ECOLOGICAL CONDITIONS AFFECT EVOLUTIONARY TRAJECTORY IN A PREDATOR–PREY SYSTEM

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The arms race of adaptation and counter adaptation in predator–prey interactions is a fascinating evolutionary dynamic with many consequences, including local adaptation and the promotion or maintenance of diversity. Although such antagonistic coevolution is suspected to be widespread in nature, experimental documentation of the process remains scant, and we have little understanding of the impact of ecological conditions. Here, we present evidence of predator–prey coevolution in a long-term experiment involving the predatory bacterium Bdellovibrio bacteriovorus and the prey Pseudomonas fluorescens, which has three morphs (SM, FS, and WS). Depending on experimentally applied disturbance regimes, the predator–prey system followed two distinct evolutionary trajectories, where the prey evolved to be either super-resistant to predation (SM morph) without counter-adaptation by the predator, or moderately resistant (FS morph), specialized to and coevolving with the predator. Although predation-resistant FS morphs suffer a cost of resistance, the evolution of extreme resistance to predation by the SM morph was apparently unconstrained by other traits (carrying capacity, growth rate). Thus we demonstrate empirically that ecological conditions can shape the evolutionary trajectory of a predator–prey system.

KEY WORDS: Antagonistic coevolution, Bdellovibrio bacteriovorus, disturbance, experimental evolution, Pseudomonas fluorescens, trade-off evolution.

Considering the ubiquity of predator–prey and host–parasite interactions in nature, antagonistic coevolution is thought to play a critical role in shaping species. Numerous theoretical (May and Anderson 1983; May 1985; Hamilton et al. 1990) and empirical studies (Cowlishaw and Mrsa 1975; Chao et al. 1977; Lenski and Levin 1985; Bohannan and Lenski 2000; Buckling and Rainey 2002; Mizoguchi et al. 2003; Webster et al. 2004; Decaestecker et al. 2007) have explored this phenomenon, but demonstrating...
antagonistic coevolution in nature has proved difficult. Long generation times and the inability to control genetic variation make natural populations hard to study. Natural conditions add variability to an already complex phenomenon. For all these reasons, bacterial populations make an ideal system for studying coevolution. Short generation times and large population sizes allow one to observe rapid evolution from initially isogenic populations (Lenski et al. 1991). Bacterial populations are easily cultivated under controlled laboratory conditions, where it is possible to dissect the impact of environmental factors on evolutionary processes.

Current interest in the use of bacterial predators as an alternative to traditional antibiotic therapy or as an environmental prophylactic (Levin and Bull 2004) also makes the study of predator–prey coevolution in bacteria relevant. Several possible outcomes might be expected from such an approach to treating bacterial disease, based on theoretical studies of predator–prey coevolution (Woolhouse et al. 2002). One outcome is the evolution of resistance to predation. Experiments performed with Escherichia coli and λ or T bacteriophages have shown that after one (Chao et al. 1977; Lenski 1984; Lenski and Levin 1985; Spanakis and Horne 1987; Forde et al. 2004) or two coevolutionary cycles (Braunbreton and Hofnung 1981; Lenski and Levin 1985; Bohannan and Lenski 2000), bacteria become completely resistant to parasitism by suppressing the expression of the receptor protein needed for viral infection. However, life-history theory (Stearns 1992) suggests that the evolution of one trait, such as resistance to predation, should be constrained by trade-offs with other life-history traits, such as growth rate or resource-utilization efficiency (i.e., conversion of a given amount of nutrient into cells, or carrying capacity; Gallet et al. 2007). Thus a second possible outcome is that resistance may not evolve because of such constraints. A third possible outcome is a coevolutionary arms race between prey and predator, with increases in both resistance and predation efficiency (Woolhouse et al. 2002). Buckling and Rainey (2002) demonstrated such a coevolutionary arms race between Pseudomonas fluorescens and the bacteriophage SBW25φ2.

More recently, experimental evolutionists have begun to develop an appreciation for the effects of ecological conditions on the dynamics of antagonistic coevolution. In bacterial populations, ecological factors like productivity (Forde et al. 2004; Lopez-Pascua and Buckling 2008), spatial structure (Brockhurst et al. 2007; Morgan et al. 2007), and population mixing (Brockhurst et al. 2003) have been shown to have an impact on antagonistic coevolution. The effect of other ecological factors, such as disturbance, is essentially unknown. Ecologists define disturbance as a massive mortality event (e.g., a forest fire or hurricane) that alters the niche opportunities available to the species in a community (Shea et al. 2004). The associated loss of biomass relaxes competitive interactions by freeing up access to key resources and, in some cases, replenishment of these resources (Shea et al. 2004). Disturbance has been shown to affect species diversity (Connell 1978; Fox 1981; Buckling et al. 2000a), and both population and community dynamics (Hughes and Connell 1999; Douhovnikoff et al. 2005). In at least one bacterial system, species diversity is maximized at intermediate frequencies of disturbance (Buckling et al. 2000b), but this relationship between diversity and disturbance breaks down in the presence of parasites (Morgan and Buckling 2004). Thus, parasitism has been shown to modify the impact of disturbance on species diversity, but the effect of disturbance on antagonistic coevolutionary interactions between a parasite and its host (or a predator and its prey) remains to be tested.

Here we evaluate how disturbance affects antagonistic coevolutionary dynamics via a long-term coevolution experiment between the predatory bacterium Bdellovibrio bacteriovorus and P. fluorescens as its prey. Bdellovibrio bacteriovorus 109J is a Gram-negative predatory bacterium that preys upon a wide range of Gram-negative bacteria (Stolp and Starr 1963; Jurkevitch et al. 2000; Rendulic et al. 2004). This very small (0.2–0.5 μm wide and 0.5–2.5 μm long) ubiquitous obligate predator only attacks bacteria in stationary phase (in contrast to most bacteriophages). Once inside its prey, it feeds on the cytoplasm and gives rise to four to five daughter cells ready to find new prey. Sockett and Lambert (2004) reported that resistance to B. bacteriovorus is extremely rare, and that the evolution of resistance to this predator is unlikely, making it a strong candidate for antibiotic therapy. Our prey species, P. fluorescens SBW25, is a Gram-negative bacterium which, in static liquid medium, diversifies into three morphs adapted to three niches of the culture environment (Rainey and Travisano 1998; Rainey 2005): (1) the SM morph (Smooth Morph) occupies the liquid medium; (2) the WS morph (Wrinkly Spreader) forms a biofilm at the air-liquid interface; (3) the FS morph (Fuzzy Spreader) is found at the bottom of the culture. When the medium is constantly agitated (as in this study), diversification does not occur in the absence of predation, and only the ancestral morph (SM) is detected. In the presence of the predator B. bacteriovorus, all morphs appear in the culture, but only one or two morphs eventually persist, depending on disturbance regime (Gallet et al. 2007). Here we report on the coevolutionary dynamics associated with the experiment of Gallet et al. (2007). Disturbance was mimicked by applying six different dilution regimes, varying in frequency and intensity, to multiple cultures (replicates) that otherwise experienced homogeneous conditions (see Methods). At the time of dilution, both predator and prey abundance were drastically reduced and the resource supply was replenished. Predator and prey populations were cultivated for approximately 200–266 prey generations.

Assays of predation efficiency at the beginning and end of cultivation, as well as at a series of samples throughout the experiment, showed that the predator–prey system followed two distinct
evolutionary trajectories. Depending on disturbance regime, we observed either antagonistic coevolution interactions between the FS morph and the predator, or the evolution of a super-resistant SM morph without predator counter-adaptation. Resistance correlated negatively with resource-utilization efficiency only in the FS morph, and not in the SM morph, suggesting that the cost of resistance involves different life-history traits under the different evolutionary trajectories.

Materials and Methods

LONG-TERM EVOLUTION EXPERIMENT

The experiment reported by Gallet et al. (2007) provided the predator and prey samples whose evolution we test here. In that experiment, 36, 60 mL polycarbonate bottles (Nalgene, Rochester, NY) containing 6 mL of diluted nutrient broth (DNB—0.8 g of nutrient broth, 1000 mL H2O, 3 mM MgCl2, 2 mM CaCl2, agar 15 g, pH = 7.2; Jurkevitch et al. 2000) were inoculated with 10^7 P. fluorescens SBW25 prey bacteria. Eighteen of these populations were cultivated without predators (control treatment), whereas the other eighteen received 10^5 B. bacteriovorus 109J predatory bacteria. All populations were propagated at 28°C under constant agitation (200 rpm). They were transferred to fresh medium 20 times, under one of six different dilution regimes: all six combinations of dilution every two, three, or four days by one hundred or one thousand fold. At the time of dilution, only 1 or 0.1% of the mixed populations were transferred to fresh medium. Twenty transfers corresponds to ~200–266 prey generations. Samples of prey and predators were taken at transfers 0, 5, 10, 15, and 20. Prey were plated out and a single prey clone was selected from each time point. Predators were isolated by filtration at 0.5 μm; prey and predators were then frozen separately in 15% glycerol and stored at −80°C.

PREDATION ASSAYS

In a series of three assays, we evaluated whether antagonistic coevolution had taken place by comparing the predation efficiency of predators sampled from the beginning and end (“initial” vs. “final” predators, respectively) of the cultivation phase using prey sampled at various time points (depending on the assay). Because predator efficiency is the result of an interaction between prey and predator, it can also be viewed as a measure of resistance to predation by the prey.

Assay 1: Evolution of predator efficiency and resistance

In a first assay, we tested predation efficiency of initial predators as well as three final predator populations in which the SM morph went to fixation and three final predator populations in which FS went to fixation (six populations identified in Table 1B). These three types of predators were offered different types of prey: naive strains (i.e., strains that had never been cultivated with the predator, including the initial SM morph [Table 1 and Fig. 1, ○], the final SM morph from the control treatment without predation [▲], or an independently derived naive strain of the FS morph [●]) and prey cultivated with predators during 20 transfers (final SM or FS, Fig. 1A, ▲, and ●).

Assay 2: Local adaptation

To test for local adaptation, we assayed predation efficiency of all 36 possible predator–prey combinations involving the six samples identified in Table 1B from the end of the cultivation phase. We contrasted predation efficiency of predator–prey pairs that had been cultivated together ("sympatric") versus not ("allopatric"), using three alternative definitions of sympathy versus allopatri. The first definition of sympathy is strict, including only those predator–prey pairs that were cocultivated (see Fig. 2B). The second definition of sympathy is broader, including any predator–prey pairing cultivated with the same prey morph (Fig. 2C). Our third comparison was between strictly sympatric (cocultivated) pairs and “allopatric” (not cocultivated) predator–prey pairs within each prey morph (Fig. 2D).

Assay 3: Evolution of resistance over time

In a third assay, we evaluated the evolution of predator efficiency (and conversely, prey resistance) over the course of the experiment by assaying prey sampled at transfers 0, 5, 10, 15, and 20 against initial and final predators.

Protocol

Predation assays were initiated with the inoculation of 10^8 prey cells (from overnight cultures) in four 60-mL bottles containing 4.8 mL HEPES buffer (25 mM HEPES, MgCl2 3 mM, CaCl2 2 mM in 1000 mL H2O, pH=7.2). Two hundred microliters of HEPES buffer containing 6 × 10^9 predator cells were introduced in three of the four bottles, to generate three independent measures of resistance to predation. Two hundred microliters of HEPES buffer without the predator was added to the control population. Prey and predator concentrations were measured before and 240 min after the introduction of predators by diluting and plating a sample on KB (20 g proteose peptone 3, 15 g agar, 10 mL ethylene glycol, 1000 mL H2O quantity sufficient for 8.6 mL K2HPO4 [1M], 6.1 mL MgSO4 [1M] added after autoclaving) or DNB plates (0.8 g of nutrient broth, 1000 mL H2O, MgCl2 3 mM, CaCl2 2 mM, [15 g agar for plates—8 g for Top agar], pH=7.2; Jurkevitch et al. 2000). Prey concentration was assayed as colony forming units (CFU) on the plates after two days of incubation at 28°C, identifying the different morphs by their colony morphology. Predator concentration was counted as plaque forming units (PFU) developing on a lawn of ancestral prey cells (in DNB Top agar) on DNB plates after five days of incubation at
Table 1. Design of the long-term evolution experiment. (A) All populations were initiated with the ancestral SM morph "◦." (B) The prey’s morph composition after 20 dilution transfers. Boxed cells identify populations that were assayed extensively to study coevolutionary processes. The dilution regimes were all possible combinations of three dilution frequencies (once every 2, 3, or 4 days) and two dilution factors (100 or 1000 fold). See Methods or Gallet et al. (2007) for more details.

<table>
<thead>
<tr>
<th>Dilution intensity</th>
<th>period (days)</th>
<th>With predators (naive prey)</th>
<th>Without predators (naive prey)</th>
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(B) Final state (t=20)

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<th>With predators (exposed prey)</th>
<th>Without predators (naive prey)</th>
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○ Initial SM ● Final SM ▲ Final FS ◯ Final SM in control treatment.

28°C. Filtration (described above) eliminated any “evolved” prey from the sample.

We measured predation efficiency in terms of absorption of the prey by the predator, where absorption rate, \( a \), was calculated as

\[
a = \frac{\ln PfC(T) - \ln PfC(0) + \ln Pf(0) - \ln Pf(T)}{T \times Bb},
\]

where \( Pf(0) \) is the concentration of \( P. fluorescens \) before predation, \( Pf(T) \) is the concentration after predation, \( PfC(0) \) and \( PfC(T) \) are the concentrations of \( P. fluorescens \) in the control treatment (without predators) at the beginning and end of the assay respectively, \( Bb \) is the \( B. bacteriovorus \) (predator) concentration before predation (constant during the assay), and \( T \) is the duration of the assay (240 min).

**EVOLUTION OF TRADE-OFFS IN PREY**

**Assay 4**

To test for the evolution of trade-offs between resistance to predation and other life-history traits in the prey, we measured growth rate and resource-utilization efficiency of prey in the six populations identified in Table 1B, using samples taken every five transfers (\( t = 0, 5, 10, 15, \) and 20). Prey growth was monitored three times independently during 7 h at 28°C with agitation, via optical density (OD) measurements every 10 min with a microplate reader (Varioskan, Thermo). The obtained growth curves were then log-transformed to linearize the exponential growth phase. The slope \( y'(x) \) – the growth rate estimator – of this linear region was calculated at the inflexion point \( (x^*, y^* \text{ where } y'' = 0, \) of the fitted cubic regression \( y(x) = a + bx + cx^2 + dx^3 \) (Kibota and Lynch 1996). Prey resource-utilization efficiency (i.e., conversion of a given amount of nutrient into cells, or carrying capacity) was measured four or more times independently by measuring prey concentration after 24 h of cultivation in DNB at 28°C with agitation in the absence of predators.

**STATISTICAL ANALYSIS**

We used hierarchical linear mixed models, implemented with the lme function from the nlme library (R ver. 2.4; Ihaka and Gentleman 1996), to analyze the data described above. Population identity of predator and prey were treated as random factors. For each dataset we began with a complex model that included fixed effects and their interactions. Models were then simplified by sequential backward selection based on AIC (stepAIC function of MASS package). Significance of fixed effects and their interactions were tested by \( F \) marginal tests (Supporting Table S1) or by comparing nested models with a likelihood-ratio test (i.e., Chi square tests). We used the latter method (\( \chi^2 \) tests) to
make specific contrasts. Use of the variance function \textit{varIdent} (library \textit{nlme}) accommodated heterogeneity in variance among levels of a stratification variable (Pinheiro and Bates 2000). After model simplification, model parameters and their confidence intervals were estimated with restricted maximum-likelihood methods.

To analyze the data from assay 1 (predator efficiency, Fig. 1) we treated predator type (initial or final from FS or SM-fixed populations) and prey type (initial FS and SM, final FS and SM cultivated with predators and final SM cultivated without predators) as categorical fixed variable. We tested for local adaptation in assay 2 by modeling predator efficiency as a function of prey type (FS and SM), predator origin (final predators from FS fixed or SM fixed populations), and prey origin as categorical fixed factors (Fig. 2). We analyzed the dynamics of predator efficiency (assay 3) and of resource-utilization and exponential growth rate (assay 4) with similar linear mixed effect models. Type of predator (initial or final) and type of prey (FS or SM) were included as categorical covariates and time (number of transfers) as continuous variable. Depending on how predator efficiency changed with time, the response of this parameter could be linear or it could reach a plateau before the end of the experiment. Thus we included the number of transfers in the model both as a linear and as a quadratic term (Fig. 3). The sign and significance of the linear versus quadratic terms (number of transfers) were used to detect linear versus nonlinear evolution of predation efficiency through time.

\textbf{Results}

\textbf{Assay 1: Evolution of prey resistance and predator efficiency}

Comparing the resistance to predation of initial versus final prey (against initial predators) illustrated that the SM morph of \textit{P. fluorescens} evolved increased resistance to predation (as indicated by the solid arrows in Fig. 1; comparing ● vs. ○, $\chi^2_1 = 160, P < 0.0001$). This was also true for SM morphs grown in the absence of predators (○ vs. ○, Fig. 1A, $\chi^2_1 = 13.2, P < 0.001$), but SM prey that had been cultivated in the presence of predators were more resistant to predation than the control SM strains (● vs. ○, Fig. 1A; $\chi^2_1 > 22, P < 0.001$). Note that although our measure of resistance showed that the final SM was extremely resistant to predation, predators were still able to persist in these populations, suggesting that our measure of resistance may not have been sensitive enough to detect very low rates of predation. The FS morph also evolved improved resistance to predation compared to the SM initial type (● vs. ○, dashed path arrows Fig. 1A; $\chi^2_1 = 103.6, P < 0.0001$), although to a lesser degree than the final SM strain (▲ vs. ●, $\chi^2_1 = 9.3, P < 0.01$).
We found no differences between the three types of predators in their efficiency at consuming SM strains (global comparison of the blocks “○–●” between Fig. 1A, B, and C: $\chi^2 = 0.002$, $P = 0.99$): initial SM (○) were highly sensitive to predation by all three types of predators and final SM (●) were highly resistant to predation by all three types of predators (Fig. 1). Initial and final predators from SM-fixed populations did not differ in their efficiency at consuming FS prey (global comparison of the blocks “△–▲” between Fig. 1A and B: $\chi^2 < 2.5$, $P = 0.12$). In contrast, the predator’s response to FS prey depended on the type of prey with which it had been cultivated. Bdellovibrio bacteriovorus cultivated in FS-fixed populations became superior predators than either of the other two B. bacteriovorus cultivars on both the naive (△) and final (▲) FS prey (global comparison of the blocks “△–▲” from Fig. 1C with “△–▲” from Fig. 1A and B: $\chi^2 > 26$, $P < 0.0001$).

**Assay 2: Local adaptation**

If local adaptation by predators has occurred, then predators should be more efficient at attacking the prey with which they were cultivated (“sympatric” prey) than prey with which they were not cultivated (“allopatric” prey). When we compared predation efficiency of strictly sympatric populations to all other populations (Fig. 2B), we found a pattern of predator local adaptation to the FS prey morph (comparison of the two ▲, $\chi^2 = 4.5$, $P = 0.03$) but not to the SM prey morph (comparison of the two ●, $\chi^2 = 0.002$, $P = 0.99$).
χ²₁ = 0.59,  P = 0.44). When sympatry was defined as including any predator–prey pairing cultivated with the same prey morph rather than strict coculturing, we found a similar pattern: predators that were cultivated on FS prey were more efficient on FS prey than those cultivated on SM prey (Fig. 2C, ▲χ²₁ = 12.0,  P < 0.001) whereas on SM prey, no difference was found between these two types of predators (Fig. 2C, ●χ²₁ = 0.42,  P = 0.51). However, we found no evidence of local adaptation when we compared strictly sympatric pairs with other predator–prey pairs within each morph (Fig. 2D; χ²₁ = 2.0,  P = 0.15).

**Assay 3: Evolution over time**

The assay of prey resistance and predation efficiency using samples from throughout the evolution experiment showed a progressive and nonlinear decline in predator efficiency in the SM-fixed populations, indicating increased resistance to predation by the SM morph (Fig. 3B). We found no effect of predator type on the mean or slope of predator efficiency (χ²₁ < 2.3,  P > 0.13; see full model for SM prey, Supporting Table S1). In contrast, predator efficiency increased in populations in which FS went to fixation; that is, the final predator line is significantly elevated above the initial predator line in Figure 3A (mean comparison between initial and final predators: χ²₁ = 7.1,  P = 0.007). At the same time, the FS prey tracked the predators’ adaptation. FS prey became more resistant to predation by the final predator (negative slope, χ²₁ = 9.5,  P = 0.002; Fig. 3A and Supporting Table S1, simplified model for FS prey), while becoming increasingly susceptible to the initial predator (positive slope, χ²₁ = 7.5,  P = 0.006).

**Assay 4: Other prey life-history traits**

Assays of prey growth rate and resource-utilization efficiency (carrying capacity) throughout the course of the evolution experiment revealed possible trade-offs with resistance to predation in the FS morph but not in the SM morph. These assays showed that initially (t = 0), higher resistance to predation by the FS morph compared to the SM morph (cf. △ vs. ○ of Fig. 1A) is associated with lower resource-utilization efficiency (Δ vs. ○, Fig. 3C, χ²₁ = 10.9,  P < 0.0001) (as in Gallet et al. 2007). Resource-utilization efficiency of the FS morph initially decreased during the experiment (Δ to ▲, Fig. 3C, χ²₁ = 9.9,  P = 0.017) followed by stabilization for the rest of the long-term evolution experiment (χ²₁ = 9.8,  P = 0.017). The growth rate of FS initially increased (Δ to ▲, Fig. 3D, χ²₁ = 4.8,  P = 0.028), followed also by
stabilization ($\chi^2_1 = 5.8, P = 0.015$). In contrast, increased resistance of the SM morph was not associated with a cost with respect to resource-utilization efficiency or growth rate (○ to ●, Fig. 3C, D). Instead, resource-utilization efficiency of the SM morph increased slightly ($+32\%$ from $t = 0$ to $t = 20$, $\chi^2 = 16, P < 0.0001$), as did its growth rate ($\chi^2 = 17.1, P < 0.0001$).

**Discussion**

**ANTAGONISTIC COEVOLUTION**

By the end of the coevolution experiment, all of the prey had evolved greater resistance to predation, but they did so following two distinct trajectories. The FS morph showed constitutive resistance to predation by *B. bacteriovorus* (Figs. 1 and 3A), whereas the ancestral SM morph was initially sensitive to predation but became progressively more and more resistant (Fig. 3B). This acquired resistance was apparently partly due to an effect of cultivation (because SM cultivated without the predator also became more resistant to predation) and partly to adaptation to the predator (because final SM cultivated in presence of predators (●) were more resistant to predation than control SM (○), Fig. 1). Evolution of increased predation efficiency, a counter adaptation to prey resistance, occurred only in populations in which the FS morph went to fixation. All of our assays and in particular the local adaptation assay demonstrated that these evolved predators were not generalists capable of attacking any kind of prey with equal efficiency; rather, they were specialized to the FS morph (Figs. 1–3). Contrasting “sympatric” versus “allopatric” predator–prey pairs in three different ways allowed us to understand that local adaptation by predators that had been cultivated with the FS morph was actually due to their higher efficiency on any final FS prey (specialization), rather than the particular culture with which they had been cultivated (local adaptation). That is, our third definition of “sympatry” versus “allopatry” failed to demonstrate local adaptation within each morph (Fig. 2D). Further, increased resistance of the FS morph to the final predator came at the cost of decreased resistance to the initial predator, and conversely, ancestral FS were more resistant to initial predators than to final predators (Fig. 3A). This constitutes a clear pattern of antagonistic coevolution between the predator and prey. Predators from populations in which the SM morph went to fixation did not evolve increased predation efficiency. Thus antagonistic coevolution was restricted to the FS morph.

**IMPACT OF DISTURBANCE ON COEVOLUTIONARY INTERACTIONS**

Elsewhere we reported that the presence of predation induces an adaptive radiation of prey (emergence and co-occurrence of SM, FS, and WS types; Gallet et al. 2007). This suggests that the FS and WS morphs have a selective advantage in the presence of predators compared to the SM morph. The FS morph is constitutively resistant to predation by *B. bacteriovorus* (Gallet et al. 2007), and the WS morph may find refuge thanks to its tendency to grow on the walls of the container. But, by the end of the experiment, the WS morph had gone to extinction and most cultures contained only the FS or only the SM type, depending on the disturbance treatment (Table 1B).

Based on both population dynamics (described above; Gallet et al. 2007) and trait dynamics (reported here), we infer that dilution regime, which mimics ecological disturbance, played a role in shaping the evolutionary trajectories of both predator and prey. When disturbance was relatively infrequent, the SM morph was not always excluded by FS (Table 1). With a low dilution rate (every four days), the SM morph’s greater efficiency at converting resources into cells (carrying capacity) allowed it to reach a density high enough to persist during the transfer. The longer the SM morph was able to persist, the greater were its chances of acquiring adaptive mutations. When disturbance frequency was higher (two or three days between two transfers), the FS morph generally outcompeted the SM morph (Table 1), precluding the evolution of SM toward super-resistance. Disturbance itself may not have directly affected predator and prey adaptation; rather, it may have influenced evolutionary trajectories by determining which prey morph the predator faced. Thus, the different disturbance regimes created different ecological conditions in which the SM morph did or did not persist, which led to alternative evolutionary trajectories between prey and predators. These results suggest that ecological conditions can influence which alternative coevolutionary outcome is realized.

**COST OF ADAPTATION IN PREY**

The evolution of predator efficiency and prey resistance is expected to be constrained or shaped by trade-offs with other traits, although these costs can sometimes be reduced or even suppressed by compensatory mutations within a few generations (Schrag and Perrot 1996). Our survey of prey resource-utilization efficiency and growth rate showed a trade-off between resistance and resource-utilization efficiency in the FS morph (Fig. 3). We found that this trade-off was not compensated even after hundreds of generations: after a drastic decline from $t = 0$ to $t = 5$, resource-utilization efficiency remained low from $t = 5$ to $t = 20$ (Fig. 3C). This kind of indirect cost of adaptation might explain why we did not observe the selection of super-resistant FS strains. Increased resistance to predation in the FS morph was temporally specific (Fig. 3A), and two hypotheses might explain this temporal adaptation of the FS morph to its current predator (Fig. 3A): (1) the direct cost of adaptation to predation is such that the FS morph is unable to resist predators from all time points, or (2) the existence of prey and predator population dynamics like Red Queen interactions. Given that genetic diversity is very low in bacterial...
populations experiencing serial dilutions, the second explanation is less plausible.

In the SM morph, the evolution of extreme resistance to predation is expected to be costly. But we found no cost either in terms of resource utilization (Fig. 3C) or in terms of growth rate (Fig. 3D) for the SM morph. In fact both of these traits increased at the same time that resistance to predation increased (predator efficiency declined). A positive correlation between these traits might explain why we observed increased resistance to predation by the naive SM morph in our control treatment (Fig. 1A, C). Indeed, adaptation to culture conditions often selects for a higher growth rate (Vasi et al. 1994) or higher carrying capacity. It may be that increased resistance in the control treatment is a secondary consequence of selection on growth rate and carrying capacity. Failing to observe a trade-off between traits is not surprising: trade-offs are usually more likely to be observed under stressful conditions (McKean et al. 2008). Resistance could also be negatively correlated with other untested traits. However, here we observed—starting with one ancestral strain—the evolution of two different morphs arising in different disturbance treatments, one showing no apparent trade-off, and the other a trade-off that was not compensated during the course of the experiment.

Conclusion
From one single ancestral strain, we observed two distinct evolutionary outcomes depending on the experimental ecological treatments. When disturbances were frequent or moderate, the FS morph usually went to fixation. When disturbances were relatively infrequent and not intense, it was possible for the SM morph to go to fixation. Antagonistic coevolution with specialization between predator and prey was observed where the FS morph went to fixation and not where the SM morph went to fixation. The predator failed to counter-adapt to the form of the SM morph that emerged. Only where the disturbance regime favored the SM morph did it persist long enough to evolve extreme resistance to predation. This suggests that there may be several alternative evolutionary or genetic pathways that a prey or host may follow to develop resistance to predation or parasitism. These alternative pathways seem to be favored by different disturbance regimes. In other words, in our experiment, disturbance seems to have driven predator–prey populations toward different features on their evolutionary landscape, with either the evolution of prey resistance (SM) or an antagonistic predator–prey coevolution (FS). This suggests that microorganisms evolve on much more complex and changing adaptive landscapes than previously thought, warranting the exploration of more than one culturing regime (in contrast to most experimental evolution designs).

Our results also provide information about the adaptive capacities of B. bacteriovorus. Thus far, this predatory bacterium was known as a generalist preying on various Gram—bacteria strain. Ours is the first report of specialization to a specific prey by B. bacteriovorus; further, we showed that the predation efficiency of B. bacteriovorus increased only on certain prey morphs. This result might be viewed as surprising and intriguing, given B. bacteriovorus’s profile as a generalist. Our results also contradict the notion that resistance to B. bacteriovorus is rare. Unfortunately, nothing is known about the mechanistic details of this prey–predator system, so we do not know if resistance to predation by B. bacteriovorus in P. fluorescens is the result of a reduced ability to enter the periplasm of the prey or less accessible cytoplasm, among other possibilities. A better understanding of the mechanisms involved in the predator–prey interaction between B. bacteriovorus and P. fluorescens might allow us to identify the cause of high resistance to predation by the SM morph and the constraints acting on resistance to predation in the FS morph.

This should also inspire caution about the use of B. bacteriovorus as an environmental prophylactic or as an alternative to antibiotic therapy (Sockeyt and Lambert 2004). Many diseases have evolved resistance to antibiotics (e.g., tuberculosis; Abdel Aziz and Wright 2005). “Living antibiotics” must also be considered carefully regarding the way their efficiency might be shaped by evolution. Successful long-term control of diseases, pests, and invasive species will require a better understanding of coevolutionary dynamics in an ecological context.

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Supporting Information

The following supporting information is available for this article:

**Table S1:** Results from some linear mixed models of the predator efficiency on FS (Fig. 3A) or SM (Fig. 3B) prey.

Supporting Information may be found in the online version of this article.
(This link will take you to the article abstract).

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