Availability of prey resources drives evolution of predator–prey interaction

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Productivity is predicted to drive the ecological and evolutionary dynamics of predator–prey interaction through changes in resource allocation between different traits. Here we report results of an evolutionary experiment where prey bacteria *Serratia marcescens* was exposed to predatory protozoa *Tetrahymena thermophila* in low- and high-resource environments for approximately 2400 prey generations. Predation generally increased prey allocation to defence and caused prey selection lines to become more diverse. On average, prey became most defensive in the high-resource environment and suffered from reduced resource use ability more in the low-resource environment. As a result, the evolution of stronger prey defence in the high-resource environment led to a strong decrease in predator-to-prey ratio. Predation increased temporal variability of populations and traits of prey. However, this destabilizing effect was less pronounced in the high-resource environment. Our results demonstrate that prey resource availability can shape the trade-off allocation of prey traits, which in turn affects multiple properties of the evolving predator–prey system.

Keywords: diversity; predator–prey interaction; productivity; *Serratia marcescens*; *Tetrahymena thermophila*; trade-off

1. INTRODUCTION

Selection for competitive and defensive traits of prey is commonly expected to have a substantial impact on the population dynamics of interacting predator and prey species. The specific dynamics and outcome of the evolutionary and ecological process can, however, be sensitive to the underlying assumptions of the models (Hochberg & van Baalen 1998; Thompson 1998; Abrams 2000). Recently, rapid evolution of prey or host defences has been shown to alter the ecological dynamics of the interacting species in laboratory experiments. These include reduction in the strength of top-down regulation on consumers in host–parasite systems (Bohannan & Lenski 1999), changes in the phase of predator–prey cycles (Yoshida *et al.* 2003) or in the course of adaptive radiation in prey (Meyer & Kassen 2007). Rapid evolution of prey traits can also have important correlated consequences on other species interactions. For example, predation by free-living protozoa has been suggested to increase survival of pathogenic bacteria outside their host (Matz *et al.* 2005) and to maintain genetic diversity required for evading the host immune system (Wildschutte *et al.* 2004). Yet, long-term studies where both evolutionary and ecological dynamics of predator–prey interaction are considered simultaneously in different environmental conditions are rare.

Availability of resources is one of the most important environmental factors affecting the fitness of organisms. When the traits affecting fitness are costly, resource availability can determine allocation between the different

traits and thus affect the potential of the traits to evolve. Trade-off between competitive (e.g. growth-related traits) and defensive traits has recently been shown to be an important mechanism driving the interplay between evolutionary and ecological dynamics of antagonistic interactions (Bohannan & Lenski 1999; Bohannan *et al.* 2002; Yoshida *et al.* 2003, 2004; Meyer & Kassen 2007). Besides genetic factors, the magnitude of this trade-off is determined by the quality of the environment, that is, how much there are available resources for the prey or host to allocate between different traits (Bohannan *et al.* 2002; Yoshida *et al.* 2004). Theory and experiments (Mole 1994; Hochberg & van Baalen 1998; Bohannan & Lenski 1999; Abrams 2000; Yoshida *et al.* 2004) suggest that when the allocation to defensive traits is costly, the competitive ability of the prey should decrease, especially in the low-resource environments (large magnitude trade-offs). By contrast, when resources are abundant, prey should be able to invest in both defensive and competitive traits simultaneously because the excess of resources cancels out the fitness cost of defence (small magnitude trade-offs). From these predictions, it follows that costly prey defence should evolve in different degrees between the low- and high-resource environments (Hochberg & van Baalen 1998). The environmental control of trade-offs also offers a potential link by which prey resource availability and predation could affect the ecological properties of a predator–prey community, such as diversity and stability (Yamauchi & Yamamura 2005).

Both productivity and predation are known to affect genotypic or species diversity when there are differences, for example, in resource use or in resource allocation between costly competitive and defensive traits. Prey resources (environmental productivity) and intensity of

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predation are independently expected to have a unimodal relationship with prey diversity (Connell 1978; Tilman 1982; Flöder & Sommer 1999; Buckling *et al.* 2000; Kassen *et al.* 2000). Moreover, models combining the two factors predict that the effect of predation on diversity depends on the productivity of the environment (e.g. Huston 1994; Kondoh 2001). However, the combined effect of predation and prey resource availability on diversity has been rarely studied (but see Worm *et al.* 2002).

The properties of predator–prey interactions can have important implications for the stability of populations and ecosystems. Predation can decrease the stability of interacting populations, for example, by causing cyclic or chaotic dynamics (Abrams 2000; Yoshida *et al.* 2003). Moreover, prey resource enrichment is predicted to lead to decreased stability of the population dynamics of prey and predator, i.e. the ‘paradox of enrichment’ (Rosenzweig 1971). These general ecological predictions can, however, change if the prey or the predator is able to evolve fast enough to have an impact on the ecological dynamics (Thompson 1998). For example, evolutionary increase in prey defence can stabilize population dynamics of prey and predator (Abrams & Matsuda 1997; Abrams 2000).

It is also possible that predators or parasitoids evolve in response to adaptations in prey species (Abrams 2000; Buckling & Rainey 2002; Brockhurst *et al.* 2003), which can, for example, lead to a sustained coevolutionary arms race (Dawkins & Krebs 1979). The theoretical predictions on the dynamics and outcome of evolution, however, differ depending on model assumptions (Abrams 2000). Currently, the mostly tentative experimental evidence suggests that the evolution of prey in response to predation is more common than the evolution of predator in response to its prey (Abrams 2000). However, more experimental evidence is still needed to unravel the importance of coevolution in predator–prey interactions.

Here we report a long-term evolutionary experiment where an initially genetically homogeneous strain of bacteria *Serratia marcescens* was exposed to ciliate predator *Tetrahymena thermophila* in the low- and high-resource environments for approximately 2400 prey generations (98 days). Control selection lines where the prey and the predator evolved alone were established aside, and changes in the prey and predator traits, genetic diversity of prey (indicated by different colony types of bacteria), trophic dynamics and stability were measured during the experiment (see §2). *Serratia marcescens* is cosmopolite heterotrophic bacteria and a common facultative pathogen with broad host range covering plants, nematodes, insects, fishes and mammals (Ding & Williams 1983; Tan 2002). *Tetrahymena thermophila* is a well-studied particle-feeding and free-swimming predator of numerous bacteria (Elliott 1974). Unlike most of the previous studies conducted with bacterial host and viral or bacterial parasitoids (Bohannan & Lenski 1999; Buckling & Rainey 2002; Gallet *et al.* 2007), our study system is a classical predator–prey system where the predator has a longer generation time than the prey and consumes multiple preys before reproducing. To our knowledge, this is the first long-term experiment where the interactive effects of productivity and predation on ecological and evolutionary properties of the predator–prey system are considered simultaneously.

2. MATERIAL AND METHODS

(a) Long-term selection experiment

A single clone of prey bacteria *S. marcescens* (ATCC strain no. 13880, capable of producing the red pigment prodigiosin) was used to establish 12 selection lines in both low- and high-resource prey culture medium that was prepared as follows: 2 g of cerophyll powder (Ward’s natural science, Rochester, NY) were boiled for 10 min in 1 l of dH₂O, filtered through a glass fibre filter (GF/C, Whatman) and diluted two- and eightfold corresponding high- and low-resource levels (2.15 and 0.54 mg l⁻¹ final concentration of plant detritus). After sterilization, buffer with pH 7.5 (1.5724 g of K₂HPO₄·3H₂O, 0.4 g of KH₂PO₄, 0.5 g of (NH₄)₂SO₄, 0.1 g of MgSO₄·7H₂O, 0.01 g of NaCl and 0.0228 g of CaCl₂·2H₂O in 1 l of dH₂O) was added to the prey culture medium. When the experiment was initiated, the prey was first grown to near carrying capacity (48 hours) after which the asexually reproducing strain of the protozoan predator, *T. thermophila* (ATCC strain no. 30008) was introduced to half of the prey selection lines (approx. 23 000 individuals) in both resource levels, and the other half was retained as control treatments without predators. As *T. thermophila* is not able to feed directly on prey culture medium, *S. marcescens* and *T. thermophila* occupied separate trophic levels. Predators were also grown in otherwise similar experimental conditions but in non-living predator culture medium (six selection lines in both low- and high-resource levels containing 2.5 or 10 g Bacto proteose peptone and 1.25 or 2.5 g of Bacto yeast extract (Becton, Dickinson and Co., Franklin Lakes, NJ) per 1 l of dH₂O, respectively). These control predators had previously been maintained under similar conditions by serial transfer since their isolation from freshwater by A. M. Elliot in 1952 (ATCC 2007). Thus, the predators had a long evolutionary history of feeding on non-living food. The control predators were used later during the experiment in prey defence assays and when the evolution of predator traits was measured. Consequently, the main experiment consisted of six different treatments each comprising six independent selection lines.

Microcosms were made of 250 ml polycarbonate Erlenmeyer flasks, capped with membrane filters (Corning) and incubated at 25°C as static cultures during the experiment. To minimize unwanted evolutionary effects resulting from different total population sizes between resource level treatments, volume of the culture medium in the microcosms was set to 120 and 40 ml for low- and high-resource level treatments, respectively. The total amount of prey biomass did not differ between low- and high-resource level control selection lines (average across all measurement times $F_{1,20}=1.6$, $p=0.226$), suggesting that our volume manipulation was of a right scale. The volume manipulation probably had little effect on oxygen availability due to the shape of the microcosms and relatively low concentration of organic matter (2.15 and 0.54 mg l⁻¹) in the prey culture medium.

Microcosms were shaken gently before weekly sampling for population sizes of prey bacteria (biomass estimated as turbidity at 600 nm) and predatory protozoa (direct count with image analysis; for details, see Laakso *et al.* 2003). After sampling, 50% of the microcosm volume was immediately renewed with fresh prey culture medium. At each sampling, subsamples were serially diluted and spread 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 hours of incubation at 25°C, the number of bacterial colonies was counted and

classified as red, intermediate or white according to the amount of prodigiosin (red, high expression; intermediate, intermediate expression and white, no expression) produced. These data were later used to estimate within-population genetic diversity from each selection line by calculating the Shannon diversity index. Colony colour resulting from expression of red pigment prodigiosin is a heritable trait in *S. marcescens* (Grimont & Grimont 1978; Coulthurst *et al.* 2006) and has been linked to various functionally important traits in *S. marcescens*, such as motility and resistance against phage Kappa (Paruchuri & Harshey 1987). Variability of bacterial colony types has been used widely in previous studies to evaluate genetic diversity of bacterial populations (e.g. Buckling *et al.* 2000; Kassen *et al.* 2000; Travisano & Rainey 2000; Meyer & Kassen 2007). Temporal stability of population sizes, prey defensive and competitive traits and diversity was estimated from the time-series data as a coefficient of variation (CV; s.d. mean⁻¹) of each microcosm (i.e. the higher the CV the lower the stability).

(b) Assays for prey traits related to resource use ability and defence against predation

Prey traits were measured a total of eight times during the main experiment in separate factorial short-term experiments. Prior to the measurements, prey clones were separated from predators by plating on agar plates. After 48 hours of incubation at 25°C, 20 clones per selection line were selected at random and mixed together in a 250 ml Erlenmeyer flask containing fresh prey culture medium, which had the same resource concentration the given selection line had previously experienced in the main experiment. To separate predators from the prey bacteria, antibiotic treatment tested to be harmless to the predator was used (10 000 units of penicillin and 10 mg of streptomycin in 1 ml of 0.9% NaCl, Sigma-Aldrich). After 24 hours of exposure to the antibiotic at 25°C, a small inoculum of predators was transferred to a high-resource level predator culture medium (proteose peptone and yeast extract) to dilute antibiotics. When measuring the prey defence 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 ml⁻¹ of streptomycin) and did not reduce bacterial growth. All the prey and predator selection lines from the main experiment were grown separately for a total of 72 hours before assessing the evolutionary changes. Seventy-two hours equal at least tens of prey and predator generations prior to the evolutionary measurements. During this time, the physiological state of study organisms is likely to reset, and the observed differences can be considered to be caused by genetic factors.

Evolutionary changes in prey resource use ability in the absence of predators were assessed as follows: small prey inoculums (less than 0.0002% of the maximum population size) were added to fresh prey culture medium at a low initial density; and the maximum population sizes and maximum growth rates of different selection lines were determined from biomass growth data recorded for 96 hours at 10 min intervals (600 nm optical density (OD)). Although a direct competitive assay between two different selection lines would be the most proper measure of prey fitness, we were not able to carry out these owing to the lack of suitable genetic markers (e.g. the commonly used sugar-based markers do not work because the prey culture medium we use contains various sugars; Sijtsma & Tan 1996). In addition, the use of markers

can have other difficulties, for example in reliability (see O'Keefe *et al.* 2006). In our assays, prey maximum growth rate at low density and long-term maximum population size indicate how well prey is able to respond to the addition of fresh resource and how efficiently resources are used to produce biomass in the long term, respectively. In our experimental setting, these traits most likely reflect prey competitive ability when the microcosms are renewed with fresh new medium ('maximum growth rate' trait) and when the resources are well consumed at the end of the weeklong renewal cycle ('maximum population size'). Thus both of these growth traits are possibly related to bacterial fitness and can have trade-offs with other traits. For example, allocation to defensive traits could decrease biomass production of prey. In addition to tracking evolutionary changes of prey in the main experiment, maximum growth rate and biomass production of randomly selected white and red ancestral prey genotypes were measured in three different resource concentrations as described above (0.22, 1.08 and 2.15 mg l⁻¹ concentration of prey culture medium). In addition, the motility of randomly selected red and white ancestral prey genotypes was measured in separate experiments (indicated as the difference in the area bacteria colonized when incubated for 48 hours on soft agar plates containing 0.7% of agar).

Evolutionary changes in prey defence were measured in separate factorial experiments as the prey ability to sustain population size in the presence of predators. This measure takes into account the overall defence of prey and does not differentiate between different defence mechanisms. Before measuring the prey defence, prey selection lines were grown to similar high densities after which small inoculums of predators (less than 0.01% of predator-carrying capacity) were introduced. The population size prey could sustain in the presence of predators was measured as OD at 600 nm for 4 days at 25°C. The evolution of prey defence was also measured from the perspective of predators as prey profitability that was determined as maximum population size predators could reach when fed on prey selection lines originating from different treatments. In addition to prey defensive ability, prey profitability can be affected, for example, by prey nutritional value. Evolutionary changes in prey profitability for the predator were measured in separate factorial experiments from the samplings in the main experiment at weeks 2 and 7. In these short-term experiments, predators that had earlier used prey bacteria or alternatively non-living predator culture medium as food were grown with prey selection lines that had evolved alone or with predators. Before measuring the prey profitability, all prey selection lines were left to grow for 3 days to reach similar high densities after the predators were introduced to the prey cultures.

(c) Predator evolution assays

To assess whether predators adapted to the use of bacterial resource by becoming more efficient consumers during the experiment, maximum growth rates and maximum population sizes of all predator selection lines were measured. The measurements were made in separate short-term experiments where predators were cultured (i) in non-living low- and high-concentration predator culture medium and (ii) on prey bacteria that had been cultured earlier in the absence or presence of predators in the low- or high-concentration prey culture medium.

Figure 1. Ecological dynamics of the prey and the predator in the low- and high-resource environments. (a) Dynamics of the prey populations in the absence (solid lines) and in the presence of predators (dotted lines) in the low (open triangles) and high (filled triangles) resource environment. (b) The dynamics of the predator populations when feeding on prey in the low (open circles)- and high (filled circles)-resource environments. The values are means of six replicates and the vertical lines show ± 1 s.e.m.

Figure 2. Evolutionary dynamics of different prey selection lines in the low- and high-resource environments. Difference in (a) prey defence (population size prey can sustain under predation) and (b) prey resource use ability (maximum population size) between prey selection lines (means of prey evolved with predator minus means of prey evolved without predator) within low (open squares) and high (filled squares) resource environment. On the y-axis, values above 0 denote stronger defence or resource use ability of the prey selection lines that were exposed to predation previously. Negative values denote stronger defence or resource use ability of control prey selection lines. The error bars in (a,b) are bootstrap estimates. The values are means of six replicates and the vertical lines show ± 1 s.e.m.

(d) Statistical analysis

All data were analysed with repeated measures ANOVA except for stability of populations and traits and diversity of the ancestral *Serratia* strain for which two-way ANOVA was used. Bonferroni adjusted *p*-values were used for multiple comparisons. If the sphericity assumption of repeated measures ANOVA was violated, Greenhouse–Geisser corrected *F*-values were used.

3. RESULTS

The fourfold increase in prey resources increased prey biomass both in the absence and presence of the predators ($F_{1,20}=93.7$, $p<0.001$; figure 1a). Predators reduced the prey population sizes on average 64 and 47% below the control treatments within the low- and high-resource levels, respectively ($F_{1,20}=250$, $p<0.001$; figure 1a). Short-term measurements of prey profitability at weeks 2 and 7 revealed that prey yielded lower predator maximum population sizes when it had been exposed to predation in the main experiment (predator biomass yield when fed on prey that had been exposed to

predation previously: $125\,044 \pm 6260$ cells ml^{-1} (± 1 s.e.m.) compared with predator biomass yield when fed on control prey; $149\,181 \pm 5955$ predator cells ml^{-1} (± 1 s.e.m.); main effect of predation, $F_{1,40}=7.9$, $p=0.008$). The interaction between prey resource level and predation was non-significant, that is, prey profitability decreased in general regardless of the resource level prey had experienced in the main experiment. However, according to the weekly prey defence assays, predators increased the population size that prey could sustain in the presence of reintroduced predators only in the high-resource environment (prey defence assays, resource level $\times$ predation interaction; $F_{1,40}=4.7$, $p=0.036$; figure 2a).

According to the growth assays where prey was grown without predators, predation history generally decreased prey maximum growth rate compared with control selection lines (growth rate measured at low density; $F_{1,20}=10.8$, $p=0.004$). Predation history decreased also the prey ability to produce biomass (measured as maximum population size of prey; $F_{1,20}=30.9$, $p<0.001$). Interestingly, this decrease was more evident in the

Figure 3. Dynamics of predator-to-prey ratio. Predator (cells ml^{-1})-to-prey (OD at 600 nm) ratio in the low- (open diamonds) and high (filled diamonds)-resource levels. The vertical lines show ± 1 s.e.m., and $n=6$ for all.

low-resource environment (resource level $\times$ predation interaction; $F_{1,20}=12.3$, $p=0.002$; figure 2b).

The interaction between the prey resource enrichment and predator-induced evolution of prey defence had significant consequences for the trophic level dynamics of predator and prey. The fourfold increase in prey resources increased mean prey biomass by approximately five times in the presence of predators. However, this increase in prey population sizes increased the population sizes of predators only by 1.6 times ($F_{1,10}=21.3$, $p=0.001$; figure 1b). Consequently, the high-resource level environment harboured more prey biomass in proportion to the number of predators (i.e. predator-to-prey ratio was considerably lower in the high-resource level; $F_{1,10}=30.5$, $p<0.001$; figure 3).

Genetic diversity of prey was higher in the presence of predators (based on the frequencies of different colony colour types; $F_{1,20}=26.8$, $p<0.001$; figure 4b). However, the diversity did not differ between resource environments in the presence of predators. The experiment was initiated from a single red colony type but intermediate and white colony types rapidly emerged in all treatments. Predation increased most prominently the frequency of white *S. marcescens* colonies whereas the red colonies dominated the control treatments (figure 4a). At the end of the main experiment, randomly selected white and red ancestral clones were compared to find out whether the colony colour could be directly related to different traits of prey. Interestingly, white colony types of the ancestral *S. marcescens* strain had poorer resource use ability in low-resource concentration (measured as maximum population size in 1.08 mg l^{-1} concentration of prey culture medium; $F_{4,81}=4.0$, $p=0.005$; figure 5b). White ancestral colony types were also four times less motile compared with red colony types ($F_{1,8}=24.3$, $p<0.001$; figure 5a). Moreover, the frequency of red colony types was observed to decrease within 48 hours by 28% after introducing predators to ancestral cultures containing even numbers of randomly selected white and red colony types ($F_{1,28}=16.6$, $p<0.001$; figure 5c).

The population dynamics of single-species prey and predator control treatments were generally more variable compared with predator–prey communities for both the prey ($F_{1,20}=145$, $p<0.001$; CV shown in table 1) and the predator ($F_{1,20}=288$, $p<0.001$; CV of the

single-species predator control treatments: 0.83 ± 0.07 and 0.49 ± 0.06 compared with CV of predator–prey community: 2.01 ± 0.07 and 1.65 ± 0.07 in low- and high-resource levels, respectively). Interestingly, predation destabilized the population dynamics of prey less in the high-resource level (resource level $\times$ predation interaction; $F_{1,20}=16.2$, $p=0.001$; table 1). Similarly, the dynamics of prey resource use ability (maximum population size: $F_{1,20}=42$, $p<0.001$) and prey diversity were destabilized less by predation in the high-resource level ($F_{1,20}=5.8$, $p=0.026$; table 1).

We found no evidence of evolution in predator traits (maximum growth rate or maximum population size when fed with non-living peptone medium or the prey bacteria) in separate short-term experiments at weeks 2 and 7, or in the weekly prey defence assays (all terms insignificant; predator selection lines were equal in their growth, regardless of their evolutionary history).

4. DISCUSSION

Prey resource availability was an important driver of the evolutionary and ecological outcomes of the predator–prey interaction. Although predation decreased prey profitability for the predator in both resource environments, evolution of prey defence was stronger in the high-resource environment, indicated by increased population size prey could sustain in the presence of predators. As expected, increasing allocation to defence was costly in terms of decreased prey growth rate in the absence of predators in both resource environments. Interestingly, the cost of defence was especially clear in the low-resource environment where the maximum population size prey could reach in the absence of the predation was reduced most. These results support the theoretical prediction that when anti-predatory adaptation is costly, evolution of the predator–prey interaction can be constrained by prey resource availability (Hochberg & van Baalen 1998; Abrams 2000; Yamauchi & Yamamura 2005). Thus, prey evolution could impact predator–prey interaction less in the low-resource environment where allocation to prey defensive traits is more strongly limited by the costs of other fitness-related traits.

We found no evidence for the evolution of predator traits (measured as maximum population growth rate or population size of predators when fed on non-living prey culture medium or prey bacteria). Weak evolution of predator traits is also supported by the population time series of predator and prey (figure 1a,b). The predator population sizes declined rapidly in both environments, probably owing to predation-driven increase in prey defence. However, that predator populations did not recover at any time point later on, suggests that predators at least partially failed to counter-adapt in response to the increase in prey defence. Therefore, it seems that our study system offers a limited potential for coevolution of predator and prey. Many properties of the predator–prey interaction could have caused this outcome. In general, the selection for prey defensive traits is thought to be stronger than that for predator efficiency, for example, the ‘life versus dinner’ dichotomy (Dawkins & Krebs 1979; Vermeij 1994). Moreover, relatively long generation times and small population sizes reduce the evolutionary potential of predators even further. For example, small

Figure 4. Prey diversity. (a) Frequencies of different prey colony types classified according to prodigiosin expression: dark grey, red colonies; light grey, intermediate colonies; white, white colonies. Letters above the bars denote for treatments where prey evolved alone in the low- and high-resource environments as A and B, respectively, and in the presence of the predators in the low- and high-resource environments as C and D, respectively. (b) Shannon diversity index of the prey populations in the absence (solid lines) and the presence (dotted lines) of predators in the low (open triangles)- and high (filled triangles)-resource environments. The values are means of six replicates and the vertical lines show ± 1 s.e.m.

Figure 5. Prey traits measured from randomly selected ancestral *S. marcescens* clones. The relationship between ancestral *S. marcescens* colony colour and (a) motility, (b) resource use ability (maximum population size) in low 1.08 mg l^{-1} concentration of prey culture medium and (c) change in the frequencies of different unselected ancestral colour morphs after 48 hours of growth in the absence (–pred) and in the presence (+pred) of predator *T. thermophila* (dark grey, red colonies; light grey, intermediate colonies; white, white colonies). In (c), both cultures contained initially an even biomass of white and red clones. The values are means of (a) 5, (b) 10 and (c) 30 ancestor clones and the vertical lines in (a,b) show ± 1 s.e.m.

Table 1. Variability of prey population sizes, prey resource use ability (maximum population size) and prey diversity. (Values are mean coefficient of variation (CV) of six replicate microcosms ± 1 s.e.m. CV is calculated from 14 consecutive data points for the prey population sizes, from nine data points for prey resource use ability (maximum population size in the absence of predation) and from five data points for prey diversity (Shannon diversity index).)

treatment	prey population size	prey resource use ability	prey diversity
predators absent, low	0.224 ± 0.003	0.546 ± 0.058	0.360 ± 0.034
predators absent, high	0.246 ± 0.009	0.249 ± 0.020	0.405 ± 0.047
predators present, low	0.970 ± 0.056	0.756 ± 0.105	0.457 ± 0.088
predators present, high	0.618 ± 0.073	0.262 ± 0.011	0.196 ± 0.071

population size results in small mutation supply rate and slower evolutionary response of the predator compared with prey (de Visser *et al.* 1999). All these features are typical to the predator–prey interaction of our study system. More generally, weak coevolution could be the characteristic property of the classical predator–prey interaction, in contrast to the tightly coupled viral parasitoid–host bacteria systems (Buckling & Rainey 2002) and trematode parasite–snail host systems (Lively & Dybdahl 2000) that frequently show coevolution.

The resource-induced differences in the evolution of the prey had profound consequences for the trophic

dynamics of the prey and the predator. In the presence of predators, a fourfold increase in prey resource availability increased the biomass of prey but did not have a long-term effect on predator numbers. This is most probably due to the emergence of less edible prey genotypes that did not markedly suffer from reduced resource use ability under a high-resource environment (Yoshida *et al.* 2004). Consequently, the strength of top-down control became weaker in the high- than in the low-resource level. Ecologists have long debated if the prey populations are controlled more by their resources or their predators. Here we show that the relative strengths of top-down and bottom-up

forces can change in productive environments where the evolution of costly defensive traits is not limited by the prey resource availability. The weak coupling of basal resources and predator biomass has been observed previously in many enrichment studies conducted in different spatial scales from microcosms to open ocean systems (Bohannon & Lenski 1999; Micheli 1999; Benndorf 2002; Henry *et al.* 2006). Thus, our results tentatively suggest that the rapid evolution of defensive traits of prey can be an important factor contributing to the weak propagation of enrichment effects to higher trophic levels in aquatic systems.

Predation generally maintained a higher diversity of prey bacteria regardless of prey resource concentration (Shannon diversity calculated from the frequencies of *S. marcescens* colour variants differing in their ability to synthesize prodigiosin). Meyer & Kassen (2007) showed that predation by *T. thermophila* can diversify *Pseudomonas fluorescens* colony morphology through negative frequency-dependent selection on the *P. fluorescens* wrinkly spreader and smooth colony types. In their experiment, *T. thermophila* preyed less effectively on the wrinkly spreader prey type forming biofilm on the water–air interface of the microcosm, and the wrinkly spreader was also less competitive than the smooth form when abundant. The synthesis of prodigiosin in *S. marcescens* is also unlikely to represent a selectively neutral trait. For example, white colonies of *S. marcescens* have been found to be resistant against phage Kappa (Paruchuri & Harshey 1987) and to be important causal agents of cucurbit yellow vine disease (Bruton *et al.* 2003). Also, opportunistic *S. marcescens* strains isolated from human patients are usually white (Grimont & Grimont 1978; Ding & Williams 1983; Tan 2002) while the red pigment has been shown to be the most important antifungal factor of *S. marcescens* (Someya *et al.* 2001). Our results suggest that the expression of prodigiosin is related to traits affecting prey resource use ability and defence against protozoan predation, which have trade-offs under resource-limited conditions. First, the white colony types of *S. marcescens* eventually became most frequent in the presence of predators in both resource levels (figure 4a). This suggests that white colonies of *S. marcescens* could be better defended against predation by *T. thermophila*. According to independent experiments carried out with the *S. marcescens* ancestor strain, predation decreased the frequency of red colonies by 28% within 2 days (figure 5c). This suggests that red *S. marcescens* colonies could be more edible compared with the white colonies. White colonies of the ancestral *S. marcescens* strain also had lower maximum population sizes under resource-limited conditions compared with red colonies (figure 5b). Selection for costly prey defence could therefore explain the lowered resource use ability detected in the low-resource environment (figure 2b). Thus, our results suggest that predation could have facilitated the coexistence of different prey types (differing in colony colour) through selection for costly anti-predatory traits (Paine 1966; Yoshida *et al.* 2004; Wildschutte *et al.* 2004; Meyer & Kassen 2007).

One possible mechanism by which *S. marcescens* could avoid predation is reduced motility, which can increase the prey defence through decrease in predator encounter rate (Monger & Landry 1992; González *et al.* 1993). Motility assays carried out with the *S. marcescens* ancestor strain

indeed showed that the white colonies were clearly less motile compared with red colonies (figure 5a). Reduced motility (decrease in flagellin expression) has been shown previously to be connected to the resistance of *S. marcescens* 274 strain against phage Kappa (Paruchuri & Harshey 1987). The formation of clumps and biofilm is another common prey defence mechanism in bacteria, which is known to reduce the risk of predation by particle-feeding ciliates (e.g. Matz *et al.* 2005; Meyer & Kassen 2007). According to our follow-up experiment, predation by *T. thermophila* can increase the biofilm formation of *S. marcescens* (V.-P. Friman, J. Laakso and T. Hiltunen 2005–2006, unpublished data). However, whether this mechanism played a significant role in this experiment remains to be tested in the future from frozen bacterial stocks. Even though it has previously been shown that the regulation of motility, biofilm formation and prodigiosin expression are interrelated in *S. marcescens* (Coulthurst *et al.* 2006), to our knowledge, this is the first study where the synthesis of prodigiosin is shown to be linked to evolution of defence against protozoa and resource use ability in *S. marcescens*.

Productivity is also often expected to have an independent effect on diversity but this was not detected, possibly because the experimental setting had only two resource levels and the relationship between diversity and productivity can be nonlinear (Connell 1978; Tilman 1982; Flöder & Sommer 1999; Buckling *et al.* 2000; Kassen *et al.* 2000). However, we found an interaction between productivity and predation: predation decreased variation in prey diversity only in the high-resource environment (table 1) and prey resource concentration modulated the initial dynamics of prey diversity (figure 4b). Although not designed to reveal resource effects on prey evolution, the experiment by Meyer & Kassen (2007) recently demonstrated that predator effects on prey diversity can be initially time dependent. They suggested that by reducing prey density the amount of prey resources increases, and as a result, predators initially slow down prey diversification. The direct manipulation of prey resources in our experiment give more support to the idea that the effect of predation on diversity is modulated by the productivity of the environment (Huston 1994; Kondoh 2001; Worm *et al.* 2002). In our experiment, predators initially reduced prey diversity in the low-resource conditions. This is consistent with our finding that prey have difficulties in allocating to both growth and defensive traits under only low-resource conditions. This resource-driven trade-off could have transiently increased the frequency of resistant and poorly growing white clones, especially as predator pressure was higher at the beginning of the experiment.

Resource enrichment and predation are both expected to destabilize food chains in non-evolving predator–prey systems (Rosenzweig 1971; Johnson & Agrawal 2003). In our case, CV of prey population sizes increased in the presence of predators as expected. However, the variability of prey populations, genetic diversity and resource use ability increased less when the prey and predator evolved together in the high-resource level. These findings could be explained, for example, by models coupling the evolution of prey defence to density-dependent population dynamics (Yamauchi & Yamamura 2005). Stabilization of prey population dynamics is expected to occur when

predation increases prey diversity by selecting for more defensive individuals (Johnson & Agrawal 2003). Moreover, these models assume that the time-scale of the evolutionary dynamics of prey is short in comparison with the ecological dynamics, and that there is a trade-off between defensive and competitive (in our case, resource use ability) traits (Abrams 2000)—conditions that are likely to have occurred in our system. Our results thus suggest that resource enrichment can stabilize the predator–prey interaction when the prey evolves and the fitness costs of anti-predatory traits diminish with resource enrichment.

Our long-term study demonstrates the profound role of prey resources and trade-offs in the ecological and evolutionary dynamics of the predator–prey interaction (Hochberg & van Baalen 1998; Thompson 1998; Abrams 2000). Our results on evolution of defence and stability are broadly similar to experiments with host–parasitoid systems (Bohannan & Lenski 1999; Bohannan *et al.* 2002; Forde *et al.* 2004, 2007) despite the fundamental differences between predator–prey and host–parasitoid interactions. This suggests that rapid resource-driven evolution of antagonistic interactions can be an important and a common factor determining the structure and dynamics of natural food webs. Interestingly, the mechanisms required for evading predators rather than parasitoids may have different and important correlated consequences for other species interactions. In our case, the eukaryotic predator selected for traits that are commonly associated with transmission and virulence of the opportunistically pathogenic prey (Ding & Williams 1983; Tan 2002), suggesting that the productivity of the environment can be important factor affecting the evolution of diseases.

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