

# Reports

*Ecology*, 91(3), 2010, pp. 629–636  
© 2010 by the Ecological Society of America

## Evolution of prey in ecological time reduces the effect size of predators in experimental microcosms

CASEY P. TERHORST,<sup>1</sup> THOMAS E. MILLER, AND DON R. LEVITAN

*Department of Biological Science, Florida State University, Tallahassee, Florida 32306-4295 USA*

**Abstract.** Ecologists have long studied the effect of predators on prey population abundance while evolutionary biologists have measured prey trait evolution in response to predation. Ecological and evolutionary processes were generally thought to occur on different time scales, but recent evidence suggests that evolution may alter the ecological effects of predation over the course of ecological experiments. We used a protozoan and its mosquito-larvae predator, naturally found in the water-filled leaves of pitcher plants, to examine the effect of prey evolution on predator–prey interactions. In experiments conducted over 12 days (approximately 50 prey generations, but less than one predator generation), we measured a decrease in the effect of mosquito larvae predators on protozoa prey populations. In a separate set of experiments, we found that the presence of predators corresponded with evolution of smaller cell size and increased population growth rate. In ecological experiments, two situations commonly occur: strong selection pressure applied by the treatment itself and discrepancies in generation times of associate species. Our results suggest that in either situation, the resulting evolutionary patterns may lead to dramatic and important changes in ecological effect size.

**Key words:** Colpoda; effect size; mosquito larvae; predator; prey; protozoa; rapid evolution; resistance; *Sarracenia purpurea*; tolerance.

### INTRODUCTION

Interactions between predators and prey are among the most-studied species interactions in ecology. Predator–prey interactions are important not only for understanding species population dynamics, but can have cascading effects on other species at lower trophic levels (e.g., Schmitz et al. 2000, Shurin et al. 2002). Meta-analyses have revealed hundreds of ecological experiments that document negative effects of predators on prey survival and abundance (e.g., Englund et al. 1999, Gurevitch et al. 2000) and theory predicts that overexploitation can lead to instability (e.g., Case 2000). Despite this, predators and prey commonly coexist in natural communities. Numerous hypotheses have been proposed to explain predator-prey coexistence, but many of these mechanisms have proven insufficient for maintaining the coexistence of predators and prey for

any significant length of time (Huffaker 1958, Fujii 1999).

A separate body of research has focused on the evolution of traits in prey in response to predation. Various traits may evolve in response to predator or herbivore selection, including coloration (Alatalo and Mappes 1996), size (Nunezfarfan and Dirzo 1994), chemical defenses (Mauricio and Rausher 1997, Shonle and Bergelson 2000), and structural defenses (Seeley 1986, Reimchen and Nosil 2002). Coevolution between defensive traits of the prey and predator traits that overcome these defenses results in an evolutionary arms race (Futuyma and Slatkin 1983), with predators continuing to have significant negative effects on prey populations (e.g., Red Queen Hypothesis; Van Valen 1973).

A growing body of work demonstrates adaptive evolution on ecological time scales (Thompson 1998, Hairston et al. 2005, Johnson and Stinchcombe 2007, Strauss et al. 2008). Slobodkin (1961) defined ecological time as the time period over which populations could maintain a steady state, thought to be hundreds of generations. Yet Hairston et al. (2005) defined rapid evolution as trait changes that have the potential to

Manuscript received 13 August 2009; revised 14 October 2009; accepted 19 October 2009. Corresponding Editor: M. C. Urban.

<sup>1</sup> E-mail: terhorst@bio.fsu.edu

affect the outcome of simultaneous ecological change, which could occur in relatively few generations. Examples of rapid evolution are important because they suggest that ecologists need to consider ongoing evolutionary change in order to explain the ecological patterns resulting from species interactions. However, the time-scale for ecological and evolutionary processes will be different for different species. For instance, prey species often have shorter generation times than predators, potentially allowing for faster evolutionary rates in prey relative to predators. This potential discrepancy in evolutionary rates between predators and prey could reduce ecological effect sizes and explain coexistence in some natural communities (Strauss et al. 2008). Conversely, an increase in the effect of predation may occur in cases where predators evolve faster than their prey (e.g., arthropods on plants). Despite some indirect evidence, few studies directly link the magnitude of responses measured in ecological experiments to the evolution of species traits (Strauss et al. 2008; but see Yoshida et al. 2003, 2007).

Here we examine if the evolution of protozoa found within the leaves of pitcher plants alters the effect size of mosquito predation on those populations. We tracked the evolution of several traits in the prey species in communities with and without predation over “ecological time” (defined here as within a predator’s life span) and determined which prey traits evolved over this time period. We predicted that the rapid evolution of anti-predator traits in prey populations over ecological time would decrease the effect of predation.

## METHODS

### *Study system*

The inquiline community found in the water-filled leaves of *Sarracenia purpurea* (purple pitcher plant; see Plate 1) has been well described elsewhere (reviewed in Miller and Kneitel 2005) and so will be only briefly described here. The host plant is widely distributed, extending from northern Florida in the United States to the Northwest Territories in Canada, and the community is representative of other types of natural microcosm communities (Srivastava et al. 2004). Pitcher plants produce cup-shaped leaves that fill with rainwater and attract insect prey, primarily ants. The energy in the prey captured by the leaves serves as a basis for a community of bacterial decomposers, bacterivorous protozoa, and rotifers, and a specialist mosquito (*Wyeomyia smithii*) that consumes bacterivores. Mosquitoes strongly affect rotifer and protozoa populations, reducing abundance by two to five orders of magnitude (Kneitel and Miller 2002, Miller et al. 2002). Mosquito larvae develop through four instar levels over the course of several weeks to several months, depending on food availability (Bradshaw and Johnson 1995).

Over the course of 12 days, we measured the ecological effect of mosquito larvae on replicate populations of a protozoan prey species (*Colpoda* sp.)

in laboratory microcosms (see Plate 1). *Colpoda* sp. is a ciliated protozoan that is intermediate in size, competitive ability, and predator tolerance relative to other bacterivores in the community (Leibold and Miller 2004). *Colpoda* reproduces asexually every 4–8 hours (Lüftnegger et al. 1990), but some sexual reproduction may occur (Dunthorn et al. 2008). Each experiment encompassed 36–72 prey generations but only a single larval stage of the predator, allowing for evolution of only the prey. To determine whether the evolution of prey traits could contribute to a change in predator effect size, we followed the evolution of several prey traits in a separate set of selection experiments.

### *Measuring ecological effects*

Because of practical limitations on the number of replicates in a given time, the experiment was conducted in two time blocks (October 2008 and January 2009), using different lines of *Colpoda* isolated from the field prior to each block to measure the ecological effect of predators. In each block, samples of water within pitcher plant leaves were collected from haphazard leaves at each of two sites in the Apalachicola National Forest in northern Florida: Crystal Bog and Nazi Borrow Pit. The evolutionary history of the collected *Colpoda* was unknown, but *Colpoda* populations in most pitcher plant leaves have almost certainly experienced some level of predation by mosquito larvae in their recent past (T. E. Miller and C. P. terHorst, *unpublished data*), as both species are frequently found in the same pitchers. In the laboratory, *Colpoda* were isolated from all possible samples, generating three lines in October and five lines in January, each isolated from an independent leaf. Mixed cultures from individual lines were used to produce ~300 individuals to inoculate laboratory microcosms (along with the associated bacteria) that mimic pitcher plants (50-mL plastic macrocentrifuge tubes). Multiple individuals were used from each line to increase genetic diversity and allow for potential evolution to occur. Protozoa in these laboratory microcosms have similar population dynamics to those in pitcher plant leaves in the field (Miller et al. 1994). Each microcosm contained 20 mL sterile well water, plastic beads (2–5 mm diameter), and 6 mg of Tetramin (Tetra Holding, Inc., Blacksburg, Virginia, USA) as a food source for the bacteria. The plastic beads served as a refuge from predation for *Colpoda* and mimic the detritus and frass found at the bottom of natural pitchers. Mosquitoes foraged in the beads as well as in the water column, but their size restricted their movement in the beads.

Replicate populations ( $n = 4$  per treatment per month) were maintained with and without predation (two third-instar *W. smithii* larvae collected from the same sites) for 12 days. Although all mosquitoes survived during the experiment, we attempted to reduce satiation of the predators and minimize competition among *Colpoda* by reinitiating the experimental microcosms every three

days with the same conditions as above, using new mosquito larvae and a random sample of 300 *Colpoda* from each replicate. On average, a sample of 300 individuals represents 2.3% and 1.7% of the total population with and without predators, respectively. Every day, *Colpoda* abundance in each population was estimated by counting a 0.1-mL subsample on a Palmer counting cell (Wildlife Supply Company, Buffalo, New York, USA) and the effect of predation was determined using the mean within each time block as: Predator effect = (mean abundance of monoculture – mean abundance with predation)/mean abundance of monoculture.

There are minor costs and benefits to using either Tetramin or dead ants as a food source, though both result in similar protozoa population dynamics. Tetramin can be added more precisely, resulting in less variance among populations, but ants are a more natural food source for bacteria in these communities. In this experiment we chose Tetramin, because we were concerned with reducing variance in the food source over time in order to test changes in effect size over time. In the second experiment (see *Selection experiments*, below) we chose dead ants to examine if traits would evolve differently across treatments given natural variation in food quality.

#### *Selection experiments*

In a separate set of experiments, we followed the evolution of traits in field-collected *Colpoda* in laboratory microcosms. In these selection experiments, replicate populations of *Colpoda* were exposed to predation by mosquito larvae for six days (18–36 prey generations) in six time blocks: February, April, May, and July of 2006, and March and June of 2007. In each time block, new independent lines of *Colpoda* were collected from the same field sites as above. Each experimental microcosm contained 30 mL of sterile deionized water, five dead sterile fire ants (*Solenopsis invicta*) as a food source for bacteria, 5 mL of sterile plastic beads, and was inoculated with ~300 *Colpoda* composed of an equal mixture of all independent lines and the associated bacteria. Two third-instar mosquito larvae were added to half of the tubes. Sample size varied among time blocks ( $n = 4$  in February 2006,  $n = 5$  in April 2006,  $n = 10$  in all other months) and was further reduced in some months because some cultures failed to grow sufficiently well to measure traits (actual sample sizes given in figures).

For both experiments, cultures were maintained in a growth chamber with a diurnal cycle (12 h:12 h) of light and temperature (day = 30°C, night = 20°C). At the end of the selection experiment, microcosms were well mixed and ~300 *Colpoda* were removed from replicates in both predator and no-predator treatments and placed into identical no-predator microcosms containing 30 mL of sterile deionized water and five dead sterile ants. *Colpoda* were grown under these common conditions

for 24 hours to minimize the role of environmental plasticity and maternal effects before measuring traits. After 24 hours (approximately three generations), two random subsamples of 300 individuals were taken from each replicate. The first was used to determine the degree to which each replicate was adapted to predation by measuring population growth rate as a proxy measure of fitness in the presence of a predator (except in February 2006). Replicates with higher growth rates in the presence of two 3rd instar mosquito larvae were deemed to be more adapted to predation. The second subsample was used to measure traits in a “common garden” without predation. Any difference in traits between treatments was assumed to be due to evolved genetic differences.

Population growth rates were measured by growing each subsample of 300 individuals in microcosms as above. Population size was estimated by counting the number of individuals in a 0.1-mL random subsample of each culture every six hours for 48 hours, or until populations peaked in abundance. Populations displayed exponential growth up to a peak and then abundance dropped off rapidly. Growth rates were determined by estimating  $r$  from the best-fit exponential growth model ( $N_0 = N_0 e^{rt}$ , where  $N_0$  is initial population size and  $t$  is time), using abundance data up to the peak. In June 2007, three additional traits were measured in each replicate: cell size, swimming speed, and refuge use. Digital videos and image analysis (NIH Image J; software *available online*)<sup>2</sup> were used to estimate cell area (mm<sup>2</sup>) and swimming speed (mm/s) of the first 30 individuals encountered in the video. Refuge use was determined by removing two 0.1-mL samples from each microcosm, one in the water column and one in the beads, and determining refuge use as  $\log(\text{number in beads}/\text{number in water})$ . This measure of refuge use provides an unbounded index, with a value of 0 indicating equal densities in the beads and water.

Predators may select for prey traits in two ways: directly, by selectively killing organisms with particular traits, or indirectly, by reducing prey densities and intraspecific competition (Schröder et al. 2009). In March and June of 2007, we used a third selection treatment to control for prey densities and attempted to elucidate the mechanism of predator selection. In this density-reduction treatment, densities were reduced every 24 hours to the median density in the predator treatment. An appropriate amount of liquid was removed from each tube, containing a random sample of *Colpoda*, and replaced with the same amount of sterile water.

#### *Statistical analyses*

All data met the assumptions of parametric tests and statistical analyses were performed in JMP version 4.0.4

<sup>2</sup> (<http://rsb.info.nih.gov/ij/>)

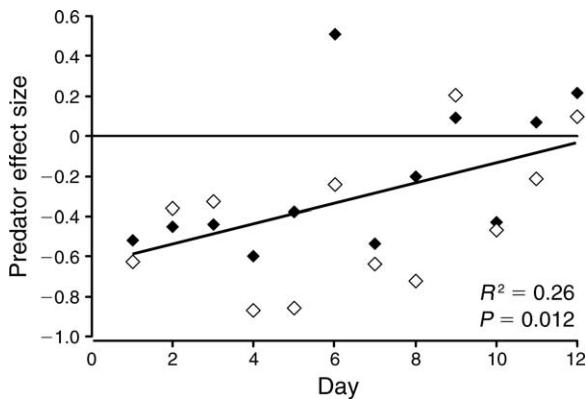


FIG. 1. The effect of predators on prey abundance over the course of 12 days in two time blocks: October (open diamonds) and January (solid diamonds). Effect size is the abundance in the predator treatment relative to the monoculture treatment. Negative values indicate a negative effect of predation on prey abundance.

(SAS Institute, Inc., Cary, North Carolina, USA). Linear regression was used to determine whether predator effect size changed over the course of the ecological effects experiment. Data within time blocks were tested for autocorrelation in JMP to ensure that they were suitable for linear regression. All ANOVAs described below were unrestricted two-way mixed-model ANOVA accounting for unbalanced design (Quinn and Keough 2003) comparing selection environment (no predator vs. predator) as a fixed factor and time block as a random factor. Population growth rates in predator environments were compared among predator and no-predator selection lines using ANOVA to examine differences in adaptation to predation. A second ANOVA compared population growth rate using all three selection treatments (no predator, predator, and density reduction). To determine which traits evolved in different selection environments, we used a series of ANOVAs (one per trait) to test for differences in trait values (measured in the no-predator common environ-

ment) between populations evolved with and without a predator. Tukey post hoc tests were used to determine significant differences among pair-wise treatment levels. An additional single factor ANOVA for each trait was used to compare trait values in all three selection treatments.

Traits are often not independent of one another and trade-offs among traits lead to the evolution of one trait in response to selection on another. To test for such effects, we used Pearson product-moment correlations to determine if there were any significant relationships between each pair-wise combination of traits in June 2007.

## RESULTS

Initially, predators had a negative ecological effect on prey abundance, reducing the abundance by approximately 50% (Fig. 1). This effect decreased significantly over the course of 12 days. By the end of the experiment, prey abundance in some replicates was higher in populations with a predator relative to control populations, consistent with an evolved response of *Colpoda* that decreased predator effect size. Effect size data were not autocorrelated with respect to any time lags.

In the selection experiment, populations that evolved with a predator had higher population growth rates in a predator environment than populations that evolved without a predator ( $F = 16.2$ ,  $df = 1, 4$ ,  $P = 0.013$ ; Fig. 2). There was no effect of time block on population growth rate ( $F = 3.03$ ,  $df = 4, 4$ ,  $P = 0.15$ ), nor was there an interaction between time block and selection environment ( $F = 1.69$ ,  $df = 4, 74$ ,  $P = 0.16$ ). In the density reduction treatment, *Colpoda* grown with predators exhibited population growth rates intermediate to that in the predator and no-predator treatments (Appendix A).

Trait evolution was measured in a common, no-predator environment. Population growth rate (assumed to be correlated with cell division rate) was again significantly higher in populations that evolved with a

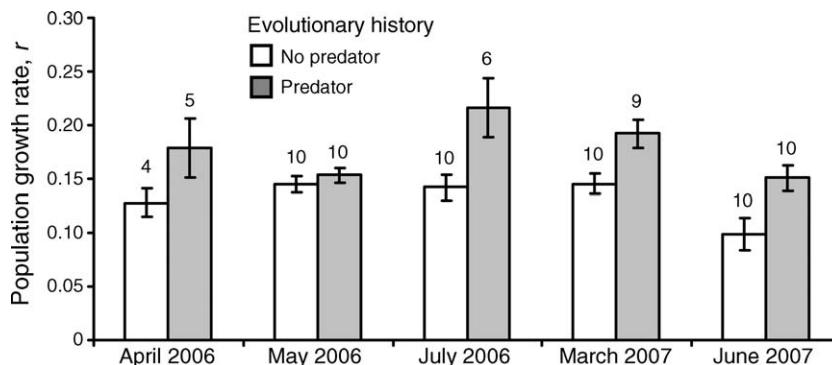


FIG. 2. Population growth rate after selection (measured in the presence of predators) of populations evolved without predation (white bars) and populations evolved with predation by mosquito larvae (gray bars) in five independent experiments. Error bars indicate  $\pm$ SE. Numbers above bars indicate the sample size (number of replicate microcosms from which measurements could be taken) for each. Note that population growth rate is a unitless measure.

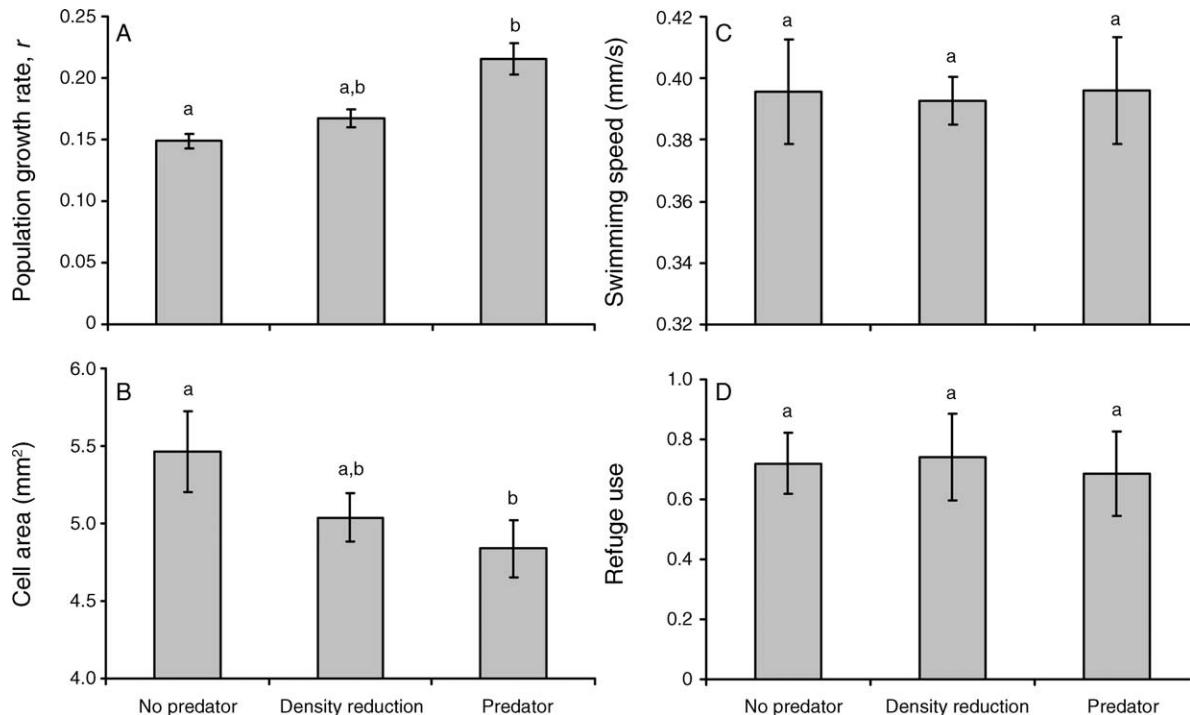


FIG. 3. Traits after selection in three environments, measured in a common environment without predators: (A) population growth rate, (B) cell area, (C) swimming speed, and (D) refuge use. A value of zero for refuge use indicates no preference for the refuge. Positive values indicate increased use of the refuge. The determination of refuge use is explained in *Methods: Selection experiments*. Error bars indicate  $\pm$ SE. Lowercase letters indicate significant pairwise differences among treatment means ( $P < 0.05$ , using Tukey post hoc tests).

predator ( $F = 9.76$ ,  $df = 1, 5$ ,  $P = 0.025$ ; Fig. 3A). There was no significant difference in growth rate among time blocks ( $F = 2.72$ ,  $df = 5, 5$ ,  $P = 0.15$ ). There was a significant block  $\times$  treatment interaction ( $F = 9.83$ ,  $df = 5, 73$ ,  $P < 0.001$ ) because the magnitude of the effect varied among time blocks. However, the mean growth rate in each block was always higher in the populations that evolved with a predator (Appendix B). Cell area decreased significantly in populations that evolved with a predator (Fig. 3B). In the density reduction treatment, population growth rate and cell area were intermediate to the predator and no-predator treatments and not significantly different from either the predator or no-predator treatment (Fig. 3). There was no difference in swimming speed (Fig. 3C) or refuge use (Fig. 3D) among treatments.

All pair-wise correlations between trait values were nonsignificant ( $P > 0.05$ ). Marginally significant correlations were found between population growth rate and refuge use ( $r = 0.34$ ,  $P = 0.066$ ) and cell size and swimming speed ( $r = 0.32$ ,  $P = 0.086$ ). The two traits that evolved in response to predation (population growth rate and cell size) were the least correlated traits among all comparisons ( $r = 0.004$ ,  $P = 0.99$ , Fig. 4).

#### DISCUSSION

Evolutionary processes have generally been ignored in ecological experiments. However, we have demonstrated

that the evolution of prey traits is associated with a qualitative and significant decrease in the ecological effect of predators on prey populations over the course of a relatively short experiment (Figs. 1 and 2). While evolution on ecological time scales has been recently

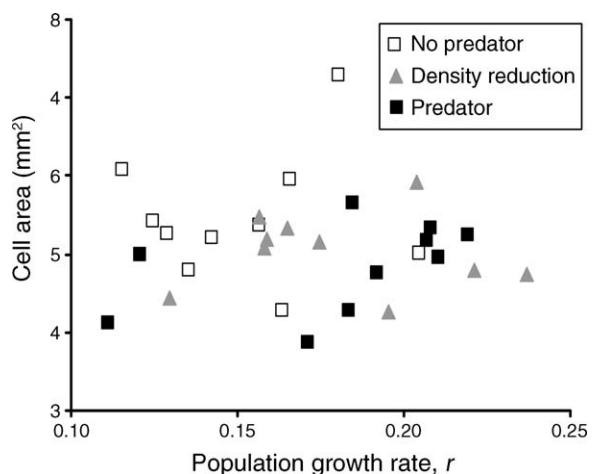


FIG. 4. Correlations between population growth rate and cell area in populations from three selection environments. Overall, there is no correlation between the two traits, nor is there any correlation within environments (no predator,  $r = 0.09$ ,  $P = 0.81$ ; density reduction,  $r = -0.05$ ,  $P = 0.90$ ; predator,  $r = 0.48$ ,  $P = 0.16$ ).



PLATE 1. (Left) Leaf of the carnivorous purple pitcher plant (*Sarracenia purpurea*). (Top right) The aquatic communities that exist inside these leaves include specialist mosquito larvae (*Wyeomyia smithii*) that consume several species of protozoa. (Right bottom) One of these species is *Colpoda* sp., seen here in the adult stage (upper right of picture) with several cysts (lower left of picture). Photo credits: Ray Stanyard; right pair, C. P. terHorst.

demonstrated in other systems, we note that the discrepancies in generation times between predator and prey, and perhaps between rates of evolution, may be particularly important for explaining ecological patterns. At the beginning of our experiment, predators had a large negative effect on protozoa abundance, reducing abundance by over 50% relative to control populations. Yet a short time later, prey reproduction was similar to the consumption rate of the predators.

Although there is an overall decrease in the ecological effect of predators over time, we note a “saw-tooth” pattern in the data that repeats every three days, corresponding to each time the tubes were reinitiated with *Colpoda*, mosquito larvae, and the associated bacteria. Two patterns might explain this result: bacterial evolution or predator satiation. Bacterial evolution, although likely to occur, is unlikely to explain the observed pattern, which would require an increase in bacteria beneficial to the *Colpoda* population. However, consumption by protozoa should select against these bacteria. Predator satiation may explain the decrease in effect over a three-day period, producing the saw-tooth pattern, but since new mosquito larvae were used every three days, the most likely explanation for the decrease in effect over the span of the experiment is evolution of the protozoa prey.

Evolution of specific traits reduced the ecological effect of predators by both increasing intrinsic population growth rates and reducing consumption through a size refuge. Our results are similar to those from

previous studies in aquatic microcosms, which also suggest that prey populations can evolve predator resistant traits that mask the effect of predation (Meyer et al. 2006, Yoshida et al. 2007). Evolution of such traits may affect the population dynamics of both predator and prey and influence the coexistence of both species (Shertzer et al. 2002, Yoshida et al. 2003).

The evolution of cell size and population growth rate conferred adaptations to predation by different mechanisms: predator resistance and predator tolerance. Predator-resistant traits are those that decrease consumption and have negative effects on the predator, such as toxin production, refuge use, or in our case, body size. Mosquito larvae in pitcher plants prefer larger prey items, with larger prey such as rotifers (*Habrotrocha rosea*) being heavily preferred by mosquito larvae over smaller protozoa such as *Poteroochromonas* or *Bodo* (Kneitel 2002, Leibold and Miller 2004). Selective predation by mosquito larvae likely drove the evolution of smaller cell sizes in our experiment (Fig. 3B). Conversely, predator-tolerant traits are those that do not reduce consumption and have little effect on the predator, but rather allow the consumed individual or population to compensate for losses to predation. In this study, selection for rapidly dividing genotypes may have resulted in the evolution of higher population growth that allowed the prey to keep pace with the rate of predation (Fig. 3A; Appendix B). We do not know all the individual traits that determine the growth rate of the population. Predator-resistant traits, for instance, may confer higher population growth rate in the presence of a predator, but we also observed higher growth rates in the absence of predators. Since population growth rate was uncorrelated with any other trait (Fig. 4), an increase in cell division rate seems the most parsimonious explanation for the increase in population growth rate.

Predator (or herbivore) tolerant traits, such as compensatory growth, have been well-studied in terrestrial plants and are thought to be constrained by trade-offs with predator resistant traits, such as physical or chemical defense (Coley et al. 1985, Mole 1994, Zangerl and Berenbaum 1997, Messina et al. 2002). Conversely, a common relationship between size and age at maturity predicts that we might find a positive association between tolerance (high growth rate) and resistance (small cell size) in our system: if cells divide before they reach their maximum size, quickly dividing cells should be smaller than slowly dividing cells. However, we found no support for either hypothesis in our *Colpoda* populations (Fig. 4). Note however that a trade-off between traits may be concealed by the different levels at which we measured such traits (growth rate at the population level vs. size at the individual level). If different individuals solve a trade-off restriction differently (e.g., grow fast or grow big), this will not be evident at the population level.

Our density-reduction treatment was intended to distinguish between two mechanisms by which predators might alter the evolution of traits in prey populations: direct selection or indirect effects on intraspecific competition (Schröder et al. 2009). However, the power of our design was insufficient to distinguish among these alternate hypotheses. This ambiguous result could have been influenced by both direct and indirect factors operating simultaneously or because the frequency and intensity of the density reduction was less than the predator effect. Ongoing work will attempt to tease apart these effects, but regardless of the mechanism, predation significantly altered the evolution of prey.

Experiments in the laboratory often differ from natural conditions. Populations with different evolutionary histories may respond differently when placed in a “common garden” in the laboratory and we can not entirely dismiss this as a possibility in explaining our results. The evolution of at least two traits in *Colpoda* (cell size and growth rate) conferred adaptation to predation in the laboratory, but these same traits may not be adaptive in all environments. Evolution of predator-resistant traits often comes at the cost of lower competitive ability (e.g., Kerfoot 1977, Wulff 2005). Previous work demonstrated a trade-off between predator tolerance and competitive ability among bacterivorous species in pitcher plant inquiline communities (Kneitel 2002, Leibold and Miller 2004). Species composition of these communities changes dramatically with leaf age. Mosquito larvae are most abundant in young leaves (younger than 10 weeks; Miller and Kneitel 2005), but both larval and bacterial abundance decline with leaf age as bacterivore abundance increases, resulting in more competition for bacteria (T. E. Miller and C. P. terHorst, unpublished data). It is unknown whether the evolution of predator tolerance and resistance traits is maladaptive in competitive communities or whether *Colpoda* is able to adapt via evolution or plasticity to these conditions.

Experimental manipulations often have unintended consequences on ecological experiments. For example, recognition that phenotypic plasticity may alter outcomes ensures that common gardens are regularly used in experiments. Likewise, strong selection imposed by ecological experiments requires that ecologists incorporate controls for evolutionary effects in ecological experiments (Strauss et al. 2008), but this is rarely the case. Our results demonstrate that evolution can have large effects on the outcome of ecological experiments. These results need not be limited to microcosm communities. Treatments in many ecological experiments impose strong selection on the manipulated population, which may result in evolution even in short-term experiments (Strauss et al. 2008). Species with long generation times may not be exempt from evolutionary effects. Although longer-lived predators were unable to evolve over the course of our experiment, their population dynamics are likely to be affected by

evolution of their food source. Discrepancies in generation times are not unique to microcosm communities. For instance, many plants have short generation times relative to mammalian herbivores, but long generation times relative to arthropod herbivores. Discrepancies in generation times increase when one considers other associate species such as microbial symbionts, mycorrhizal fungi, or pathogenic bacteria or viruses. Evolution in any associate species may affect ecological measurements on the focal species. We caution experimental ecologists to begin recognizing evolution as a possible explanation of ecological results and to incorporate appropriate controls into experimental protocols.

#### ACKNOWLEDGMENTS

We thank A. Jenkins and C. Stokes for their help in measuring population growth rates in the laboratory. D. Houle, D. Reznick, J. A. Stallins, and J. Wulff provided valuable advice on this work. The manuscript was improved by comments from two anonymous reviewers. This work was supported by a FSU Dissertation Research Grant and Kingsbury Fellowship (FSU English Department) to C. P. terHorst and grants from the National Science Foundation to T. E. Miller (DEB 0519170 and DEB 0716891).

#### LITERATURE CITED

- Alatalo, R. V., and J. Mappes. 1996. Tracking the evolution of warning signals. *Nature* 382:708–710.
- Bradshaw, W. E., and K. Johnson. 1995. Initiation of metamorphosis in the pitcher-plant mosquito: effects of larval growth history. *Ecology* 76:2055–2065.
- Case, T. J. 2000. An illustrated guide to theoretical ecology. Oxford University Press, New York, New York, USA.
- Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. *Science* 230:895–899.
- Dunthorn, M., W. Foissner, and L. A. Katz. 2008. Molecular phylogenetic analysis of class Colpodea (phylum Ciliophora) using broad taxonomic sampling. *Molecular Phylogenetics and Evolution* 46:316–327.
- Englund, G., O. Sarnelle, and S. D. Cooper. 1999. The importance of data-selection criteria: meta-analyses of stream predation experiments. *Ecology* 80:1132–1141.
- Fujii, K. 1999. Overview of S. Utida's research. *Researches in Population Ecology* 41:11–13.
- Futuyma, D. J., and M. Slatkin. 1983. *Coevolution*. Sinauer Associates, Sunderland, Massachusetts, USA.
- Gurevitch, J., J. A. Morrison, and L. V. Hedges. 2000. The interaction between competition and predation: a meta-analysis of field experiments. *American Naturalist* 155:435–453.
- Hairton, N. G., Jr., S. P. Ellner, M. A. Geber, T. Yoshida, and J. A. Fox. 2005. Rapid evolution and the convergence of ecological and evolutionary time. *Trends in Ecology and Evolution* 8:1114–1127.
- Huffaker, C. B. 1958. Experimental studies on predation: dispersion factors and predator-prey oscillations. *Hilgardia* 27:343–383.
- Johnson, M. T. J., and J. R. Stinchcombe. 2007. An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology and Evolution* 22:250–257.
- Kerfoot, W. C. 1977. Competition in cladoceran communities: cost of evolving predation against copepod predation. *Ecology* 58:303–313.
- Kneitel, J. M. 2002. Species diversity and trade-offs in pitcher plant (*Sarracenia purpurea*) inquiline communities. Dissertation. Florida State University, Tallahassee, Florida, USA.

- Kneitel, J. M., and T. E. Miller. 2002. Resource and top-predator regulation in the pitcher plant (*Sarracenia purpurea*) inquiline community. *Ecology* 83:680–688.
- Leibold, M. A., and T. E. Miller. 2004. From metapopulations to metacommunities. Pages 133–150 in I. Hanski and O. Gaggiotti, editors. *Ecology, genetics, and evolution of metapopulations*. Academic Press, San Diego, California, USA.
- Lüftnegger, G., W. Foissner, and H. Adam. 1990. *r*- and *K*-selection in soil ciliates: a field and experimental approach. *Oecologia* 66:574–579.
- Mauricio, R., and M. D. Rausher. 1997. Experimental evidence of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution* 51:1435–1444.
- Messina, F. J., S. L. Durham, J. H. Richards, and E. D. McArthur. 2002. Trade-off between plant growth and defense? A comparison of sagebrush populations. *Oecologia* 131:43–51.
- Meyer, J. R., S. P. Ellner, N. G. Hairston, L. E. Jones, and T. Yoshida. 2006. Prey evolution on the time-scale of predator-prey dynamics revealed by allele-specific quantitative PCR. *Proceedings of the National Academy of Sciences (USA)* 103:10690–10695.
- Miller, T., D. Cassill, C. Johnson, C. Kindell, J. Leips, D. McInnes, T. Bevis, D. Mehlman, and B. Richard. 1994. Intraspecific and interspecific competition of *Wyeomyia smithii* (COQ) (Culcidae) in pitcher plant communities. *American Midland Naturalist* 131:136–145.
- Miller, T. E., and J. M. Kneitel. 2005. Inquiline communities in pitcher plants as prototypical metacommunities. Pages 122–145 in M. Holyoak, M. A. Leibold, and R. D. Holt, editors. *Metacommunities: spatial dynamics and ecological communities*. University of Chicago Press, Chicago, Illinois, USA.
- Miller, T. E., J. M. Kneitel, and J. H. Burns. 2002. Effect of community structure on invasion success and rate. *Ecology* 83:898–905.
- Mole, S. 1994. Trade-offs and constraints in plant-herbivore defense theory: a life history perspective. *Oikos* 71:3–12.
- Nunezfarfan, J., and R. Dirzo. 1994. Evolutionary ecology of *Datura stramonium* L. in central Mexico: natural selection for resistance to herbivorous insects. *Evolution* 48:423–436.
- Quinn, G., and M. Keough. 2003. *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge, UK.
- Reimchen, T. E., and P. Nosil. 2002. Temporal variation in divergent selection on spine number in threespine stickleback. *Evolution* 56:2472–2483.
- Schmitz, O. J., P. A. Hamback, and A. P. Beckerman. 2000. Trophic cascades in terrestrial systems: a review of the effect of carnivore removals on plants. *American Naturalist* 155:141–153.
- Schröder, A., L. Persson, and A. M. de Roos. 2009. Culling experiments demonstrate size-class specific biomass increases with mortality. *Proceedings of the National Academy of Sciences (USA)* 106:2671–2676.
- Seeley, R. H. 1986. Intense natural selection caused by a rapid morphological transition in a living marine snail. *Proceedings of the National Academy of Sciences (USA)* 83:6897–6901.
- Shertzer, K. W., S. P. Ellner, G. F. Fussmann, and N. G. Hairston. 2002. Predator-prey cycles in an aquatic microcosm: testing hypotheses of mechanism. *Journal of Animal Ecology* 71:802–815.
- Shonle, I., and J. Bergelson. 2000. Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae). *Evolution* 54:778–788.
- Shurin, J. B., E. T. Borer, E. W. Seabloom, K. Anderson, C. A. Blanchette, B. Broitman, S. D. Cooper, and B. S. Halpern. 2002. A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters* 5:785–791.
- Slobodkin, L. B. 1961. *Growth and regulation of animal populations*. Holt, Rinehart and Winston, New York, New York, USA.
- Srivastava, D. S., J. Kolasa, J. Bengtsson, A. Gonzalez, S. P. Lawler, T. E. Miller, P. Munguia, T. Romanuk, D. C. Schneider, and M. K. Trzcinski. 2004. Are natural microcosms useful model systems for ecology? *Trends in Ecology and Evolution* 19:379–384.
- Strauss, S. Y., J. A. Lau, T. W. Schoener, and P. Tiffin. 2008. Evolution in ecological field experiments: implications for effect size. *Ecology Letters* 11:199–207.
- Thompson, J. N. 1998. Rapid evolution as an ecological process. *Trends in Ecology and Evolution* 13:329–332.
- Van Valen, L. 1973. A new evolutionary law. *Evolutionary Theory* 1:1–30.
- Wulff, J. L. 2005. Trade-offs in resistance to competitors and predators, and their effects on the diversity of tropical marine sponges. *Journal of Animal Ecology* 74:313–321.
- Yoshida, T., S. P. Ellner, L. E. Jones, B. J. M. Bohannan, R. E. Lenski, and N. G. Hairston. 2007. Cryptic population dynamics: rapid evolution masks trophic interactions. *PLoS Biology* 5:1868–1879.
- Yoshida, T., L. E. Jones, S. P. Ellner, G. F. Fussmann, and N. G. Hairston. 2003. Rapid evolution drives ecological dynamics in a predator-prey system. *Nature* 424:303–306.
- Zangerl, A. R., and M. R. Berenbaum. 1997. Cost of chemically defending seeds: Furanocoumarins and *Pastinaca sativa*. *American Naturalist* 150:491–504.

#### APPENDIX A

Population growth rate (measured in the presence of predators) of populations evolved without predation, with predation, and under low-density conditions (*Ecological Archives* E091-046-A1).

#### APPENDIX B

Population growth rate (measured in the absence of predators) of populations evolved without predation and populations evolved with predation by mosquito larvae in six independent experiments (*Ecological Archives* E091-046-A2).