underwent a 1:10 periodic bottleneck for every 12 hr. The ten-fold growth between successive bottlenecks corresponded to ~3.3 generations. In contrast, the small (S) populations were bottlenecked 1:10⁴ for every 48 hr. The 10,000-fold growth between successive bottlenecks in these populations corresponded to ~13.3 generations. This bottlenecking protocol

ensured that for a given environmental regimen, both the large and the small populations spent similar durations in the stationary phase. We also ensured that the starting concentration and composition of the liquid media were identical for the small and the large populations.

Fitness measurement

At the end of the experiment, we measured the fitness of each of the 96 independently evolving populations in five different environments (where the sole carbon source was one of Acetate, Thy, Gal, Sor, or Ara). To this end, we first revived samples derived from the endpoint cryo-stocks by allowing them to grow a hundredfold in a glucose-based M9 minimal medium. We then grew all the 96 revived populations in each of the five environments and measured their optical density at 600 nm for every 20 min using an automated well-plate reader (Synergy HT, BIOTEK Winooski, VT, USA). The physical conditions during the growth measurement were identical to those during the evolution experiment. As a single 96-well plate was insufficient for all the fitness assays, we used a randomized complete block design (RCBD) and assayed one replicate of each of the 16 regimens in each environment on a given day (Milliken & Johnson, 2009). We used the maximum slope of the growth curves, calculated over a moving window of 10 readings, as the measure of fitness (Chavhan et al., 2021; Karve et al., 2015; Leiby & Marx, 2014).

Statistical analysis

We determined if our experimental populations had adapted or maladapted significantly to their habitual and marginal niches. To this end, we used single-sample *t*-test against the ancestral fitness (scaled to 1 in each of the five assay environments). This statistical approach has been widely used in bacterial experimental evolution where a single ancestral clone founds all the evolving populations (Bennett & Lenski, 1996; Buckling et al., 2007; Dillon et al., 2016; Kassen, 2014). Since the ancestor is a single clone, its fitness measures are highly reproducible and show negligible variation, making it a suitable benchmark for single sample *t*-tests. Indeed, the ancestral fitness showed almost no variation in replicated measurements in our experiments (Supplementary Figure S2). We corrected for family-wise error rates using the Holm–Šidák procedure, categorizing fitness >1 (corrected $p < .05$) as adaptations and cases with fitness <1 (corrected $p < .05$) as maladaptations. For the eight regimens with fluctuating habitual niches, we used the geometric means of the fitness values in Thy, Gal, Sor, and Ara (the habitual niche components) and compared them to the ancestral value (=1) using single sample *t*-tests followed by Holm–Šidák correction.

To investigate the effects of population size and the habitual niche's identity on fitness in the marginal niche, we used a mixed model ANOVA (RCBD) with “population size” (two levels: L and S) and “habitual niche” (eight levels: Ara, Gal, Sor, Thy, PF, PS, UF, and US) as fixed factors crossed with each other and “day of assay” as the random factor. We also used partial η^2 as the measure of effect size (Cohen, 1988), where small, medium, and large effects were identified with partial $\eta^2 < 0.06$, $0.06 < \text{partial } \eta^2 < 0.14$, and $0.14 < \text{partial } \eta^2$, respectively.

We used a similar mixed model ANOVA (RCBD) to analyze how population size and the identity of the constant habitual niche shaped fitness in the latter. Specifically, we treated “population size” (two levels: L and S) and “constant habitual niche

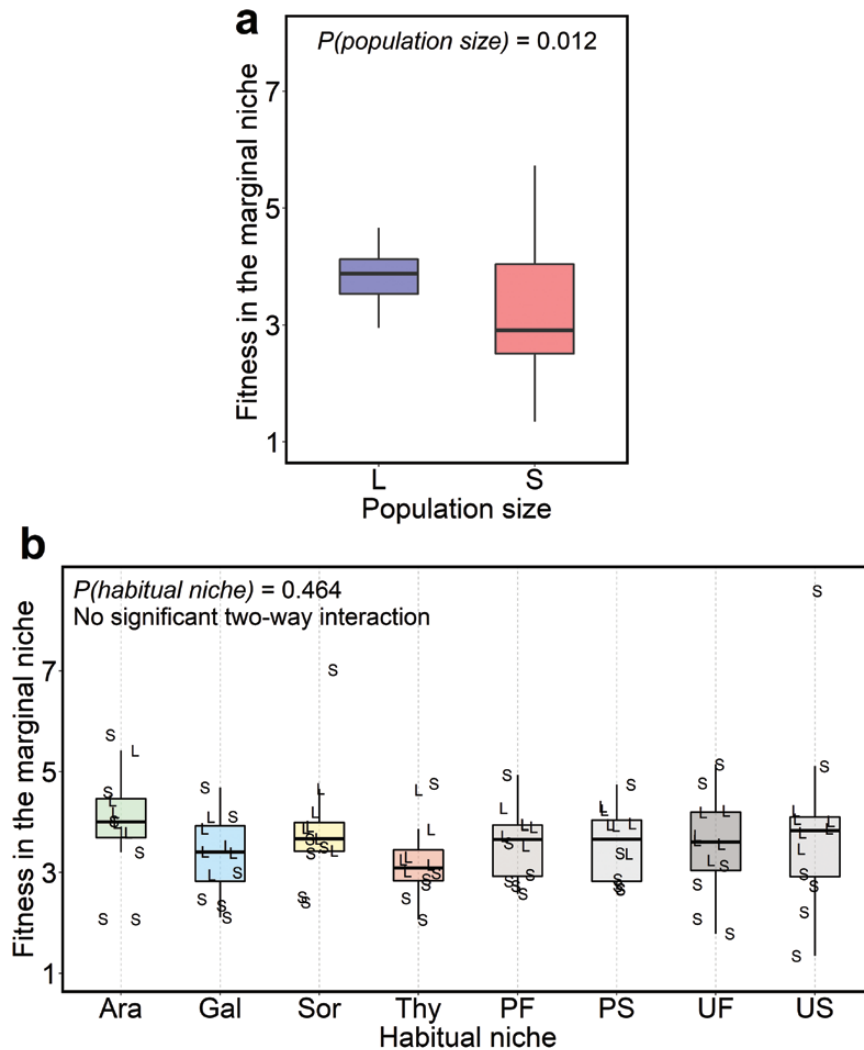


Figure 2. Fitness in the marginal niche after ~480 generations. (A) Larger populations had significantly better fitness in the marginal niche than smaller populations. (B) The habitual niche’s identity did not significantly influence fitness in the marginal niche. The colored plots correspond to regimens with a constant habitual niche, black and white plots correspond to regimens with a fluctuating habitual niche. L and S represent large and small populations, respectively. In both the plots, the lower and upper hinges of boxes show the 25% and 75% quantiles, respectively. The horizontal line within boxes represents the median. The whiskers denote the two extremes within the data that are less than or equal to 1.5 times the length of the box, extending away from the box. While the larger populations adapted more to the marginal niche, surprisingly, such adaptation was unconstrained by the composition of the habitual niche.

identity” (four levels: Thy, Gal, Ara, and Sor) as fixed factors crossed with each other and “day of assay” as the random factor.

Analogously, to analyze how population size and the fluctuating niche’s identity shaped fitness in the latter, we conducted a mixed model ANOVA (RCBD) on geometric mean fitness values over the four component environments of the fluctuating habitual niche. Here we treated “population size” (two levels: L and S), “fluctuation predictability” (two levels: predictable and unpredictable), and “fluctuation speed” (two levels: fast and slow) as fixed factors crossed with each other and “day of assay” as the random factor.

Results

Adaptation to the marginal niche was shaped by the population size, not by the habitual niche’s identity or stability

Our evolution experiment resulted in widespread adaptation to exploit the marginal ecological opportunity presented by

Acetate. Specifically, 15/16 regimens underwent significant adaptation to Acetate during the ~480 generations of evolution (Figure 2; see Supplementary Table S1 for statistical details). For the regimens with constant habitual niches, we measured the fitness values in the following carbon sources: Ara (for Ara-L and Ara-S); Gal (for Gal-L and Gal-S); Sor (for Sor-L and Sor-S); and Thy (for Thy-L and Thy-S). In contrast, for the fluctuating regimens (PFL, PFS, PSL, PSS, UFL, UFS, USL, and USS) fitness in the habitual niche was measured as the geometric mean fitness across Ara, Gal, Sor, and Thy. We found that while 15/16 of these regimens avoided maladaptation to their respective habitual niches, 12 of them also showed significant habitual niche adaptation (Supplementary Tables S2 and S3). In other words, in terms of fitness in the habitual niche, 12/16 regimens showed adaptation, 3/16 regimens showed no significant difference, and 1/16 showed maladaptation. Next, we determined the population genetic and ecological factors that could explain the extent of adaptation to the marginal niche.

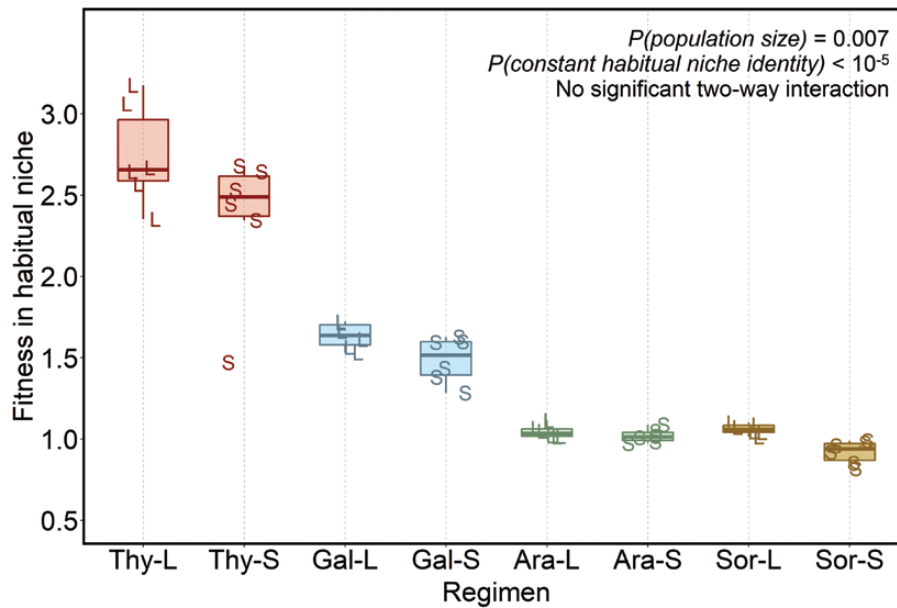


Figure 3. Fitness in the constant habitual niche after ~480 generations. All data points show fitness values relative to the ancestor. L and S represent large and small populations, respectively. The box and whisker notations are similar to those in Figure 2. Overall, fitness in the constant habitual niches was influenced significantly by the population size and the constant habitual niche's identity. However, the effects of either of these factors were not contingent on the other.

We found that the population size had a significant effect on fitness in the marginal niche (mixed model ANOVA: population size [fixed effect] $F_{1,75} = 6.62$; $p = .01$; partial $\eta^2 = 0.081$ [medium effect]). Specifically, larger populations gained higher fitness in Acetate (Figure 2A). This aligns with our first hypothesis that the larger populations should show greater adaptation to the marginal niche.

The identity of the habitual niche (constant [Ara, Gal, Sor, or Thy] or fluctuating [PF, PS, UF, and US]) did not have a significant effect on fitness in the marginal niche (mixed model ANOVA: habitual niche identity [fixed effect] $F_{7,75} = 0.96$; $p = .46$) (Figure 2B). Moreover, we did not find any significant statistical interaction of population size and the habitual niche identity in shaping the fitness in the marginal niche ($F_{7,75} = 0.20$; $p = .98$). Both these observations contradict our second hypothesis that the adaptation to the marginal niche should be influenced by the habitual niche's identity. Taken together, we found that population size shaped marginal niche adaptation unconstrained by the identity of the sympatric habitual niche.

Before presenting a population genetic explanation of this unexpected pattern of niche expansion and discussing its implications, we describe the fitness changes in the habitual niches of our experimental regimens. Since the constant habitual niches in our study were qualitatively different from the fluctuating habitual niches, we conducted separate analyses of the drivers of adaptation in them.

Fitness gains in constant habitual niches were shaped by their identities and the population size

We found that larger populations tended to have significantly greater fitness in their constant habitual niches (mixed model ANOVA: population size [fixed effect] $F_{1,35} = 8.099$; $p = .007$; partial $\eta^2 = 0.188$ [large effect]) (Figure 3). We had shown earlier that the habitual niche identities had no effect on fitness in the marginal niche (Figure 2B). In contrast, the identities of the habitual niches significantly affected fitness changes in them (mixed model ANOVA: identity of

the constant habitual niche [fixed effect] $F_{3,35} = 136.08$; $p < 10^{-5}$; partial $\eta^2 = 0.921$ [large effect]) (Figure 3). There was no statistically significant interaction of population size and constant habitual niche's identity in influencing fitness in the habitual niche ($F_{3,35} = 1.532$; $p = .223$). Specifically, the extent of adaptation to habitual niches showed the following trend: Thy > Gal > Ara \approx Sor (based on post hoc test using Tukey's HSD) (Figure 3; Supplementary Table S2). We also found that absolute ancestral fitness in habitual niches had an inverse trend relative to the extent of adaptation in them (Supplementary Figure S3).

Adaptation to the fluctuating habitual niche was not affected by the predictability or speed of fluctuations

Analogous to the observation in the constant habitual niches (Figure 3), larger populations gained significantly higher fitness in their fluctuating habitual niches (Figure 4). Specifically, we found that larger populations gained greater geometric mean fitness across Ara, Gal, Sor, and Thy (mixed model ANOVA: $F_{1,35} = 104.43$; $p = 4.792 \times 10^{-12}$). Moreover, the predictability ($F_{1,35} = 0.003$; $p = .954$) and speed ($F_{1,35} = 0.526$; $p = .473$) of fluctuations failed to significantly shape fitness changes in the fluctuating habitual niche (Figure 4). This trend was so unambiguously evident that neither the three possible two-way interactions nor the three-way interaction between the three fixed effects were significant: population size \times predictability ($F_{1,35} = 1.191$; $p = .283$); population size \times speed ($F_{1,35} = 0.409$; $p = .527$); predictability \times speed ($F_{1,35} = 0.230$; $p = .635$); and population size \times predictability \times speed ($F_{1,35} = 4.9 \times 10^{-4}$; $p = .983$).

Two negatively correlated habitual niches can each show positive fitness correlations with the marginal niche

A previous study had shown that Gal and Thy show negative fitness correlations in *E. coli* (Chavhan et al., 2020).

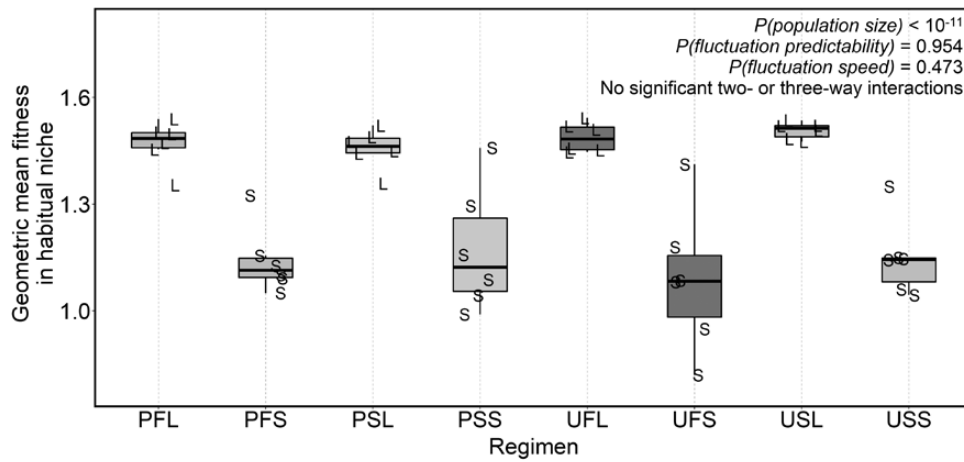


Figure 4. Fitness in the fluctuating habitual niche after ~480 generations. All data points show geometric mean fitness values relative to the ancestor. L and S represent large and small populations, respectively. The box and whisker notations are similar to those in Figure 2. The fitness in fluctuating habitual niches was significantly shaped by the population sizes but not by either the predictability or the speed of habitual niche fluctuations. In the three-letter labels on the x-axis, the first letter refers to the predictability of environmental fluctuation (P for predictable and U for unpredictable), the second letter stands for fluctuation speed (F for fast and S for slow), while the third letter represents the population size (L for large and S for small).

Agreeing with this notion, we also found a strong negative Gal-Thy fitness correlation in regimens with either Gal or Thy in their constant habitual niche (Figure 5A; Gal-Thy fitness correlation calculated over Gal-L, Gal-S, Thy-L, and Thy-S: Pearson’s $r = -0.924$; $p = 6.021 \times 10^{-11}$). Thus, the presence of the ever-present marginal niche (Acetate) could not prevent the realization of such negative Gal-Thy fitness correlations. Moreover, over these four regimens, the marginal niche (Acetate) did not show any significant fitness correlation with either of the habitual niches (Gal for Gal-L and Gal-S; Thy for Thy-L and Thy-S; Figure 5B and C). Specifically, over the four regimens, we did not find any Acetate-Gal correlation (the gray shaded region and dashed line in Figure 5B: Pearson’s $r = 0.021$; $p = .461$). Analogously, over these four regimens, there was no Acetate-Thy correlation (the gray shaded region and dashed line in Figure 5C: Pearson’s $r = 0.118$; $p = .290$).

Notwithstanding the lack of an Acetate-Gal correlation over Gal-L, Gal-S, Thy-L, and Thy-S described earlier, we found that the regimens with Gal as their constant habitual niche (Gal-L and Gal-S) showed a significant positive Acetate-Gal correlation (Pearson’s $r = 0.573$; $p = .026$; the pink shaded region and solid line in Figure 5B). Furthermore, there was no significant Acetate-Gal correlation over Thy-L and Thy-S ($p = .108$).

Similarly, despite the lack of an Acetate-Thy correlation over Gal-L, Gal-S, Thy-L, and Thy-S described earlier, the regimens with Thy as their constant habitual niche (Thy-L and Thy-S) showed a significant positive Acetate-Thy correlation (Pearson’s $r = 0.670$; $p = .009$; the blue shaded region and solid line in Figure 5C). Moreover, we found no significant Acetate-Thy correlation over Gal-L and Gal-S ($p = .850$).

Taken together, the two negatively correlated habitual niches (Gal and Thy) do not show an overall fitness correlation with the marginal niche (Acetate). However, regimens with one of these constant habitual niches can still exhibit a positive correlation between Acetate and their habitual niche (Acetate-Gal for Gal-L and Gal-S; Acetate-Thy for Thy-L and Thy-S). This way, two negatively correlated habitual niches can individually exhibit a positive correlation with the ever-present marginal niche.

Having demonstrated widespread adaptation to a newly available marginal niche shaped by the population size but unconstrained by the composition of the sympatric habitual niche, we turn to the ecological and population genetic explanations of the observed fitness trends.

Discussion

The rapid adaptation to a new, low-productivity (marginal) niche observed in our study reveals a potential for widespread bacterial niche expansion in the face of novel ecological opportunities. Such rampant adaptation to the marginal niche aligns with the “rule of declining adaptability” (Couce & Tenaillon, 2015). Specifically, this rule predicts that our experimental populations should adapt much more to Acetate than the habitual carbon sources, in which the ancestral genotype had a relatively greater absolute fitness (Supplementary Figure S1). In other words, the fitness gains in the marginal niche should be much greater than fitness changes in the habitual niches. Our observations match this general expectation (Supplementary Figure S3; also compare Figure 2B with Figure 3, noting the difference of the scale on the Y-axis).

We found that the extent of marginal niche adaptation was consistently greater in larger populations (Figure 2). This observation can be explained by the relatively greater supply of variation and better efficiency of natural selection in larger populations (Chavhan et al., 2019, 2020; Desai & Fisher, 2007; Desai et al., 2007). Moreover, the larger populations showed a relatively lower variation in fitness than the smaller populations (Figures 2A, 3, and 4). This observation aligns with the theoretical prediction that the stochastic effects of drift would be weaker in larger populations, which would lead to more repeatable fitness changes in them (Chavhan et al., 2019; Sniegowski & Gerrish, 2010). Although contrasting habitual niches could have led to different population sizes at saturation (N_f), we note that the pairwise difference in N_f was never greater than an order of magnitude across the four habitual niches (Supplementary Figure S1). As pointed out by Chavhan et al. (2019), N_f has a much weaker influence on the dynamics of adaptation than the periodic bottleneck ratio. Moreover, differences in N_f

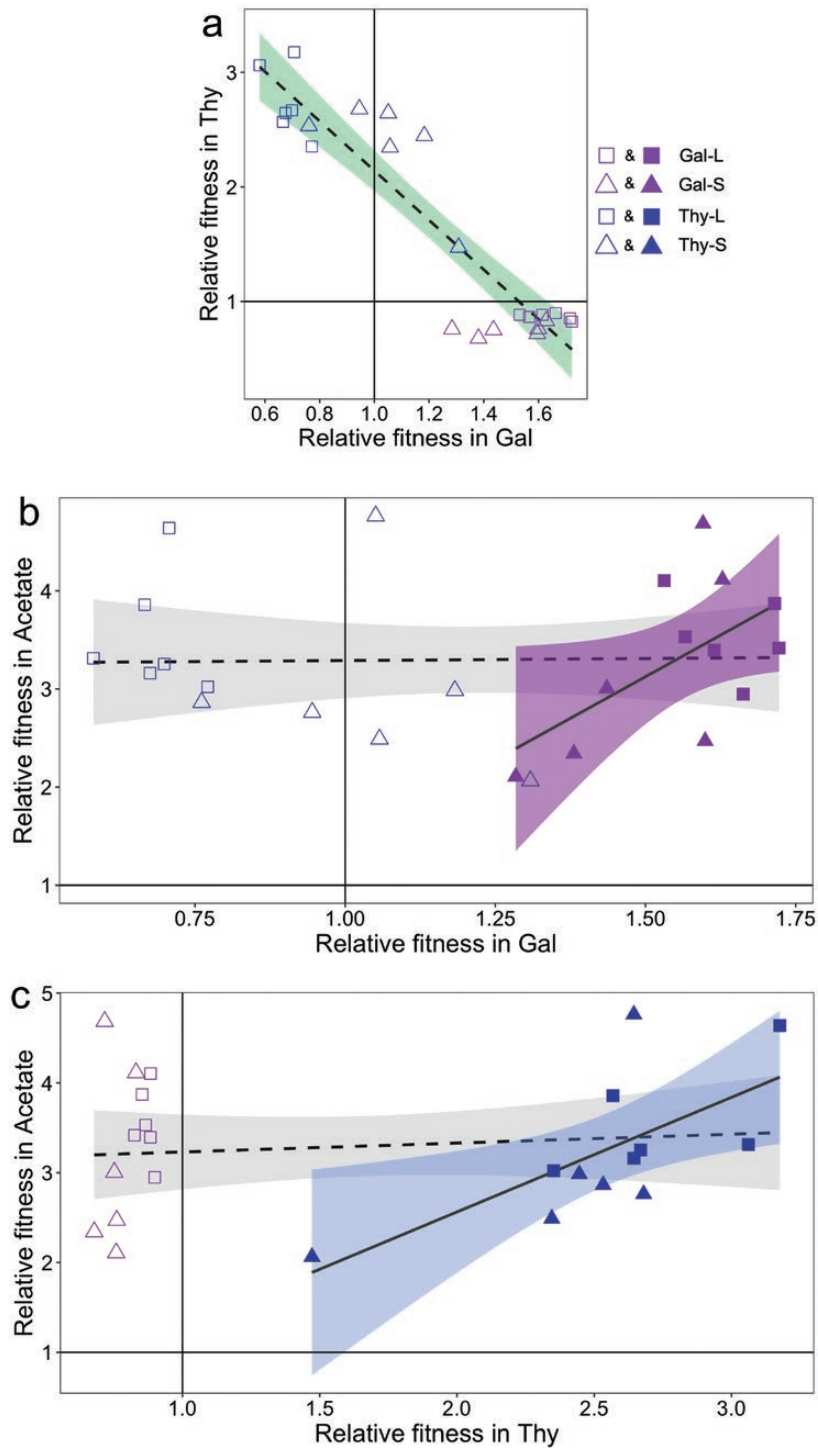


Figure 5. Fitness correlations across Gal, Thy, and Acetate. See the text for statistical details. (A) Fitness was negatively correlated across Gal and Thy in regimens that had either Gal or Thy as the only habitual carbon source. (B) When calculated over Gal-L, Gal-S, Thy-L, and Thy-S, there was no significant fitness correlation between Acetate and Gal (*gray shaded region and dashed line*). However, the regimens with Gal as their constant habitual niche (Gal-L and Gal-S, depicted with filled *pink symbols*) showed a significant positive Acetate-Gal correlation. (C) When calculated over Gal-L, Gal-S, Thy-L, and Thy-S, there was no significant fitness correlation between Acetate and Thy (*gray shaded region and dashed line*). However, the regimens with Thy as their constant habitual niche (Thy-L and Thy-S, depicted with filled *blue symbols*) showed a significant positive Acetate-Thy correlation (*blue shaded region and solid line*). Also see [Supplementary Figure S4](#).

smaller than an order of magnitude are unlikely to result in significantly different adaptive dynamics. Our experiment was designed in such a way that the influence of such minor population size differences across distinct habitual niches could still be captured by the effects of habitual niche

identities. Hence, our observation that the habitual niche identity did not have a significant influence on marginal niche adaptation strengthens the notion that minor differences across different habitual niches should not affect marginal niche adaptation.

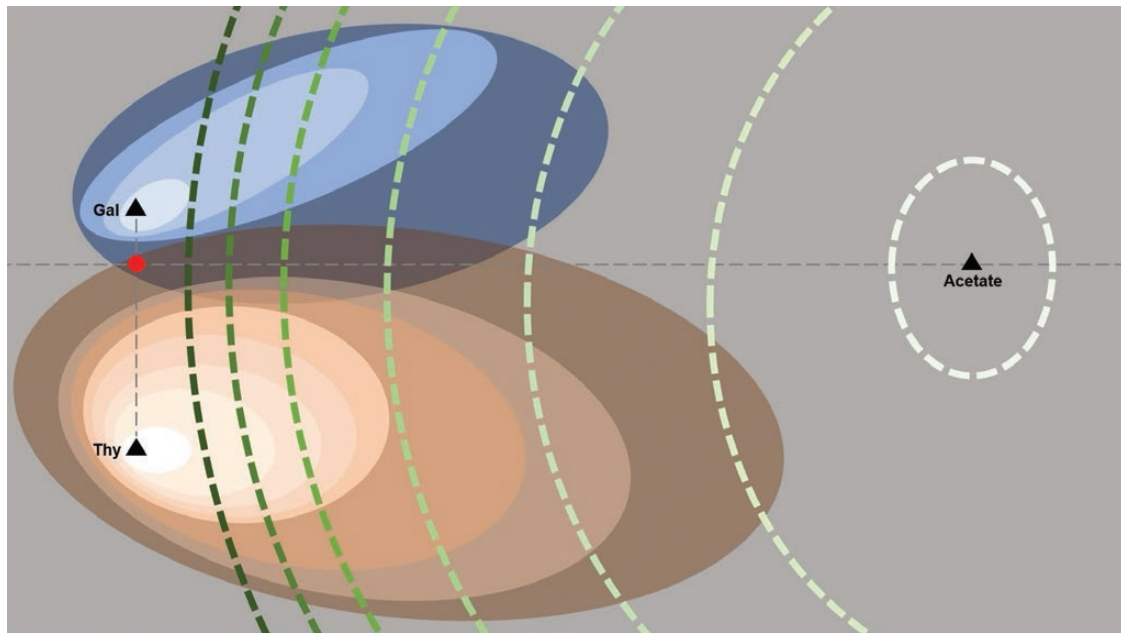


Figure 6. A schematic fitness landscape based on Fisher’s geometric model with three niches (Acetate, Gal, and Thy). The *darkest blue* and *brown* areas represent genotypes with ancestral level of fitness in Gal and Thy, respectively. The *red spot* represents the ancestral genotype. The lighter the blue shade the higher the fitness in Gal. Similarly, lighter shades of brown correspond to higher fitness in Thy. *Black triangles* represent fitness peaks in the niche in question. All gray regions that are outside the blue area have lower fitness in Gal as compared to the ancestor. Similarly, all regions outside the brown area have lower fitness than the ancestor in Thy. The *dashed green curves* show the fitness gradient in acetate (the brighter the green shade, the higher the fitness in acetate). We emphasize that while our placement of the three fitness optima is based on the rule of declining adaptability, it is merely schematic as our data does not allow us to have the precise knowledge of the locations of these optima.

In contrast, our other key observation is surprising: we found that adaptation to the marginal niche (Acetate) was unaffected by the identities of the several distinct constant and fluctuating sympatric habitual niches in our study (Figure 2B). We use the following chain of arguments to emphasize why this observation was unexpected. First, as the marginal niche could not support the growth on its own at the outset (Supplementary Figure S1), our experimental regimens faced a strong selection for maintaining (or gaining) fitness in their respective habitual niches. Indeed, we found that 15/16 regimens either gained or maintained fitness in their respective habitual niches (Supplementary Tables S2 and S3). Second, the selection pressures to maintain (or gain) fitness in the habitual niches were expected to be qualitatively different across our distinct experimental regimens. In other words, the fitness optima relevant to our different habitual niche regimens were not expected to align with each other. For example, Gal-S and Gal-L adapted to Gal (i.e., moved toward the Gal optimum) but became maladapted to Thy (i.e., moved farther away from the Thy optimum as compared to the ancestor), which shows that the Gal and Thy optima were likely not aligned with each other. Moreover, the extent of adaptation to the unchanging habitual niche was significantly different across the different regimens with a constant habitual single-carbon source (Figure 3). Finally, the regimens with a single unchanging habitual carbon source faced markedly different selection pressures as compared to those with fluctuating habitual carbon sources. Since the location of the habitual niche optimum should influence adaptation to the marginal niche (Figure 1) and given that our regimens experienced multiple constant and fluctuating habitual niches whose optima were likely unaligned, it is surprising that adaptation to the marginal niche was unaffected by the identities of the contrasting

habitual niches (Figure 2B). In other words, our observations did not match the expectations based on the simplistic FGM scenarios presented in Figure 1, which included two distinct fitness optima, one each for the habitual and marginal niches, respectively. To fix this, we drew upon a more nuanced extension of FGM that includes three distinct fitness optima, two in habitual niches and one in the marginal niche.

Although our experiment deals with four distinct habitual niches, we prefer an FGM with two habitual niches and one marginal niche for two reasons: (1) It is relatively easier to visualize in 2D images. (2) Two of the habitual niches (Ara and Sor) showed a much smaller fitness changes than Gal and Thy (Figure 3). Based on the four key notions described below, we created a schematic fitness landscape with Acetate, Gal, and Thy, and tested the predictions emerging from it using our experimental observations in both constant and fluctuating environment regimens (Figure 6):

1. Gal and Thy are expected to show reciprocal fitness trade-offs, indicating antagonistic pleiotropy across these two niches (Chavhan et al., 2020). Thus, (based on the theory presented by Martin and Lenormand (2015)), the ancestral genotype should be located between the Gal and Thy optima.
2. Based on the rule of declining adaptability (Couce & Tenaillon, 2015), we expect the following trends for the scope of adaptation: Acetate >> Thy > Gal. Therefore, the distance of the ancestor from the fitness optima in the three niches should also have the same order.
3. Based on the fitness correlations shown in Figure 5, it was likely to simultaneously increase fitness in Gal and Acetate or Thy and Acetate, but not in Gal and Thy. Therefore, the Acetate optimum cannot be along the

axis joining the Gal and Thy optima; instead, the Acetate optimum should be substantially distant from it.

4. The large scope of adaptation offered by Acetate can pull genotypes away from the trade-off axis connecting the Gal and Thy optima, thereby diluting the Gal-Thy trade-offs.

Combining the above notions, we created the schematic fitness landscape shown in [Figure 6](#). We then used our empirical observations to test several distinct predictions emerging from it. We emphasize that the FGM schematic of [Figure 6](#) applies to the triplet of optima used here (Acetate, Gal, and Thy); like most other evolution experiments, it may not work in a generalizable manner. Moreover, we use this extended FGM to evaluate and understand our many different results using a single framework. Thus, the model should be looked at as an interpretative guide to our observations and not as another standalone result of our study.

The FGM predicts that Gal-L and Gal-S should travel to the blue region with higher fitness in Gal and Acetate, which should lead to their maladaptation to Thy. This is indeed what we observed in our experiments ([Supplementary Tables S1 and S2](#)).

Since the regimens with fluctuating habitual niches are shaped by selection pressures in both Gal and Thy, the FGM predicts that they should adapt to Acetate without losing significant fitness in either Gal or Thy (the overlap between blue and brown regions allows this possibility). Agreeing with this prediction, we found that none of the regimens with fluctuating habitual niches became maladapted to either Gal or Thy.

Since the ancestral genotype is situated between the Gal and Thy optima, the FGM predicts that no single mutation is likely to increase fitness simultaneously in both Gal and Thy. Thus, simultaneous adaptation to Gal and Thy, if it happens at all, should require the enrichment of multiple distinct mutations. Since such an event is expected to be relatively rare, adaptation to both Gal and Thy should occur primarily in larger populations with high enough mutational supply for enriching multiple mutations. This is similar to the scenario presented by [Chavhan et al. \(2021\)](#), where large populations evolved in fluctuating environments could adapt to both Gal and Thy by fixing multiple distinct mutations (at least one linked to Gal and another to Thy), but small populations could not do so. Indeed, we found that all the large population regimes with a fluctuating habitual niche (PFL, PSL, UFL, and USL) became adapted to both Gal and Thy but the analogous small population regimens (PFS, PSS, UFS, and USS) could adapt to neither ([Supplementary Table S2](#)).

Finally, the FGM predicts that Thy-L and Thy-S should adapt to both Acetate and Thy, but either maladapt to or do not change their fitness in Gal. Agreeing with this, we observed that Thy-L gained fitness in Acetate and Thy but became maladapted to Gal. Thy-S adapted to Acetate and Thy as predicted. However, its fitness remained unchanged in Gal ([Supplementary Table S2](#)). Among TL and TS, TS should end up closer to the axis joining the ancestral genotype with the Acetate optimum. This is because due to its lower mutation supply than Thy-L, Thy-S should adapt via the most readily accessible mutations, which should be more frequent for Acetate than for Thy, as predicted by the rule of declining adaptability ([Couce & Tenaillon, 2015](#)). Consequently, the fitness of Thy-S in Gal may not be statistically distinguishable from the ancestral fitness, as we observed in our experiment

([Supplementary Table S2](#)). Taken together, the extended FGM of [Figure 6](#) can explain the fitness trends in both the marginal and habitual niches in both constant and fluctuating environments in our study.

In our study, adaptation to the fluctuating habitual niche was not significantly shaped by the predictability or speed of fluctuations ([Figure 4](#)). This observation contrasts with some previous studies that have found the predictability and/or the speed of environmental fluctuations to be important determinants of fitness changes ([Boyer et al., 2021](#); [Hughes et al., 2007](#)). In contrast, another recent study has found that the predictability of environmental fluctuations does not significantly influence the extent of adaptation in *E. coli* ([Karve et al., 2018](#)). We note that a crucial difference between these previous studies and our experiment is the sustained presence of a marginal niche that the bacteria could adapt to. Adaptation to this unchanging marginal niche likely masked the subtle effects of the speed and predictability of habitual niche fluctuations, particularly given that Acetate diluted the Gal-Thy trade-offs. Indeed, we found another manifestation of trade-off dilution in the presence of the marginal niche in the fluctuating habitual niche regimens. In a previous experimental evolution study on *E. coli* that did not involve Acetate, the small populations evolving in an environment that fluctuated across several sole carbon sources (including Gal and Thy) adapted to Thy but became significantly maladapted to Gal ([Chavhan et al., 2021](#)). In contrast, in the present study (where Acetate was always available), all the small populations evolving in the fluctuating environment regimens (PFS, PSS, UFS, and USS) maintained their fitness in both Gal and Thy ([Supplementary Table S2](#)).

Every independently evolving population in our experiment had simultaneous access to equal quantities (w/vol) of two distinct carbon sources, one habitual (high-productivity) and the other marginal (low-productivity). Whether two simultaneously available carbon sources are used hierarchically is straightforward to explain if one of them is a PTS sugar (i.e., a sugar whose uptake involves the PTS; e.g., glucose) and the other is a non-PTS sugar. In such a scenario, which has been studied extensively, the PTS sugar tends to be utilized preferentially before the non-PTS sugar ([Brückner & Titzmeyer, 2002](#)). In contrast, the utilization hierarchies (if any) within mixtures of two non-PTS sugars remain much less understood ([Aidelberg et al., 2014](#)). Moreover, the hierarchical utilization (if any) among the five non-PTS carbon sources (four habitual and one marginal) used in our study has not been established in the current literature. While speculating in this regard is out of scope of our study, it is worth noting that the presence or absence of such utilization hierarchies does not change the interpretation of our key results. Regardless of the presence or absence of hierarchical carbon source utilization, our experimental populations faced selection for maintaining (or gaining) fitness in the habitual niche while adapting to the marginal niche. This is because although our experimental populations had access to equal quantities (w/vol) of both the habitual and marginal carbon sources, at the outset of our experiment, this quantity of the marginal carbon source could not sustain substantial bacterial growth on its own ([Supplementary Figure S1](#)). In other words, bacterial growth was largely supported by the habitual niche at the beginning of our evolution experiment. Moreover, our interpretation of the observed fitness changes relies on two distinct notions, none of which depend on the presence/absence of resource

utilization hierarchies. The first notion concerns the rule of declining adaptability in microbial populations, which posits that within a fixed time frame, the extent of adaptation to an environment varies inversely with the ancestral fitness in it (Couce & Tenaillon, 2015). The second notion involves the FGM with distinct fitness optima in three different environments presented in Figure 6. Since none of these two notions are affected by carbon utilization hierarchies, our interpretations are robust to the presence/absence of such hierarchies.

Finally, although our experimental populations had simultaneous access to both the marginal and habitual niches without any spatial constraints, one of our key results can also be interpreted using the relatively simple framework of source-sink dynamics, which was primarily designed to understand evolution in spatially constrained systems involving immigration from one environment (the source) into another (the sink) (Holt, 1985; Perron et al., 2007; Pulliam, 1988). Specifically, the habitual niche (which supported most of the bacterial growth in the beginning of our experiment) can be imagined as a “source” for supporting genetic variation that can lead to adaptation to the marginal niche (which can be imagined as the sink). In this framework, adaptation to the marginal niche would only depend on the amount of variation the source can provide, which would be a simple function of the population size. This can explain why adaptation to the marginal niche was shaped by the population size in our experiment but was unconstrained by the identity of the sympatric habitual niche. The biological insignificance of differences in N_f across the different sympatric habitual niche regimens (described earlier in the Discussion) also agrees with this notion. Specifically, the differences in the amounts of beneficial variation supplied by the different habitual niche sources were unlikely to be biologically important because the differences in N_f across the habitual niche regimens were smaller than an order of magnitude. Thus, the relatively more elaborate scaffold of the FGM may not be required to explain the result that adaptation to the marginal niche is unconstrained by the habitual niche’s identity. However, we also note that such simple explanations based merely on the population size would be inadequate for simultaneously accounting for fitness changes in both habitual and marginal niches. Specifically, the important result that adaptation to the habitual niches observed in our experiment was shaped by both the identity of the habitual niche and the population size (but not by their statistical interaction; see Figure 3) cannot be explained based on the population size alone. In contrast, a more nuanced setup like the FGM in Figure 6 can help interpret all our results simultaneously.

Our observations imply a pervasive evolutionary potential for the expansion of bacterial niche width that is unconstrained by the composition of the habitual niche, even at low population sizes. Furthermore, despite employing four different habitual niches with different unchanging (constant) sole carbon sources and four others with different fluctuations, only one out of the 16 regimens showed fitness trade-offs across their habitual and marginal niches (Supplementary Table S4). Such a pervasive and costless manner of niche expansion in the face of a new ecological opportunity can potentially explain why *E. coli* tends to show a substantial metabolic niche breadth (Sajed et al., 2016). We note that notwithstanding the “immediately costless” nature of niche expansion observed in our study, there can be costs of such niche expansion in terms of evolvability, as proposed recently by Bono et al. (2019). Determination of such evolvability

costs of niche expansion represents a key future direction. We also note that most of the 16 regimens in our study adapted to both the marginal and the habitual niches (Supplementary Table S4). This observation contrasts with the results of an experimental evolution study with *Pseudomonas fluorescens*, where the populations adapted largely to the low-productivity niche but not to the high-productivity niche (Jasmin & Kassen, 2007).

To the best of our knowledge, this is the first study to propose that fitness trade-offs between two habitual environments can be diluted by the presence of a marginal ecological opportunity. This finding can be a critical step in understanding ecological specialization in bacteria.

Conclusion

Our explanations are likely to work generally in the presence of a marginal niche with a large scope of adaptation. Moreover, unlike Gal and Thy, if the environments in the habitual niche do not show reciprocal trade-offs, relatively greater niche expansion is expected. Given the relatively short span of our study (~480 generations), our results demonstrate that bacteria can quickly adapt to multiple environmental components to expand their niches if a new opportunity becomes available, regardless of the properties of the habitual niche still at their disposal. Our observations and their population genetic explanations should act as stepping-stones for more nuanced tests of niche expansion theories by targeting direct and pleiotropic mutational effects in large vs. small populations in constant vs. fluctuating habitual niches.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

All the data relevant to this study are available on Dryad at DOI: 10.5061/dryad.k0p2ngff8

Author contributions

Y.C. and S.D. designed the study. Y.C. and S.M. conducted the experiments. Y.C. and S.M. analyzed the data. Y.C. and S.D. wrote the manuscript with inputs from S.M.

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Conflict of interest: Editorial processing of the manuscript was done independently of S.D., who is an Associate Editor of *Evolution*. The other authors declare no conflict of interest.

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