Form of an evolutionary tradeoff affects eco-evolutionary dynamics in a predator–prey system

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Major category: Biological Sciences

Minor category: Ecology

Short title: evolutionary tradeoffs and eco-evo feedbacks

Keywords: allele-specific quantitative PCR, *Chlorella vulgaris*, clonal models, grazing resistance, rapid evolution

Abstract

Evolution on a time scale similar to ecological dynamics has been increasingly recognized for the last three decades. Selection mediated by ecological interactions can change heritable phenotypic variation (i.e., evolution), and evolution of traits, in turn, can affect ecological interactions. Hence, ecological and evolutionary dynamics can be tightly linked and important to predict future dynamics, but our understanding of eco-evolutionary dynamics is still in its infancy and there is a significant gap between theoretical predictions and empirical tests. Empirical studies have demonstrated that the presence of genetic variation can dramatically change ecological dynamics, whereas theoretical studies predict that eco-evolutionary dynamics depend on the details of the genetic variation, such as the form of a tradeoff among genotypes, which can be more important than the presence or absence of the genetic variation. Using a predator–prey (rotifer–algal) experimental system in laboratory microcosms, we studied how different forms of a tradeoff between prey defense and growth affect eco-evolutionary dynamics. Our experimental results show for the first time to our knowledge that different forms of the tradeoff produce remarkably divergent eco-evolutionary dynamics, including near

fixation, near extinction, and coexistence of algal genotypes, with quantitatively different population dynamics. A mathematical model, parameterized from completely independent experiments, explains the observed dynamics. The results suggest that knowing the details of heritable trait variation and covariation within a population is essential for understanding how evolution and ecology will interact and what form of eco-evolutionary dynamics will result.

Significance Statement

Rapid evolution on an ecological time scale has been increasingly recognized. Ecological and evolutionary dynamics can be tightly linked and important to predict future dynamics, but there is a significant gap between theoretical predictions and empirical tests, especially on the effects of the nature of genetic variation such as the form of a fitness tradeoff. Using a predator–prey experimental system, we show for the first time to our knowledge that different forms of a fitness tradeoff produce remarkably divergent eco-evolutionary dynamics. A mathematical model supports the observed dynamics. Our results suggest that without knowing the details of genetic variation that is usually variable among wild populations, it is difficult to understand how evolution and ecology interact and what form of eco-evolutionary dynamics results.

Evolutionary dynamics, changes in intraspecific genotype frequency over generations, can have a time scale similar to that of ecological dynamics (1–3). Selection mediated by ecological interactions causes evolutionary dynamics, and evolution of traits, in turn, changes ecological interactions. Thus, understanding population dynamics needs to take account of the feedbacks between trait evolution and ecological interactions (i.e., eco-evolutionary feedbacks). These feedbacks have increasingly attracted ecologists' attention since Pimentel (4) proposed genetic feedback as a mechanism regulating animal populations (e.g., ref. 5–11). This integration of evolutionary biology and ecology has important implications in both basic and applied problems in biology (12–17).

Empirical studies have shown that rapid evolution can affect many ecological interactions, including predator–prey (18–20), host–parasite (21), herbivore–plant (22), competitive interactions (23), and interactions with abiotic environments (24–27). Previous empirical studies on eco-evolutionary feedbacks have usually compared the dynamics of populations with and without genetic variation, but recent theoretical models predicted that not only the presence or absence of genetic variation (28–30) but

also the form of the evolutionary tradeoff among genotypes is important in generating qualitatively different dynamics (31–35). Indeed, the forms of evolutionary tradeoffs within populations are known to be remarkably variable in plants and microbes (36–38). Thus, there should be various eco-evolutionary dynamics depending on the form of evolutionary tradeoffs existing in wild populations. Nevertheless, to our knowledge, no empirical study has directly demonstrated the theoretically predicted effects of the evolutionary tradeoff on eco-evolutionary dynamics, and it is still unclear how different forms of an evolutionary tradeoff in real organisms can result in different eco-evolutionary dynamics.

Here, using a predator–prey (rotifer–algal) system cultured in continuous flow-through microcosms (chemostats), we examined how different forms of an evolutionary tradeoff between defense and growth in algal prey (*Chlorella vulgaris*) affect the population dynamics of the predator–prey system and the evolutionary changes in the clonal frequency of the algal prey. Experimental studies using laboratory microcosms have been a powerful approach in exploring eco-evolutionary dynamics and testing theoretical predictions because of the constant environment and simple

community structure (39–41). We used two different pairs of algal clones originally obtained from the University of Texas (UTEX) algal collection that showed different forms of a fitness tradeoff between antipredator defense and competitive ability to obtain the resource limiting population growth in the experimental system (inorganic nitrogen). Each pair of algal clones was cultured with an obligately asexual lineage of rotifer predators (*Brachionus calyciflorus*). Population dynamics of the predators and prey and clonal frequency changes in the algal pair were observed in long-term chemostat runs. We recorded evolutionary dynamics (genotype frequency change) by using an allele-specific quantitative PCR (AsQ-PCR) technique based on microsatellite DNA that allowed us to measure the relative abundance of algal clones (42). We also developed a mathematical model for the experimental system, based on a model of Jones and Ellner (43), parameterized the model using data from separate experiments, and compared the model's predictions to the observed population and genotype dynamics.

Results

Both pairs of algal clones that we used showed an evolutionary tradeoff between defense against rotifer predation and reproductive ability (Fig. 1). The pair of UTEX1809 and UTEX1811 clones had a relatively "costly defense" tradeoff: Defense is not very effective despite a huge reduction in growth rate. UTEX1809 is a fast-growing but undefended alga, and UTEX1811 is a more defended but slowly growing alga (maximum growth rate, *t* test, $t = 4.992$, $P < 0.01$; defense, *t* test, $t = 3.683$, $P < 0.05$; Fig. 1). The pair of UTEX396 and UTEX265 clones, which was already known to have a tradeoff (42), had a relatively "cheap defense" tradeoff compared with the UTEX1809–1811 pair: Defense is effective, even though the difference in growth rate is small. UTEX396 has higher population growth rate, whereas UTEX265 is more defended against rotifer predation (maximum growth rate, *t* test, $t = 2.138$, $P < 0.05$; palatability, *t* test, $t = 3.338$, $P < 0.05$; Fig. 1). Meyer et al. (42) showed that the rotifers fed on the algal clones unselectively, but the defended clone was defecated in a viable state by rotifers much more frequently than the undefended clone in the UTEX396–265 pair.

In the costly defense tradeoff pair, population and evolutionary dynamics

were similar among the three replicate experiments (Fig. 2). Before rotifers increased in abundance at the beginning of the experiment, the competitive clone (UTEX1809) was dominant in the algal population. As rotifers increased, the defended clone (UTEX1811) became advantageous and increased in frequency, whereas the total abundance of algae declined dramatically. However, the dominance of the defended clone was temporary. The competitive clone eventually increased again and went to near fixation (remarkably dominant in the population), probably because of the high cost of defense in this pair of algal clones (Fig. 1). Meanwhile, rotifer abundance gradually decreased after 30 d, whereas the competitive, undefended clone increased slightly more, which might suggest that the undefended algal clone evolved to be less palatable. Then, rotifer and algal densities stayed almost constant. Note that one of the replicates had to be terminated owing to bacterial contamination of the inflowing fresh medium before reaching equilibrium of rotifers and algae (Fig. 2*E* and *F*).

In the cheap defense tradeoff pair, we observed two different types of population and evolutionary dynamics (Fig. 3), both of which were quite different from those with the costly defense tradeoff pair. One type of dynamics was characterized by

coexistence of the two algal clones at similar frequency (Fig. 3*B* and *D*) and relatively low abundance of rotifers (Fig. 3*A* and *C*). At the beginning of the experiment, when algal abundance quickly declined as rotifers increased, the algal clonal frequencies fluctuated greatly. This was followed by dampening of the fluctuations to some extent and resulted in the coexistence of the algal clones. Fluctuation of rotifer abundance followed the fluctuations in algal genotype frequency rather than the fluctuations in total algal abundance in Fig. 3*C* and *D*, suggesting the influence of algal clonal frequency on rotifer population growth (Fig. S1). The second type of dynamics with the cheap defense pair was characterized by dominance or near fixation of the defended algal clone (Fig. 3*F* and *H*), probably because of the cheap defense. Rotifer density tended to be higher when the defended clone was selected for, followed by decline of rotifer density as the defended clone continued to be dominant in the algal population (Fig. 3*E*–*H*). This type of dynamics with this pair of algal clones was consistent with previous results for the same pair (42), whereas the first type of the dynamics (Fig 3*A*–*D*) was not observed in the previous study.

To understand the experimental results, we analyzed a mathematical model

based on a model of Jones and Ellner (43). Here we briefly explain the model and results (see *SI Text* for details). The model describes the population and evolutionary dynamics of the rotifer–algal system cultured in a chemostat as in our experiment. The algal population consists of two clones that have a tradeoff between defense against predation and reproductive ability. The model was parameterized completely from previous and present experimental results separate from the chemostat runs (Table S1), except for the parameter representing algal "palatability" (vulnerability to rotifer predation). Our measured palatability cannot be used directly as the palatability parameter in the model; however, the ratio of measured palatabilities provides an estimate for the relative values of the palatability parameter (see *SI Text* for details). We therefore assumed that the palatability parameters of the algal strains were proportional to the experimentally measured values (Fig. 1). We calculated the palatability parameters of the four clones so that the relative palatability values were the same in the model as in the observed data. We found a set of palatability parameters (Fig. S2), subject to this constraint, such that the model reproduced the observed population and evolutionary dynamics of both clone pairs (Fig. 4).

With the costly defense tradeoff between UTEX1809 and UTEX1811, the model predicted the fixation of the undefended, competitive clone (UTEX1809) (i.e., competitive exclusion of the defended clone UTEX1811) and equilibrium of rotifer and algal densities (Fig. 4). The fixation of the undefended clone allows the rotifer population to persist, which would go extinct only if the defended clone is present (Fig. S3). With the cheap defense tradeoff between UTEX396 and UTEX265, the tradeoff parameters were very near the border of two different types of dynamics (Fig. 4). One type is the fixation of defended clone (competitive exclusion of undefended clone) and the equilibrium of predators and prey, and the other type is the coexistence of two clones and the equilibrium of predators and prey (Fig. 4). This suggests that an experimental system could display either type of dynamics (as we observed in our experiments with this clone pair), depending on slight changes in conditions such as the chemostat dilution rate (i.e., the rate at which nutrient is continuously added to the chemostat and all components are removed). Thus, the model analysis suggests that the form of the tradeoff is important in determining the resulting eco-evolutionary dynamics. This is supported by the additional analysis of the model assuming the scaled

tradeoffs with the same mean trait values and the different forms, showing the consistent results with the model having the original, unscaled tradeoffs (Fig. S4). Also, the model predicts that the system will reach equilibrium irrespective of whether the algal population can evolve or not (Fig. S3). Overall, the model predictions are qualitatively consistent with the experimental data, capturing some quantitative aspects as well (*Discussion*).

Discussion

Our experimental and theoretical results showed that different forms of an evolutionary tradeoff result in qualitatively different eco-evolutionary dynamics. Although theoretical models have often suggested that the details of evolutionary tradeoffs are important in determining eco-evolutionary dynamics, as was predicted in our predator–prey system (43, 44), empirical studies using real organisms have not tested this prediction so far. Here we show, for the first time to our knowledge, that intraspecific genetic variation within an algal species can be large enough to produce different consequences in eco-evolutionary dynamics as a result of differences in the

slope of a tradeoff curve. This confirms that not only the presence or absence of genetic variation but also the actual components of the genetic diversity are important to understand eco-evolutionary feedbacks (45–47). Intraspecific trait variation has been often measured quantitatively, such as the frequency distribution of trait values (14, 24, 48). However, even when the variation of trait values is the same, the form of a tradeoff between different traits can be variable (i.e., the same trait means and variances can be associated with different genetic covariances), and this can result in distinct eco-evolutionary outcomes, as in our study. Thus, measurements of intraspecific trait variation need to include not only the variation of each trait but also the relationships between different traits.

Evolutionary dynamics (clonal frequency changes) were especially different between the two pairs of algal clones showing different forms of tradeoff. For the costly defense tradeoff pair that had relatively large difference in reproductive ability, the palatable, undefended clone became dominant toward the end of the experiment (Fig. 2). In contrast, the defended clone became dominant, or the two clones coexisted with comparable frequencies, for the cheap defense tradeoff pair (Fig. 3). These results make

sense because a high cost of defense favors the undefended clone, whereas cheap defense favors the defended clone. This intuitive understanding was supported by the mathematical model that showed the influence of the tradeoff form on eco-evolutionary dynamics (Fig. 4).

Two qualitatively different dynamics were observed for the cheap defense tradeoff pair. The defended clone was dominant when rotifer density was relatively high, whereas the two clones coexisted with comparable frequencies when rotifer density was relatively low. This can be explained by the mathematical model if the cheap defense tradeoff lies at the boundary of the two different dynamics in the phase diagram shown in Fig. 4. Then, which dynamics the predator–prey system takes can depend on the slight change in the dilution rate of chemostat, which influences the pattern of the phase diagram as well (43, 49, 50). Indeed, the dilution rate was slightly different among the replicated runs of chemostats, as a result of small but unavoidable fluctuations in dilution rate over time. The higher rotifer density when the defended clone was dominant than when the two clones coexisted (Fig. 3) would not be intuitively understandable because rotifer density was lower when palatable, undefended clone was

more abundant. Our model predicts that the rotifer density is higher when the defended clone is dominant than when the two clones coexist (Fig. 4), suggesting that the higher rotifer density should have selected the defended clone.

An alternative explanation of the different dynamics with the cheap defense tradeoff pair would be a dependence on the initial densities of the algal clones and rotifers. Initial clonal frequencies were slightly different among the replicated chemostats, even though the two clones were inoculated into the chemostats with almost identical densities. If the difference in the initial condition affects the following dynamics, it means that the predator–prey system has a bistability (i.e., there are two locally stable states or attractors). However, our mathematical model did not show the bistability corresponding to the observed dynamics. Stage- or age-structured models often show complex multistability, and our results of the UTEX396–265 pair may be explained by alternative stable states driven by structured interactions. For example, McCauley et al. (51, 52) demonstrated that small- and large-amplitude cycles coexisted in *Daphnia*–algal microcosm systems owing to resource-dependent mortality and a dynamic development delay in consumers (*Daphnia*). However, in our case, consumers

are rotifers that do not have as distinct an age structure as daphnids have. Also, previous theoretical studies found that age structure of rotifers (senescence) did not change the dynamics substantially (43, 44). Therefore, the different dynamics with the cheap defense tradeoff pair were likely due to the slight change in the dilution rate, although it remains a challenge for future research to investigate bistability in the predator–prey system (35). It should be noted that the observed different eco-evolutionary dynamics between the two pairs with the different tradeoff forms cannot be explained by the slight change in the dilution rate we had (Fig. S5), although the dilution rate has the significant influence on dynamics.

With respect to eco-evolutionary dynamics, the equilibria of rotifer and algal densities can be seen as qualitatively different depending on the form of the tradeoff. For the costly defense tradeoff, rotifer persistence depends on the evolution of algal prey, in which the palatable clone is selected for and the defended one is selected against (Fig. 4). The defended clone itself cannot support the rotifer population (Fig. S3). However, for the cheap defense tradeoff, the persistence of rotifer population is independent from the algal evolution (Fig. S3).

Our results were in accord with previous studies showing that rapid evolutionary changes can affect the ecological interaction and population dynamics in a predator–prey system (39, 42, 44, 53). Our study provides a previously unidentified insight into the importance of the details of genetic diversity. The details are likely to be very variable owing to intraspecific variation in evolutionary tradeoffs (36–38, 54). Theory predicts that numerous details can greatly affect eco-evolutionary dynamics (55): tradeoffs between defense cost and resource availability (32, 34), interactions between phenotypic plasticity and evolution (33, 56–58), and spatial heterogeneity and gene flow (59, 60). However, empirical studies were lacking. Our experiments demonstrate that the details of genetic diversity can be more important in understanding ecological and evolutionary dynamics in nature than we assumed before. The form of fitness tradeoffs matters.

Materials and Methods

The predator–prey system we used in this study consisted of *B. calyciflorus* (asexually reproducing rotifer predator) and *C. vulgaris* (asexually reproducing algal prey), which

was the same system used in previous studies (42, 49, 53). Because the original algal strains can be composed of multiple clones (50), we isolated a single clone from each strain for the tradeoff and chemostat experiments described below. The all-algal cultures were kept axenic. To examine the clonal frequency changes in the algal population (i.e., natural selection in the population), we used the AsQ-PCR (Allele-specific Quantitative PCR) technique developed by Meyer et al. (42), in which the frequencies of a pair of clones can be quantified by using a microsatellite-DNA marker. Because a pair of algal clones had different microsatellite-DNA sequences, the amount of PCR products amplified from each allele can be used to quantify their frequencies. Note that this method cannot be applied to any arbitrarily chosen pair of clones, but it works for some specific pairs of clones. We used two pairs of clones, UTEX396 and UTEX265, and UTEX1809 and UTEX1811, for each of which the clonal frequency can be accurately quantified by the AsQ-PCR, because the correlation between known and estimated frequencies was highly significant ($r^2 > 0.97$ for the UTEX396–265 pair and $r^2 > 0.98$ for the UTEX1809–1811 pair).

Measuring a Tradeoff Between Palatability and Reproductive Ability

We examined the evolutionary tradeoff for each pair of algal clones. First, we measured the reproductive ability of each clone in the culture medium that was used for the chemostat experiments. The medium was the same as in previous studies (42, 49, 53) and had the limiting nutrient (nitrate) at 80 $\mu mol \cdot L^{-1}$. We inoculated algal cells of each clone (1×10^4 cells per milliliter) into 50 mL of fresh medium with nine replicates per clone, and maintained at 24°C in continuous light (120 $\mu E \cdot m^{-2} \cdot s^{-1}$). Algal density was monitored daily until population growth saturated. Algal densities exponentially increased from low but observable density to nearly saturation, and we estimated the maximum growth rate as the slope of a linear function fitted to log (algal density) versus time using the data during the exponential growth.

To measure the vulnerability to predation ("palatability") of algal clones, we inoculated algal cells of each pair of clones (3.5×10^6 cells per milliliter for each clone) into 50 mL of fresh medium with 100 rotifers. To prevent algal growth, the medium lacked nitrate and the culture was kept in darkness. Three replicates for each clone pair were continuously mixed at 1 rpm on a rotary shaker at 24°C. Algal density was

monitored daily, and we used the data during the period of exponential decline. Clonal frequencies for each pair were determined by using AsQ-PCR at the beginning and end of the exponential decline. Three additional replicates without rotifers were used for the control. Mortality rate *d* was calculated by

$$d = \frac{\log(C_{\text{end}}) - \log(C_{\text{start}})}{t}, \quad [1]$$

where C_{end} and C_{start} were densities of each clone at the end and beginning of the exponential decline (calculated from total algal density and the clone frequencies), respectively, and *t* is time period of the experiment (days). The palatability of each clone was estimated as the difference between the *d* with rotifers present and the *d* with rotifers absent.

Ecological and Evolutionary Dynamics: Chemostat Experiment

We ran rotifer–algal chemostat experiments following the methods of previous studies (44, 49, 53). Our rotifer population consisted of a strain that reproduced only asexually in the chemostat (40). For the algal population, we used two pairs of algal clones (UTEX396 and UTEX265; UTEX1809 and UTEX1811) that showed the different

forms of tradeoff (*Results*). The culture medium was the same as used for the tradeoff experiment, and the dilution rate of chemostat was 0.5 ± 0.1 per day. Chemostats were held at 24°C in continuous light (120 $\mu E \cdot m^{-2} \cdot s^{-1}$). The rotifer and algal densities were measured at 1- to 2-d intervals using a microscope and a cell counter (CASY Model TTC; Roche), respectively. We checked bacteria contamination by monitoring the particle size distribution in fresh samples using the cell counter, but no significant sign of bacteria contamination was detected during the experiments. The frequencies of algal clones were determined by AsQ-PCR as described above.

Acknowledgements

We thank R. Iwadate, O. Mashita, K. Miura, T. Muranaka, M. Ohue, M. Saido, M. Seki, M. Shinya, K. Suzuki, K. Yokoi, and K. Yoshimura for their assistance in laboratory experiments and S. P. Ellner, N. G. Hairston Jr., B. E. Miner, and members of the Ellner laboratory for their helpful comments on the manuscript. We also thank the editor and the anonymous reviewers for their comments that improved the manuscript. This study is supported by a research fellowship of the Japan Society for the Promotion of Science (JSPS) for young scientists (23-8508) (to M.K.), a JSPS postdoctoral fellowship for research abroad (24-869) (to M.Y.), and the Nakajima Foundation, Japan Science and Technology Agency Precursory Research for Embryonic Science and Technology, JSPS Grants-in-Aid for Scientific Research (Grants 19687002, 20370009, and 26291088) (to T.Y.).

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Figures

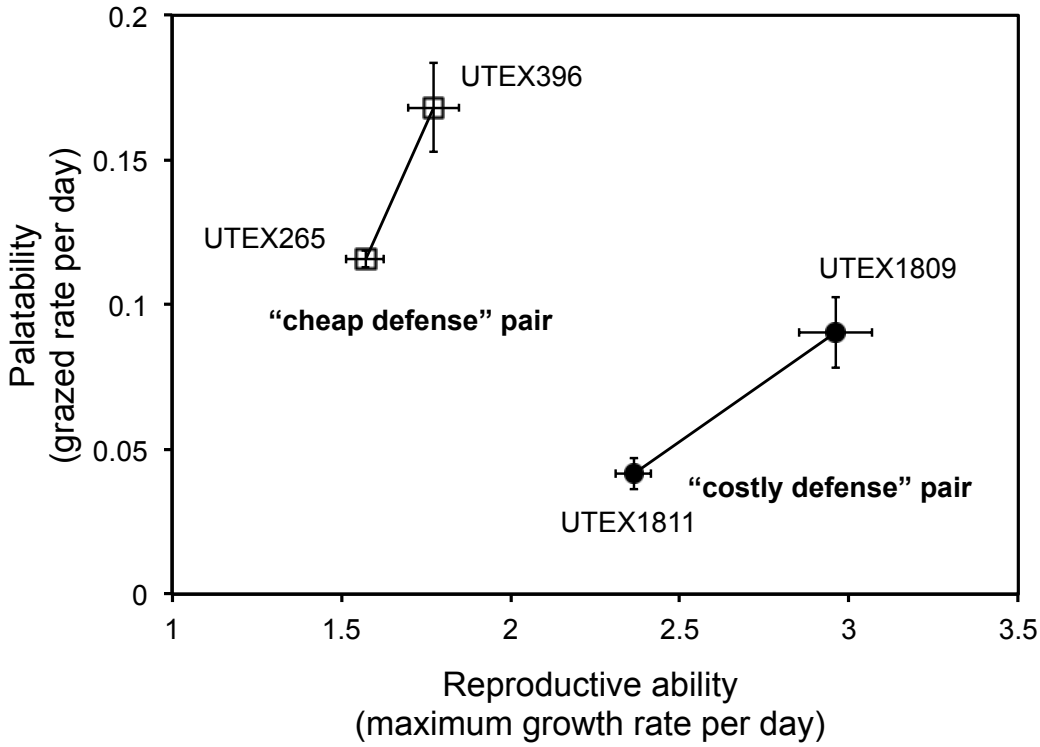

Fig. 1. Different forms of an evolutionary tradeoff between two pairs of algal clones. The pair of UTEX396 and UTEX265 had a cheap defense tradeoff (better defended clone has only slightly lower maximum growth rate) compared with the pair of UTEX1809 and UTEX1811 showing a costly defense tradeoff. Error bars represent SD (n = 3 for palatability, n = 9 for maximum growth rate).

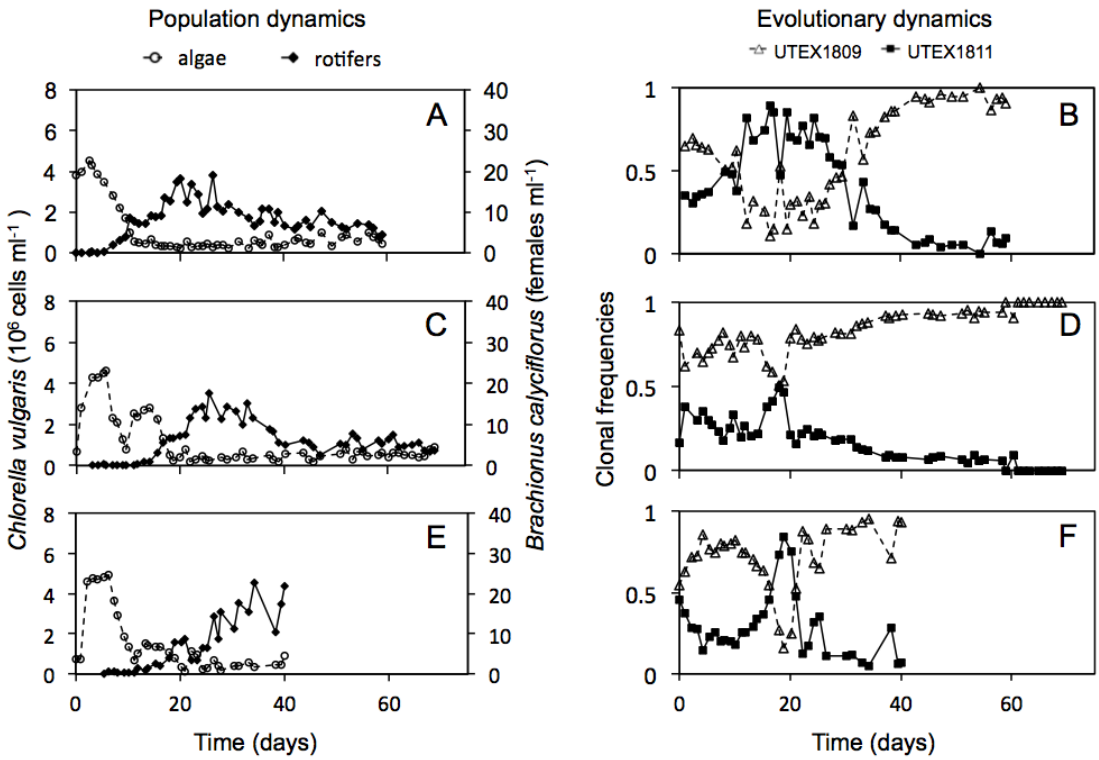

Fig. 2. Population dynamics and evolutionary dynamics for the pair showing a costly defense tradeoff (panels in the same row are data from the same run of chemostat). *A*, *C*, and *E* show rotifer and algal population dynamics, corresponding to *B*, *D*, and *F*, respectively, which show the changes in algal clonal frequencies in the same chemostat. The mean and range of dilution rates during the experiments were 0.55 (0.49–0.58) d^{-1} (*A* and *B*), 0.52 (0.49–0.55) d^{-1} (*C* and *D*), and 0.52 (0.50–0.54) d^{-1} (*E* and *F*).

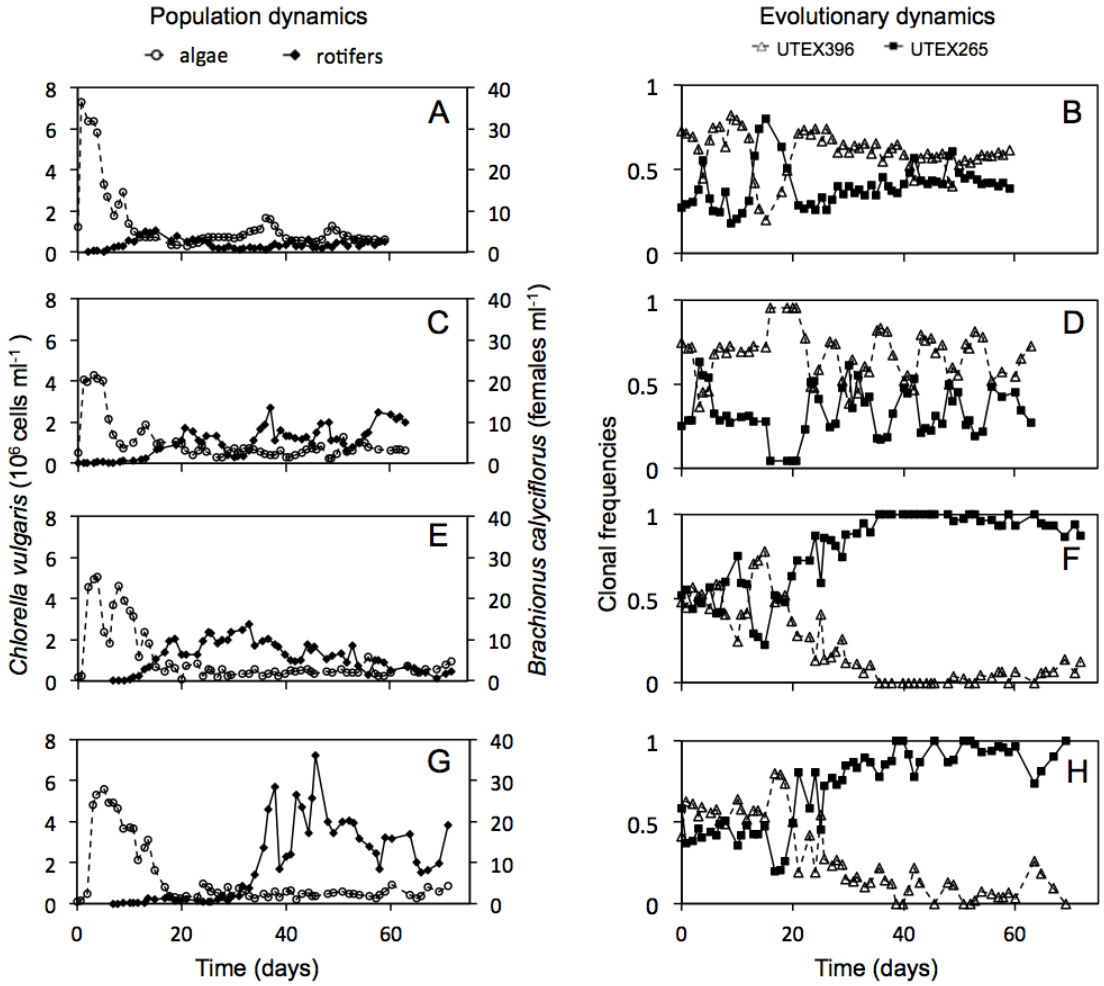

Fig. 3. Population dynamics and evolutionary dynamics for the pair showing a cheap defense tradeoff (panels in the same row are data from the same run of chemostat). *A*, *C*, *E*, and *G* show rotifer and algal population dynamics, corresponding to *B*, *D*, *F*, and *H*, respectively, which show the changes in algal clonal frequencies in the same chemostat. The mean and range of dilution rates during the experiments were 0.44 (0.40–0.50) d^{-1} (*A* and *B*), 0.49 (0.46–0.52) d^{-1} (*C* and *D*), 0.48 (0.42–0.56) d^{-1} (*E* and *F*), and 0.49 (0.48–0.51)d^{-1} (*G* and *H*).

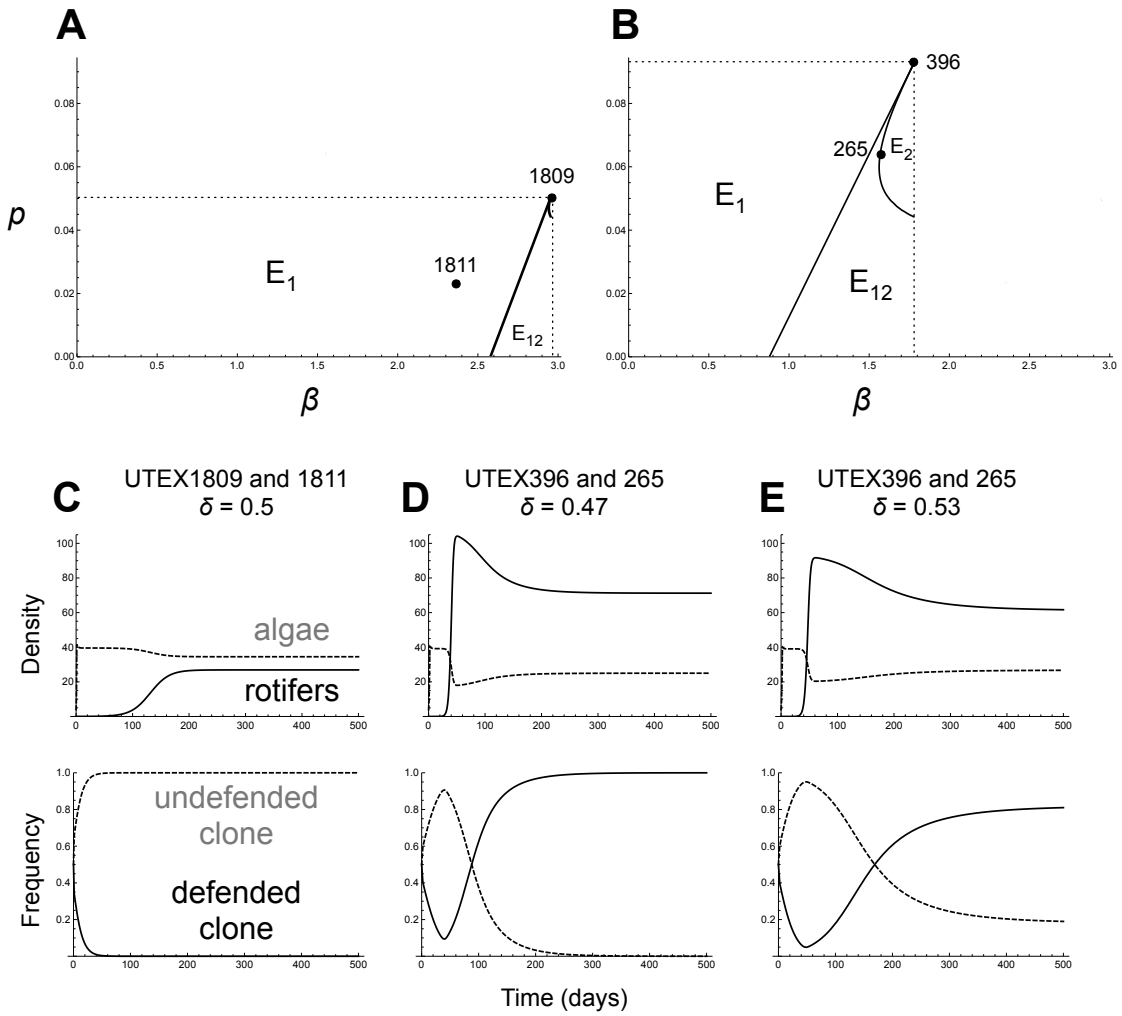

Fig. 4. (*A* and *B*) Phase diagram for each pair of algal clones showing different eco-evolutionary dynamics. Parameters p and β are palatability and maximum recruitment rate, respectively, in the mathematical model (*SI Text*). Black circles represent the estimated parameters based on the experimental results. E_1, stable equilibrium with undefended prey; E_2, stable equilibrium with defended prey; E_{12}, stable equilibrium with coexisting undefended and defended prey. (*C*–*E*) Population

and evolutionary dynamics when p_{11} = 0.055 (*SI Text*) for the UTEX1809–1811 pair when δ = 0.5 (*C*) and the UTEX396–265 pair when δ = 0.47 (*D*) and δ = 0.53 (*E*). Eco-evolutionary dynamics shown in *C*, *D*, and *E* correspond to E_1 in *A* and E_2 and E_{12} in *B*, respectively. Solid lines in upper panels, rotifers (individuals per milliliter); dashed lines in upper panels, total algae (10^5 cells per milliliter); dashed lines in lower panels, frequency of undefended clone; solid lines in lower panels, frequency of defended clone.

SI Text

According to a model of Jones and Ellner (1), dynamics of nitrogen (micromoles per liter) N, density of the jth algal clone (10^9 cells per liter or 10^6 cells per milliliter) C_{ij}, (undefended, j = 1, or defended, j = 2) in the ith pair (where i = 1 for the UTEX1809–1811 pair and i = 2 for the UTEX396–265 pair), and total population density of rotifer predator (individuals per liter) B are

$$\frac{dN}{dt} = \delta\left(N_I - N\right) - \frac{\omega_c}{\varepsilon_c}\sum_{j=1}^{2}\frac{\beta_{ij}NC_{ij}}{K_c + N},$$

$$\frac{dC_{ij}}{dt} = C_{ij}\left[\chi_c\frac{\omega_c}{\varepsilon_c}\frac{\beta_{ij}N}{K_c + N} - \frac{Gp_{ij}B}{K_b + \sum_j p_{ij}C_{ij}} - \delta\right], \qquad (j = 1, 2) \quad \textbf{[S1]}$$

$$\frac{dB}{dt} = B\left[\chi_b\frac{G\sum_j p_{ij}C_{ij}}{K_b + \sum_j p_{ij}C_{ij}} - \left(\delta + m\right)\right],$$

where UTEX1809 is C_{11}, UTEX1811 is C_{12}, UTEX396 is C_{21}, and UTEX265 is C_{22}. We assume a tradeoff between prey palatability p_{ij} and maximum recruitment rate β_{ij} as in the study of Meyer et al. (2). Definitions, units, and estimated values are shown in Table S1. We also assume m = 0 because the experimentally estimated value is negligibly smaller than dilution rate δ according to the model of Jones and Ellner (1). Based on the results of our experiment, β_{11} = 2.96, β_{12} = 2.36, β_{21} = 1.77, and β_{22} = 1.57 where the growth rate parameters of undefended clones UTEX1809 and UTEX396 are β_{11} and β_{21}, and those of defended clones UTEX1811 and UTEX265 are β_{12} and β_{22}, respectively. Note that $x_{1\#}$ is for the UTEX1809–1811 pair and $x_{2\#}$ is for the UTEX396–265 pair, and $x_{\#1}$ is for the undefended clone and $x_{\#2}$ is for the defended clone, respectively, where parameter x is either p or β.

We try to find out a parameter set of palatability that matches the experimental results for measuring the tradeoff (Fig. 1) and the predator–prey dynamics (Figs. 2 and 3). We denote the defense parameters of the undefended clones UTEX1809 and UTEX1811 as p_{11} and p_{12}, and those of defended clones UTEX396 and UTEX265 as p_{21} and p_{22}, respectively. From the Eq. **S1**, algal dynamics in the experiment to measure palatability is

$$\frac{dC_{ij}}{dt} = -\frac{Gp_{ij}BC_{ij}}{K_b + \sum_j p_{ij}C_{ij}}, \qquad \textbf{[S2]}$$

where algae did not grow because the medium lacked nitrate and the culture was kept in darkness (*Materials* and *Methods*). This can be rewritten as

$$\frac{dC_{ij}}{dt} = -C_{ij}p_{ij}f_i(t), \qquad \textbf{[S3]}$$

by defining a time-dependent function: $f_i(t) = GB \Big/ \left(K_b + \sum_j p_{ij}C_{ij} \right)$. Then,

$$\begin{aligned} \frac{d\log C_{ij}}{dt} &= -p_{ij}f_i(t), \\ \log C_{ij}(t) &= \log C_{ij}(0) - p_{ij}\int_0^t f_i(s)s, \\ d &\equiv \frac{\log C_{ij}(t) - \log C_{ij}(0)}{t} = -p_{ij}\left[\frac{1}{t}\int_0^t f_i(s)s\right]. \end{aligned} \qquad \textbf{[S4]}$$

Hence, Eq. **S1** implies that the ratio of measured palatabilities (d values) should equal the ratio of p_{ij} values, at least within the pairs. Adding background mortality of green algae owing to the experimental condition of darkness results in the same conclusion (note that green algae decreased even without rotifers because of the background mortality, thus the palatability of each clone was estimated as the difference between the d with rotifers present and the d with rotifers absent). This may not be the case between the pairs, so we examine effects of two parameters (p_{11} and p_{21}) independently below. However, it turned out that the observed population and evolutionary dynamics in the chemostat experiment can arise by keeping the ratio of p_{ij} values as the ratio of measured palatabilities even between pairs [and it means that $f_1(t) \approx f_2(t)$]. We assume the relative relationships of prey palatabilities as

$$\begin{aligned} p_{12} &= 0.460\,p_{11}, \\ p_{22} &= 0.688\,p_{21}, \end{aligned} \qquad \textbf{[S5]}$$

to give the same ratios among palatabilities as in the experimental results. We search for appropriate parameter values of p_{11} and p_{21} to match the observed eco-evolutionary dynamics when $\delta = 0.5$.

First, we consider the condition where the UTEX1809–1811 pair shows a stable equilibrium with predator and undefended prey genotype (as in Fig. 2). For the

state to be stable, per-capita growth rate of the defended clone when it is rare should be negative:

$$\frac{1}{C_{12}}\frac{dC_{12}}{dt} = \chi_c \frac{\omega_c}{\varepsilon_c}\frac{\beta_{12}\bar{N}}{K_c + \bar{N}} - \frac{Gp_{12}\bar{B}}{K_b + p_{11}\bar{C}_{11}} - \delta < 0, \qquad \textbf{[S6]}$$

where $\bar{N}$, $\bar{C}_{11}$, and $\bar{B}$ are equilibrium densities without defended clone, obtained by solving $dN/dt = 0$, $dC_{11}/dt = 0$, and $dB/dt = 0$ (with $C_{12} = 0$; see refs. 1 and 3). We found that defended clone cannot invade the system when undefended clone's defense is effective (solid line in Fig. S2*A*). The equilibrium density of predator without defended clone ($\bar{B}$) shows the similar pattern: when undefended clone's defense is effective, predator goes extinct (i.e., predator density is negative: dashed line in Fig. S2*A*). For the system to show a stable equilibrium with predator and undefended clone, $1/C_{12}(dC_{12}/dt) < 0$ and $\bar{B} > 0$ (green zone in Fig. S2*A*).

Second, we consider the condition where the UTEX396–265 pair shows a stable equilibrium with predator and defended clone or that with the coexistence of two clones (as in Fig. 3). This kind of dynamics arises when per-capita growth rates of the undefended clone when it is rare is close to zero, thus we calculated

$$\frac{1}{C_{21}}\frac{dC_{21}}{dt} = \chi_c \frac{\omega_c}{\varepsilon_c}\frac{\beta_{21}\hat{N}}{K_c + \hat{N}} - \frac{Gp_{21}\hat{B}}{K_b + p_{22}\hat{C}_{22}} - \delta \approx 0, \qquad \textbf{[S7]}$$

where $\hat{N}$, $\hat{C}_{22}$, and $\hat{B}$ are equilibrium densities without undefended clone, obtained by solving $dN/dt = 0$, $dC_{22}/dt = 0$, and $dB/dt = 0$ (with $C_{21} = 0$). The condition is met when $p_{21} \approx 0.1$. If we assume the relative relationship $p_{21} = 1.86\ p_{11}$ measured in the experiments, the green zone in Fig. S2*A* corresponds to that in Fig. S2*B*, and the observed eco-evolutionary dynamics for the UTEX396–265 pair can be reproduced when p_{21} is within the green zone. Therefore, we found that the observed dynamics can arise, for example, when $p_{11} = 0.055$ and $p_{21} = 0.102$ (red lines in Fig. S2) by keeping the relative relationship between the algal clone pairs measured in the experiments (i.e., $p_{21} = 1.86\ p_{11}$).

With this parameter set, the UTEX1809–1811 pair shows a stable equilibrium with predator and undefended prey genotype (Fig. 4*C*), whereas the UTEX396–265 pair shows a stable equilibrium with predator and defended prey (Fig. 4*D*) or a stable equilibrium with coexisting clones (Fig. 4*E*) depending on the dilution rate of chemostat, under the condition of Eq. **S2** (see also Fig. 4*A* and *B*).

To explore how the population dynamics interacts with the evolutionary

dynamics, we analyzed the model when algal evolution is stopped by assuming the algal population to consist of a single clone (i.e., no clonal frequency change allowed) (Fig. S3). If the algal population consists of only UTEX1811 (defended clone), rotifers cannot persist because of the low food quality. However, rotifers establish their population if the algal population consists of only UTEX1809 (undefended clone). Thus, the persistence of rotifer population when the pair of UTEX1809–1811 consists of the algal population should result from the selection against defended clone and the dominance of undefended one.

Rotifers can persist their population if the algal population consists of either UTEX396 or UTEX265, although equilibrium rotifer density is higher with the undefended clone (Fig. S3). However, population dynamics are different from when the algal population consists of the two clones (Fig. 4). When the algal population consists of either clone, rotifers smoothly reach the equilibrium density at the beginning (Fig. S3), whereas when the algal population consists of the two clones, the increase of rotifer density shows overshooting and rotifers gradually decrease to the equilibrium level. This is because of the initial selection for the undefended clone and the later selection for the defended clone, which changes the quality of algal food as rotifers increase. Thus, the evolutionary dynamics of algal clones can produce ecological dynamics different from those when no clonal diversity is assumed in the algal population.

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SI Figures

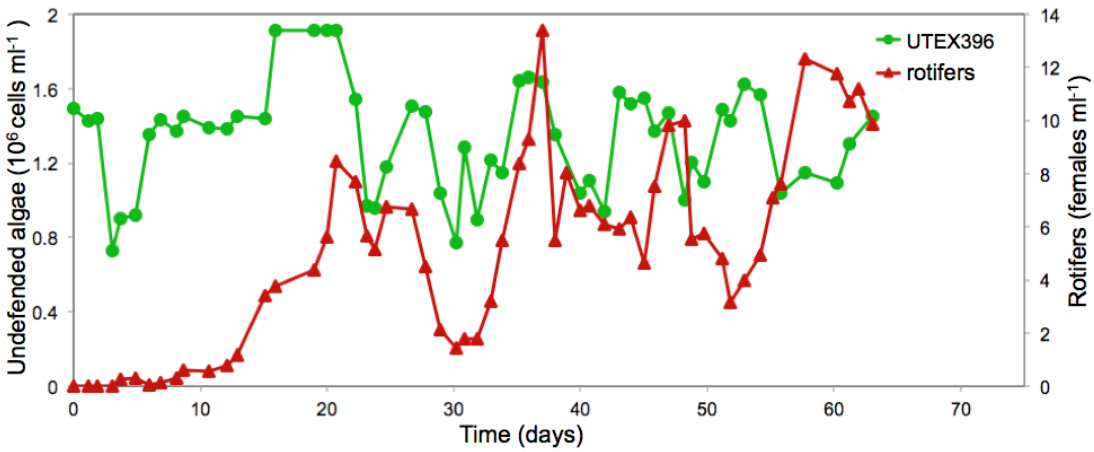

Fig. S1. Close-up of population dynamics of rotifers and undefended algae (UTEX396) in Fig. 3*C* and *D*. Red triangles, rotifers (individuals per milliliter); green circles, undefended algae (10^6 cells per milliliter).

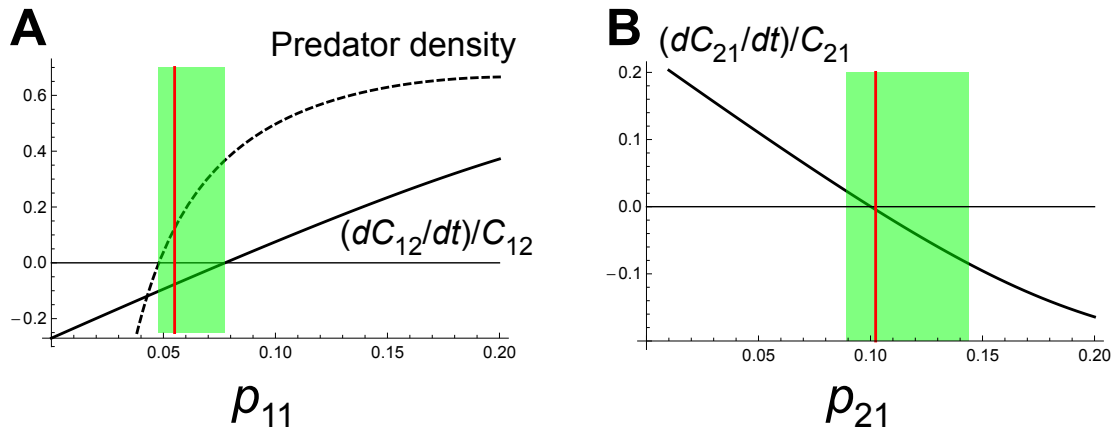

Fig. S2. (*A*) Per-capita growth rate of defended clone when it is rare (black solid line) and predator equilibrium density (black dashed line) for the UTEX1809–1811 pair. The green zone indicates the condition for the observed chemostat dynamics. (*B*) Per-capita growth rate of undefended clone when it is rare for the UTEX396–265 pair. The green zone indicate the case where p_{21} = 1.86 p_{11}. Red lines indicate the parameter condition for Fig. 4 and Fig. S3.

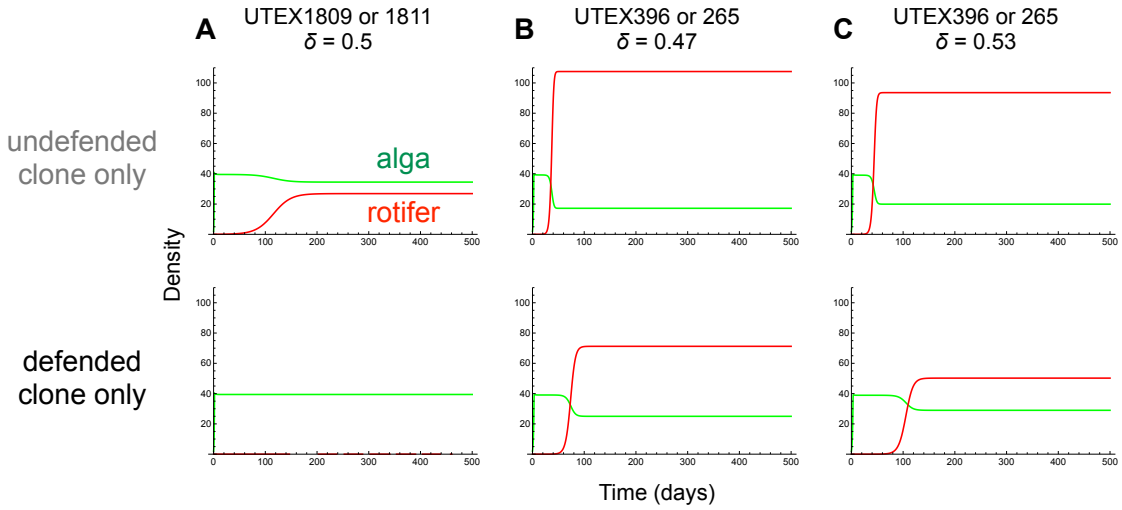

Fig. S3. Predator–prey dynamics of rotifers and algae consisting of a single clone. (*A*) The UTEX1809 (undefended) or UTEX1811 (defended) when $\delta = 0.5$. (*B*) The UTEX396 (undefended) or UTEX265 (defended) when $\delta = 0.47$. (*C*) The UTEX396 or UTEX265 pair when $\delta = 0.53$. Red lines, rotifers (individuals per milliliter); green lines, total algae (10^5 cells per milliliter).

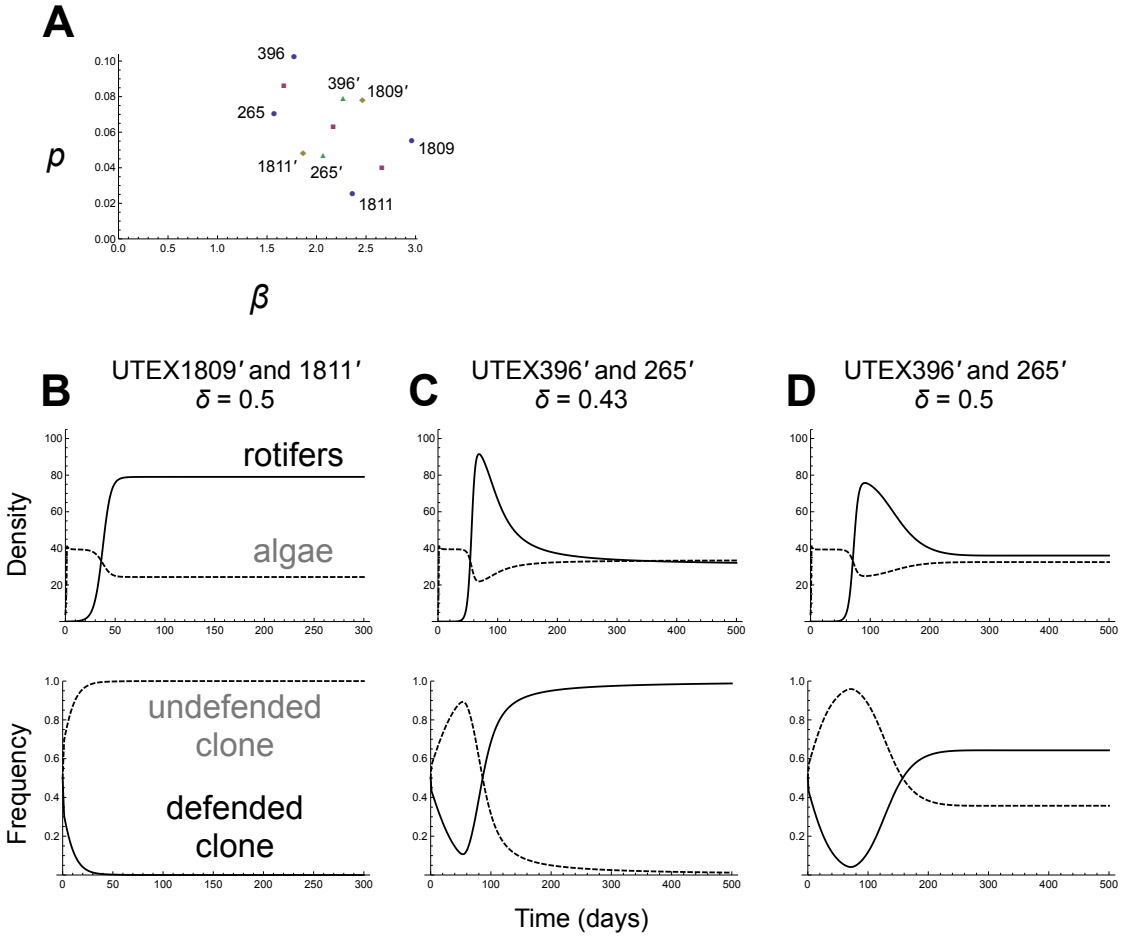

Fig. S4. Predator–prey and evolutionary dynamics predicted by the model with the scaled tradeoffs of the two pairs of algal clones to have the same mean trait values. (*A*) The scaled tradeoff forms; 1809'–1811' and 396'–265' are scaled from original 1809–1811 and 396–265, respectively. (*B–D*) The eco-evolutionary dynamics that are qualitatively consistent with those predicted with the original tradeoff forms (Fig. 4), although the dilution rate for *C* should be slightly lower than that in the original model to show the same dynamics.

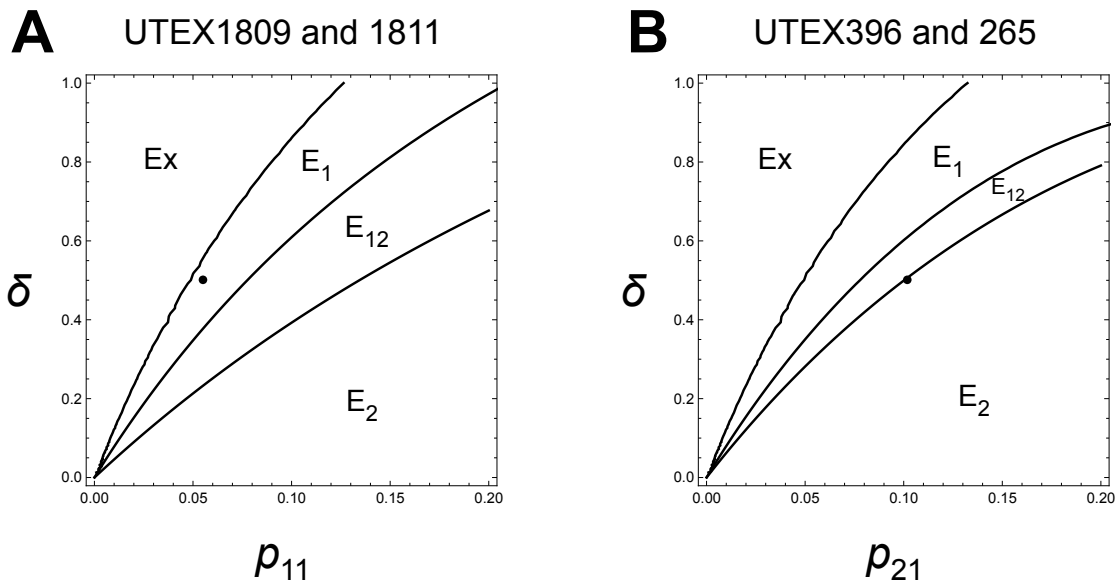

Fig. S5. Phase diagrams of eco-evolutionary dynamics for dilution rate δ and $p_{\#1}$ (palatability of undefended algal clone). (*A*) UTEX1809–1811 pair. (*B*) UTEX396–265 pair. Ex, predator extinction; E_1, stable equilibrium with undefended algal clone; E_2, stable equilibrium with defended algal clone; E_{12}, stable equilibrium with coexisting undefended and defended clones. Black circles represent the parameters used in this study. For the UTEX1809–1811 pair to show the same experimental results as the UTEX396–265 pair did, the dilution rate should be less than 0.37 d^{-1} for an equilibrium with coexisting clones (E_{12}) or less than 0.23 d^{-1} for an equilibrium with defended clone (E_2). However, for the UTEX396–265 pair to show the same experimental results as the UTEX1809–1811 pair did, the dilution rate should be higher than 0.61 d^{-1}. This range of dilution rate contrasts to our experimental setting of the dilution rate (0.52–0.55 d^{-1} for UTEX1809–1811 and 0.44–0.50 d^{-1} for UTEX396–265). Thus, it is unlikely that observed different eco-evolutionary dynamics were due to the different dilution rate between the two algal pairs.

Table S1. Parameters for the *Chlorella–Brachionus* microcosm model

Parameter	Description	Value	Reference
N_I	Limiting nutrient inflow	80 (μmol N/l)	Set
δ	Dilution rate	Variable (/day)	Set
χ_c	Algal conversion efficiency	0.05 (10^9 algal cells/μmol N)	(1)
χ_b	Rotifer conversion efficiency	54000 (rotifers/10^9 algal cells)	(2)
m	Rotifer mortality	0.055 (/day)	(1)
K_c	Minimum algal half-saturation	4.3 (μmol N/l)	(1)
K_b	Rotifer half-saturation	0.835 (10^9 algal cells/l)	(2)
β_{ij}	Maximum algal recruitment rate	Variable (/day)	Measured
p_{ij}	Palatability	Variable	Partly measured
ω_c	N content in 10^9 algal cells	20 (μmol/10^9 algal cells)	(1)
ε_c	Algal assimilation efficiency	1	(1)
G	Rotifer maximum consumption rate	5.0×10^{-5} [10^9 cells/(day×rotifers)]	(2)

Set, adjustable parameters set by experimenter.

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