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Reviewed work(s):
Source: The American Naturalist, Vol. 177, No. 3 (March 2011), pp. 334-345
Published by: The University of Chicago Press for The American Society of Naturalists
Stable URL: http://www.jstor.org/stable/10.1086/658364
Accessed: 22/02/2012 14:46

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Pulsed-Resource Dynamics Constrain the Evolution of Predator-Prey Interactions

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Submitted June 9, 2010; Accepted November 15, 2010; Electronically published January 28, 2011

ABSTRACT: Although temporal variability in the physical environment plays a major role in population fluctuations, little is known about how it drives the ecological and evolutionary dynamics of species interactions. We studied experimentally how extrinsic resource pulses affect evolutionary and ecological dynamics between the prey bacterium Serratia marcescens and the predatory protozoan Tetrahymena thermophila. Predation increased the frequency of defensive, nonpigmented prey types, which bore competitive costs in terms of reduced maximum growth rate, most in a constant-resource environment. Furthermore, the predator densities of the pulsed-resource environment regularly fluctuated above and below the mean predator densities of the constant environment. These results suggest that selection favored fast-growing competitor prey types over defensive but slower-growing prey types more often in the pulsed-resource environment (abundance of resources and low predation risk). As a result, the selection for prey defense fluctuated more in the pulsed-resource environment, leading to a weaker mean response in prey defense. At the ecological level, the evolution of prey defense weakened the relative strength of top-down regulation on prey community. This was more evident in the constant-resource environment, whereas the slow emergence of defensive prey types gradually decreased the amplitude of predator peaks in the pulsed-resource environment. Our study suggests that rapid evolution plays a smaller role in the ecological dynamics of communities dominated by resource pulses.

Keywords: antagonism, ecology, productivity, resource competition, temporal variation, trade-off.

Introduction

It has long been recognized that temporal variability in the physical environment is one of the major drivers of population fluctuations (Chesson 2003). For example, externally driven fluctuations in basal resources have been shown to govern the ecological dynamics of many terrestrial and aquatic food webs (Ostfeld and Keesing 2000; Polis et al. 2004; Yang et al. 2008). One interesting form of extrinsically driven resource variability is the pulsed-resource fluctuations commonly found in both terrestrial and aquatic ecosystems (Rosenzweig 1995; Ostfeld and Keesing 2000; Yang et al. 2008). The influence of resource pulses on ecological processes at the level of individuals, populations, and communities has been investigated in many studies (reviewed in Yang et al. 2008). However, only a few studies have investigated how the ecological transients created by resource pulses influence the evolution of species interactions (Holt 2008; Yang et al. 2008). In this study, we explored how extrinsic resource pulses influence the ecological and evolutionary dynamics of predator-prey interaction in a model of an aquatic microbial food chain consisting of nonliving resources, a consumer bacterium, and its protozoan predator.

Theory predicts that the effect of extrinsic resource pulses on the fluctuations in prey and predator numbers depends on the frequency, magnitude, and duration of the pulse (Holt 2008; Yang et al. 2008). The magnitude of the resource pulse could be an especially important factor because it is expected to strongly increase the amplitude of population fluctuations (Holt 2008). Assuming that the predator-prey system displays dampening dynamics (e.g., because of resource depletion), the ecological events after the pulse could be roughly divided into three phases. The resource pulse is first likely to numerically favor the prey species if it has a shorter generation time than its predators and if the predator satiates (e.g., has a type 2 functional response; Abrams 2000). Second, after the initial increase in prey density and consequent depletion of resources, the predator is likely to respond numerically, creating high predation risk conditions for the prey (Abrams 2000; Holt 2008). If the frequency between resource pulses is long and the prey or predator does not respond evolutionarily, the population densities of both species will likely decline at the end of the third phase, when the resources are depleted (Holt 2008).
The resource pulse–driven ecological transients in prey and predator population densities could also influence the evolutionary dynamics of the predator–prey interaction. For example, selection for prey defensive and competitive (e.g., growth) traits could fluctuate depending on predation risk (Levins 1968; Hairston and Dillon 1990; Yoshida et al. 2003, 2007) and resource availability (Bohannan and Lenski 1999, 2000; Friman et al. 2008; Hall et al. 2008). The first phase of the resource-pulse transient is likely to select for “competitor” prey types that grow fast under conditions of resource abundance (Holt 2008). However, the resulting increase in predation risk is likely to tip the selection in favor of defensive prey types that can escape predation (Yoshida et al. 2003; Meyer et al. 2006; Friman et al. 2008). If the predator is not able to evolve to better capture its prey, the increase in the frequency of defensive prey types will lead to a subsequent decrease in predator numbers (Abrams 2000). This resulting decrease in predation risk could subsequently strengthen the relative importance of resource competition, especially if defensive prey types bear a competitive cost in terms of reduced ability to use resources (Bohannan et al. 2002; Yoshida et al. 2003, 2004; Meyer et al. 2006; Friman et al. 2008). Consequently, resource pulses could favor the fast-growing competitor prey types over the more defensive but slower-growing prey types during both the beginning (abundance of resources and low predation risk) and ending (low predation risk) phases of the resource pulse. As a result, the selection for prey defense could be strong only when the predation risk is high (Leibold 1996; Yoshida et al. 2003, 2004; Meyer et al. 2006)—that is, in the middle phase of the pulse. Compared with the constant-resource environment, the resource pulse–mediated alternating selective conditions could decrease the overall strength of selection for prey defensive traits, even though these conditions could allow the fluctuating coexistence of different prey types (Yoshida et al. 2004, 2007). Evolution of defensive prey genotypes could also select for more efficient predators, leading to the coevolutionary dynamics often observed between parasites and their hosts (Buckling and Rainey 2002). In contrast to parasites, however, it is less likely that predators could coevolve because they usually have relatively smaller population sizes and longer generation times than do prey (Yoshida et al. 2003; Meyer and Kassen 2007; Friman et al. 2008; Hall et al. 2008).

From the perspective of trophic dynamics, the weak defensive response of prey could result in a situation where the resource pulses more effectively increase the biomass of the predators (Oksanen et al. 1981; Kaunzinger and Morin 1998). In contrast, a strong defensive response could lead to a bottom-up-regulated community, where the resources turn more effectively into a biomass of the defensive prey (Bohannan and Lenski 1999; Friman et al. 2008). While effort has been made recently to develop models that consider the community- and population-level responses to environmental variability (Chesson 2003; Holt 2008), there are still few experimental studies that have also included the effect of evolutionary dynamics with these hypotheses.

Here we report the results of a 42-day-long microcosm experiment in which an initially genetically homogeneous strain of bacterium, Serratia marcescens, either was left to evolve alone or was exposed to a ciliate predator, Tetrahymena thermophila, for approximately 1,000 bacterial generations under both constant- and pulsed-resource environments. In the constant-resource environment, prey resource availability was held close to steady by means of daily small-magnitude renewals (12% of microcosm volume). In the pulsed-resource environment, the daily small-magnitude renewal (6% of microcosm volume) was contrasted with short, high-magnitude weekly resource renewal (24% of microcosm volume). The average resource renewal rate was equal in both resource environments. The microcosms were sampled daily for prey and predator population sizes, and the evolutionary changes in prey defensive and competitive (growth-related) traits were measured weekly in separate short-term experiments. Because it is also possible that predators evolve in response to changes in prey (Abrams 2000), the coevolutionary changes in prey defense and predator counteradaptations were measured at the end of the experiment as the predator’s ability to feed on different prey-selection lines. To monitor changes in the adaptive radiation of prey, changes in bacterial prodigiosin (red pigment) expression were scored during the experiment. The T. thermophila growth on red and white S. marcescens colony mixes were measured at the end of the experiment to determine whether prodigiosin was related to defense. According to previous experiments, predation by T. thermophila selects for S. marcescens bacteria that are able to form predator-resistant biofilm (V.-P. Friman, L. Mikonranta, and J. Laakso, unpublished data). To investigate the evolution of this bacterial defense mechanism in more detail, half of the microcosm replicates were replaced with new culture vessels every week, whereas the same culture vessels were used for the whole duration of the experiment for the other half of replicates. The amount of biofilm formed in discarded microcosms was measured every week. The evolutionary changes in bacterial biofilm-formation ability were also measured weekly in separate short-term experiments.

We hypothesized that increasing the magnitude of resource pulses would create a fluctuating selection that weakens the overall evolutionary increase in prey defense. This hypothesis was based on theoretical (Holt 2008) and experimental (Meyer et al. 2006; Yoshida et al. 2007) work.
that suggests that (1) basal resource pulses will initially favor the fast-growing (competitor) prey types; (2) the resulting increase in predator densities will dampen when the frequency of defending prey types increases; and (3) the trade-off between prey defense and the growth rate will again favor the fast-growing competitor prey types at the ending phase of the resource pulse, when predation risk is low. The resulting differences in evolutionary dynamics could further lead to different ecological dynamics in prey diversity and population variability between the constant- and pulsed-resource environment.

Methods

Microcosm Experiment

_Serratia marcescens_ is a cosmopolitan, heterotrophic bacterium found in aquatic and soil environments (Grimont and Grimont 1978). The predator _Tetrahymena thermophila_ is a well-studied, free-swimming, particle-feeding protozoan that preys on numerous bacteria (Elliott 1974). The experiment was conducted in 50-mL plastic centrifuge tubes (VWR) with loosened screw caps to maintain aerobic conditions (hereafter referred to as “culture vessels”). At the beginning of the experiment, a single clone of prey bacterium _S. marcescens_ (ATCC strain 13880) capable of producing the red pigment prodigiosin was grown to late log phase in sterile prey culture medium (pH 7.5) containing plant detritus at a final concentration of 2.15 mg L\(^{-1}\) (Friman et al. 2008) in a batch-culture setting of approximately 3.9 \(\times\) 10\(^7\) cells mL\(^{-1}\). Sixteen prey replicates were established in both constant- and pulsed-resource environments as follows: 24 mL of prey culture medium containing _S. marcescens_ was measured into the microcosms, and thereafter 1 mL of an asexually reproducing strain of the protozoan predator _T. thermophila_ (ATCC strain 30008) was introduced to half of the prey replicates in both resource environments (1 mL containing approximately 56,800 predator individuals). The predator culture was centrifuged, washed with buffer (Friman et al. 2008), and thereafter starved for 24 h before insertion. The other half of the microcosms (containing only _S. marcescens_) was retained for control treatments, and 1 mL of fresh prey culture medium was added instead of predators to ensure an equal total volume (25 mL) in both cultures. _Serratia marcescens_ and _T. thermophila_ occupied separate trophic levels in our experimental community because _T. thermophila_ cannot feed directly on prey culture medium. Control predators were also grown under otherwise similar experimental conditions, except that they were grown axenically on nonliving predator culture medium: four replicates in both constant- and pulsed-resource environments containing 5 g of Bacto proteose peptone and 1.25 g of Bacto yeast extract (Becton, Dickinson) per 1 L of dH\(_2\)O, respectively. These control predators were used at the end of the experiment to estimate the coevolutionary changes in prey defense and predator counterdefenses as the growth of different predator treatments on different prey treatments. In addition, half of the microcosm replicates were replaced with new culture vessels every week, whereas the same culture vessels were used for the whole duration of the experiment for the other half of the replicates. Thus, the main experiment consisted of a total of 10 different treatments where the prey and the predator evolved alone or together in the constant- or pulsed-resource environment. Moreover, in the replicates that included bacteria, the culture vessels were either retained or replaced with a new one weekly. Each treatment combination comprised four replicates.

In the constant-resource environment, the prey resource renewal was 3 mL day\(^{-1}\) (12%) throughout the experiment, whereas in the pulsed-resource environment it was periodic, alternating between 6 days of 1.5 mL renewal day\(^{-1}\) (6%) and 1 day of 12 mL renewal day\(^{-1}\) (24%). Thus, the average resource renewal was equal in both environments for the entire duration of the experiment (21 mL week\(^{-1}\)). Resource renewal was generated manually with a pipette by replacing part of the experimental population with fresh resources. The time scale of variation in the pulsed-resource environment, equating approximately 168 prey and 11 predator generations, was chosen on the basis of our previous experiments showing that protozoan predation led to evolutionary increases in prey defense (Friman et al. 2008). The contents of the microcosms were mixed gently before sampling and addition of new resources. The total volume of the microcosms was kept at 25 mL for the entire duration of the experiment. To prevent bacterial contamination, all microcosms were kept in a laminar flow chamber. Microcosms were incubated at 25°C and sampled daily during the experiment.

Prey population biomasses were estimated by measuring optical density (OD) with a spectrophotometer (Bioscreen C, Oy Growth Curves; wideband option at 420–580 nm). Population sizes and biomasses of protozoa were determined using image analysis (for details see Laakso et al. 2003). The temporal stability of population sizes was estimated from the time-series data as the coefficient of variation (SD mean\(^{-1}\)) of each microcosm (i.e., the higher the coefficient of variation, the lower the stability).

Measuring the Evolutionary Changes in Prey Defense, Competitive Ability, and Biofilm Formation

Evolutionary changes in prey traits were measured five times during the main experiment in separate factorial short-term experiments. Before the measurements, prey
clones were separated from predators by plating on agar plates containing 2.5 g of yeast extract, 10 g of nutrient broth, and 15 g of agar in 1 L of dH2O. After 48 h of incubation at 25°C, 20 clones per selection line were selected at random and mixed together in two 50-mL centrifuge tubes (VWR) containing 20 mL of fresh prey culture medium. Antibiotic treatment, tested to be harmless to the predator, was used to separate predators from the prey bacteria (10,000 units of penicillin and 10 mg of streptomycin in 1 mL of 0.9% NaCl; Sigma-Aldrich). After 24 h of exposure to the antibiotics at 25°C, a small inoculum of predators was transferred to predator culture medium to dilute the antibiotics. When measuring the prey defense against predation, the amount of antibiotic transferred to the bacterial culture with the predator inoculum was negligible (final concentration of 0.43 units of penicillin mL⁻¹ and 0.44 µg of streptomycin mL⁻¹) and did not reduce bacterial growth. All prey and predator selection lines from the main experiment were grown separately for 72 h before assessing evolutionary changes; 72 h equaled at least 10 prey and predator generations before the trait measurements. During this time, the possible treatment-induced differences in the physiological state of study organisms is likely to reset, and the observed differences can be considered to be caused by genetic factors. However, it is possible that the predator-free 72-h growth period could select for less defensive prey genotypes if these clones bear competitive fitness disadvantages in the absence of predators. However, that we observed evolutionary differences between different treatments proves that selection during this growth period was not strong enough to eradicate genetic differences driven by experimental treatments used in the long-term microcosm experiment (see “Results”).

Evolutionary changes in prey defense were measured in separate factorial experiments as the prey bacterium’s ability to sustain population size in the presence of predators. This measure takes into account the overall defense of prey and does not differentiate between different defense mechanisms. Before the prey defense measurements, prey selection lines were grown to high densities in prey culture medium. During this time, the prey consumed most of the available resources in the batch culture, and the subsequent addition of approximately 100 predator individuals resulted in reduction in prey densities only. The population size prey that could be sustained in the presence of predators was measured in terms of the OD at 420–580 nm for 4 days at 25°C.

Evolutionary changes in prey competitive ability were assessed as the maximum growth rates and population sizes of different prey selection lines in the absence of predators (biomass growth data recorded for 96 h at 5-min intervals, OD at 420–580 nm). Prey maximum growth rate at low density and long-term maximum population size indicate how well prey respond to the addition of fresh resources and how efficiently resources are used to produce biomass in the long term, respectively. The prey maximum growth rate presumably best reflects the prey competitive ability in our experimental setting (i.e., prey growth response to the addition of fresh resources). However, a maximum growth rate could also correlate with maximum population size (Friman et al. 2008) and be indicative of prey competitive ability, especially under low-resource conditions.

Prey biofilm-formation ability was measured in the absence and in the presence of predators by a method modified from O’Toole and Kolter (1998). After 4 days of bacterial growth, 100 µL of 1% crystal violet solution (Sigma-Aldrich) was added to each well of a microtiter plate (Honeycomb 2, Thermo Electron Oy). After 10 min, all wells were rinsed with distilled water three times followed by the addition of 450 µL of 96% ethanol to all wells to dissolve the crystal violet–stained bacteria from the walls of the microtiter plate. The amount of biofilm was estimated by measuring the OD of the crystal violet–ethanol solution for 24 h at 420–580 nm.

Measuring the Coevolutionary Changes of Predator Resource Use Ability on Bacterial and Nonliving Resources

To determine whether predators adapted for more efficient use of nonliving or bacterial resources, the maximum growth rates and population sizes of all predator selection lines were measured at the end of the experiment (week 6). The measurements were made in separate short-term experiments where predators that had previously evolved in the presence or absence of prey were grown under control conditions or with prey-evolved prey bacteria that had evolved in the constant- or pulsed-resource environment. Predator growth was also measured on nonliving predator culture medium. Before measuring predator growth on bacterial resources, all prey replicate populations were grown to similar high densities for a total of 72 h from isolation to beginning of measurements after approximately 100 predator individuals were introduced to the cultures.

Role of Prodigiosin in Bacterial Defense against Protozoan Predation

The S. marcescens bacterial strain ATCC 13880 normally produces the red pigment prodigiosin on agar plates at room temperature, but exposure to protozoan predation has been shown to select for nonpigmented colony types (Friman et al. 2008). To determine whether the loss of
pigment production is directly linked to prey defense, we measured predator growth on the red and white *S. marcescens* colonies (random mixture of 20 clones per colony color per replicate) at the end of experiment, using the methods described above.

**Measuring the Amount of Biofilm Formed in the Microcosms**

Half of the culture vessels (microcosms) were replaced with new ones every week. The amount of biofilm in the discarded microcosms was measured as described above with several minor modifications. Because the discarded microcosms were empty, they were first filled with 30 mL of distilled water before 1.2 mL of 1% crystal violet solution (Sigma-Aldrich) was added to the microcosms. After 24 h, the microcosms were rinsed with distilled water, 96% ethanol was used to dissolve the crystal violet–stained bacteria, and the amount of biofilm produced was measured as the OD of crystal violet–ethanol solution for 24 h at 420–580 nm.

**Results**

**Population Dynamics**

Predation decreased prey population sizes ($F_{1,24} = 1.938$, $P < .001$; fig. 1). Resource pulses and culture vessel replacement decreased prey population sizes in the absence of predation (resource pulses: $F_{1,24} = 100.3$, $P < .001$; culture vessel replacement: $F_{1,24} = 47.9$, $P < .001$; fig. 1A). However, culture vessel replacement had no effect on prey population sizes in the presence of predators (predation × culture vessel replacement: $F_{1,24} = 100.3$, $P < .001$; fig. 1). In addition, resource pulses did not decrease the prey population sizes as clearly in the presence of predators (predation × resource environment: $F_{1,24} = 7.1$, $P = .014$; fig. 1).

The predator-to-prey ratio was higher in the pulsed-resource environment ($F_{1,12} = 5.5$, $P < .037$; figs. 1A, 2B). Resource pulses increased the variability of predator populations that were coevolving with bacteria ($F_{1,12} = 6.3$, $P = .027$; coefficient of variation of 0.47 ± 0.02 and 0.53 ± 0.01 in constant- and pulsed-resource environments, respectively). Resource pulses induced clear week-long predator cycles (fig. 2B), with decreasing amplitude toward the end of the experiment (fig. 2B). Culture vessel replacement had no effect on the predator population sizes ($F_{1,12} = 0.3$, $P = .576$). Resource pulses had different ef-
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Figure 2: Predator population sizes (biomass) on (A) nonliving and (B) bacterial resources in constant-resource (open symbols) and pulsed-resource (filled symbols) environments. The diamonds in B depict culture vessel replacement in constant- and pulsed-resource environments. Vertical lines show means ± 1 SEM. Note the different scales on the Y-axis.

Effects on predator population sizes depending on the resource type used (resource type × resource environment: $F_{1,43} = 43.9, P < .001$); resource pulses decreased the mean predator population sizes when cultured on nonliving resources ($F_{1,6} = 18.8, P = .005$; fig. 2A) but increased predator population sizes when cultured on bacterial resources ($F_{1,14} = 4.6, P = .049$; fig. 2B).

**Evolution of Prey Defense, Resource Use Ability, and Biofilm Formation**

Predators increased prey defense in general ($F_{1,43} = 7.4, P = .01$; fig. 3A). According to the weekly measurements, prey defense tended to increase more when prey coevolved with predators in the constant environment (predation × resource environment: $F_{1,43} = 2.9, P = .095$).

Increasing prey allocation to defense was costly in terms of reduced prey maximum growth rate in the absence of predators ($F_{1,24} = 21.9, P < .001$; fig. 3B). Moreover, predation tended to decrease prey maximum growth rate more in the constant-resource environment (predation × resource environment: $F_{1,24} = 3.5, P < .074$). Predation, culture vessel replacement, or resource environment had no effects on prey maximum population sizes ($P > .05$ for all).

Predation, culture vessel replacement, and resource environment did not affect the prey bacterium’s ability to form biofilm in the absence of predation ($P > .05$ for all). However, previous exposure to predation increased the prey bacterium’s ability to form biofilm in the presence of predators ($F_{1,80} = 17.14, P < .001$; 0.123 ± 0.01 and 0.135 ± 0.01 OD at 600 nm for alone and coevolved prey, respectively). This effect was clearer when the prey had coevolved with predators in the constant-resource (0.139 ± 0.01 OD at 600 nm) than in the pulsed-resource (0.131 ± 0.01) environment (predation × resource environment: $F_{1,40} = 5.0, P = .03$).

**Formation of Biofilm in the Microcosms during the Main Experiment**

Both predation and weekly replacement of the culture vessel decreased the amount of biofilm prey formed in the microcosms (predation: $F_{1,24} = 1.355, P < .001$; culture vessel replacement: $F_{1,24} = 309, P < .001$; data not shown). Predation also decreased the amount of biofilm prey formed in the microcosms during treatment when the microcosms were replaced with new ones every week ($F_{1,12} = 7.491, P < .001$; data not shown). Moreover, this effect was clearer in the pulsed-resource environment (predation × resource environment: $F_{1,12} = 9.5, P = .009$; data not shown).

**Evolution of Predator Growth on Bacterial and Nonliving Resources**

We did not find evidence for predator evolution; predators grew equally well on bacterial or nonliving resources regardless of the evolutionary history of predators during the main experiment ($P > .05$ for all). However, predators had smaller maximum population sizes ($F_{1,61} = 100.7, P < .001$) and lower growth rates ($F_{1,61} = 86.1, P < .001$) when grown on bacterial selection lines that had evolved in the presence of predators (fig. 4). Moreover, this effect
was especially clear in the constant-resource environment (predation × resource environment: $F_{1,61} = 62.8$ and $P < .001$ for maximum population size, $F_{1,61} = 27.1$ and $P < .001$ for maximum growth rate; fig. 4A, 4B).

**Prey Diversification into Red and White Colony Morphs**

Predation caused rapid diversification of the ancestral prey genotype by increasing the frequency of small and white colonies, which were incapable of expressing the red pigment prodigiosin ($F_{2,4} = 27.3$, $P < .001$). The frequency of white colonies especially increased in the constant-resource environment (predation × resource environment: $F_{1,61} = 16.4$, $P < .001$; fig. 5A). According to separate short-term measurements, the white clones yielded considerably lower maximum predator population sizes ($F_{1,61} = 61.7$, $P < .001$) and maximum growth rates ($F_{1,61} = 52.5$, $P < .001$) than did the red clones (a randomly selected clone mix of white and red clones previously exposed to predation in the constant environment; fig. 5B). Evolutionary history, such as food type or resource environment, did not affect the predators’ ability to consume white or red prey clones ($P > .05$ for both clones).

**Discussion**

Our results suggest that pulsed-resource dynamics can constrain the evolution of predator-prey interactions. According to population dynamics data, the prey bacteria population sizes were clearly larger in the absence of predators across all treatments (fig. 1), suggesting that predators were effective in consuming prey. Interestingly, the population dynamics of coevolved prey varied more (measured as the coefficient of variation) and were clearly cyclic in the pulsed-resource environment (fig. 2B). Basal resources must flow through the prey to reach the predator trophic level in our experimental setting, suggesting that the weekly high-magnitude resource renewals first turned into a biomass of fast-growing competitive prey types, which were subsequently consumed by the predators (fig. 2B). However, these high predator density peaks were very short lived, and the predator numbers consistently fell below the average densities observed in the constant-resource environment (fig. 2). These population dynamics data thus suggest that selection for prey defense fluctuated more in the pulsed-resource environment, which could have caused only a small net increase in prey defense in the long term.

According to the weekly prey defense measurements, predation generally increased prey investment in defense regardless of resource environment (fig. 3A). However, the measurements performed at the end of the long-term microcosm experiment showed that prey defense evolved more strongly in the constant-resource environment because predators reached smaller population sizes when grown with prey that had coevolved with predators in the constant-resource environment compared with the pulsed-resource environment (fig. 4). No evidence of predator evolution was found (see also Friman et al. 2008), suggesting that coevolutionary dynamics cannot explain the difference in the mean strength of prey defense between the constant- and pulsed-resource environments. The prey
Figure 4: Prey defense measured as predator maximum population size and population growth rate on prey selection lines that had evolved in the absence (open symbols, open bars) or presence (filled symbols, filled bars) of predators in (A, B) constant-resource and (C, D) pulsed-resource environments. A and C show the means of predator growth dynamics; B and D show the calculated values for predator maximum growth rates (two uppermost bars) and maximum population sizes (two lowermost bars). The vertical and horizontal lines show treatment means ± 1 SEM.
Figure 5: A, Predator-induced increase in frequencies of small white *Serratia marcescens* colonies in the constant-resource (solid lines) and pulsed-resource (dotted lines) environments. B, C, Prey defense measured as predator maximum population size and population growth rate on white (open squares, open bars) and red (filled squares, filled bars) clone mixes isolated from the constant-resource environment. The means of predator growth dynamics are shown in B, and the calculated values for predator maximum growth rates (two uppermost bars) and maximum population sizes (two lowermost bars) are shown in C. The vertical and horizontal lines show treatment means ± 1 SEM.
defense was costly in terms of decreased prey maximum growth rate (fig. 3B). In addition, this cost tended to be greater for prey that had coevolved with predators in the constant-resource environment compared with the pulsed-resource environment. We found no differences in prey maximum population sizes, presumably because the resource renewal cycle was not long enough to cause selection for prey types that are good at growing in resource-limited conditions. Taken together, our results demonstrate that the evolution of prey defense was weaker in the resource-pulsed environment and that the prey’s defensive ability was traded off with its maximum growth rate.

Prey bacteria have been shown to use many different defensive strategies against protozoan predation (reviewed in Matz and Kjelleberg 2005). We found that the prey bacterium’s ability to form predator-resistant biofilm on the walls of microcosms could act as a direct prey defense mechanism in our study system. The most defensive prey, which coevolved with predators in the constant-resource environment, formed the most biofilm in the presence of predators. In addition, the formation of the measured trait, predator-resistant biofilm, was positively correlated with the amount of biofilm found on the walls of weekly discarded microcosms. However, the mean differences in prey biofilm-formation ability were not very large, and thus it is possible that the prey used other defense mechanisms in our experiment (Matz and Kjelleberg 2005).

On the basis of the results described above, we propose that the resource pulses weakened the selection for prey defense through two mechanisms: by increasing the fluctuations in predator densities (selection for prey defense) and in resource availability (selection for prey competitive ability). The incoming resource pulse probably first favored the fast-growing competitor prey types. However, even though the resulting increase in predation pressure likely exerted strong selection for prey defense, only a weak mean defensive prey response was observed in the evolutionary measurements (fig. 4). This suggests that the high peaks in predator density selected for more defensive prey types periodically during the middle phase of the resource pulse. However, as the predators were not able to coevolve, the increase in the frequency of defensive prey types led to a subsequent decrease in predator numbers. The decline in predation risk probably intensified the competition for resources between different prey types, leading to selective conditions that again favored fast-growing competitor prey types because the defensive prey types bore competitive cost in terms of reduced maximum growth rates. As a result, resource pulses presumably favored the fast-growing competitor prey types over the more defensive and slower-growing prey types during both the beginning (abundance of resources and low predation risk) and ending (low predation risk) phases of the resource pulse. Consequently, the evolutionary dynamics of prey fluctuated more in the pulsed-resource environment, leading to a weaker mean increase in prey defense. However, because we always sampled microcosms at the time point when the predation risk was low in the pulsed-resource environment (every seventh day), it is possible that the mean strength of defense was actually more or less equal between the environments but only varied more in the pulsed-resource environment according to the changes in competitor and defender genotype frequencies.

It is also possible that the more effective flow of energy from the basal to the predator trophic level led to more severe depletion of prey resources in the pulsed-resource environment. This lack of resources could have constrained the evolution of the defensive prey types regardless of the periodically high predation risk because defensive adaptations are often energetically demanding to produce and maintain. For example, producing bacterial biofilm is energetically costly (Spiers et al. 2003; MacLean et al. 2004), and the strength of prey biofilm defense is positively dependent on environmental productivity (Meyer and Kassen 2007; Hall et al. 2008). Therefore, in addition to suffering a competitive cost in terms of reduced maximum growth rate, it is possible that the lack of resources constrained the evolution of defensive prey types more severely in the pulsed-resource environment.

Consistent with the results of a previous experiment conducted under similar conditions (Friman et al. 2008), predation favored the white *Serratia marcescens* colony types, which are incapable of expressing the red pigment prodigiosin (fig. 5A). The white colony types were clearly less edible for predators (fig. 5B), and, as expected, their frequency increased more in the constant-resource environment, giving more support to the idea that resource pulses constrained the evolution of prey defense (fig. 5A). This result shows that prodigiosin expression is directly connected to the defense against protozoan predation in *S. marcescens*. Interestingly, the defensive white prey types also increased gradually toward the end of the experiment to a very low frequency in the pulsed-resource environment (fig. 5A). One possible explanation for this is that a low frequency of defending prey types were able to survive the first resource pulse and consume their share of resources from the next resource pulse. If this process was continued in every pulse, it could have led to a very slow increase in the frequency of defending prey clones (fig. 5A). Our results thus suggest that the resource pulses considerably weakened but did not prevent the evolution of defending prey types.

Recent studies have shown that rapid evolutionary dynamics can have considerable effects on the ecological dynamics of interacting species (Yoshida et al. 2003, 2007; Thompson 2005; Meyer and Kassen 2007; Friman et al. 2008; Me
We found that the evolution of prey defense considerably changed the trophic dynamics and the variability of the experimental predator-prey system. The constant-resource environment harbored less prey biomass than did the pulsed-resource environment, in both the absence and the presence of predators (fig. 1). However, the high prey densities did not turn linearly to high predator densities. Instead, the predators reached higher mean population sizes when feeding on prey bacteria in the pulsed-resource compared with the constant-resource environment, even though the pulsed-resource environment harbored less prey biomass (figs. 1B, 2B). We propose that the repressor in the transfer of energy from basal to higher trophic levels occurred because the frequency of defensive prey types increased more clearly in the constant-resource environment (fig. 5A, 5B). The delayed emergence of defensive prey types could have caused the gradual decline in predator density peaks toward the end of the experiment in the pulsed-resource environment (fig. 2B), as the defensive prey types started to siphon off the resources that otherwise would have been converted into predator biomass. More generally, this result suggests that the evolution of prey defense weakens the environmental variation–induced destabilization of predator population dynamics.

The ecological and evolutionary dynamics of predator-prey interactions has seldom been studied experimentally in resource-pulsed environments, even though the terrestrial and aquatic ecosystems are often characterized by temporal resource fluctuations (Rosenzweig 1995; Ostfeld and Keesing 2000; Yang et al. 2008). Our experiment demonstrates that extrinsic resource pulses can constrain the evolution of the predator-prey interactions by imposing fluctuating selection for prey defense and competitive traits. As a result, the relative strength of the top-down regulation decreased more clearly in the constant-resource environment, whereas the slow emergence of defensive prey types mainly weakened the resource pulse–induced destabilization of predator population dynamics in the temporally varying productivity environment. Evidence for predator evolution was not found in this study. This result is consistent with the current theoretical predictions (Dawkins and Krebs 1979; Vermeij 1994; Hochberg and van Baalen 1998; de Visser et al. 1999; Abrams 2000) and suggests that the prey’s ability to respond to predation is more likely to be an evolutionary outcome of predator-prey interactions (Friman et al. 2008).

Acknowledgments

We thank A. Hall, T. Hiltunen, and K. Lehmann for helpful comments and K. Viipale for conceptual assistance. This study was funded by the Academy of Finland (project 1106993 and 1130724) and the Centre of Excellence in Evolution Research of Jyväskylä University.

Literature Cited


MacLean, R. C., G. Bell, and P. B. Rainey. 2004. The evolution of a...
pleiotropic fitness tradeoff in *Pseudomonas fluorescens.* Proceeding of the National Academy of Sciences of the USA 101:8072–8077.


