博士論文（要約）

Dynamic change of biological interactions and its consequences in ecological systems

(生態システムにおける動的な生物間相互作用とその影響)

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Chapter 1

General introduction

Dynamic change of biological interactions

Evolutionary process is often thought to have very different timescales from those of ecological dynamics. Indeed, the processes of speciation and niche diversification occur on a timescale that is much longer than that for ecological dynamics (Hutchinson, 1965; Sepkoski, 1998), and in most of the "Adaptive Dynamics" literature, a timescale separation is assumed, for the sake of theoretical simplicity, between ecology and evolution (Abrams, 2005; Metz et al., 1996). Recently, however, scientists have paid more attention to rapid adaptations such as phenotypic plasticity and evolution on a shorter timescale (e.g., Shimada et al., 2010; Thompson, 1998). This rapid adaptation, unlike the adaptation on long timescales, can strongly interact with ecological dynamics because the timescales of rapid adaptation overlap those of ecological dynamics (Carroll et al., 2007; Hairston et al., 2005; Johnson and Agrawal, 2003). Population density and community structure may influence natural selection through ecological processes such as density effect, competition, and predation pressure, resulting in changes in trait variation within the population. The change in trait variation may, in turn, affect population dynamics and community structure. Thus, the feedback between ecological and adaptation dynamics can arise. Although this concept of feedback is mainly discussed in the context of "eco-evolutionary dynamics" (Kokko and López-Sepulcre, 2007; Pelletier et al., 2009; Pimentel, 1961; Post and Palkovacs, 2009; Schoener, 2011), feedback can occur in all forms of adaptation, from behavioral change, through phenotypic plasticity, to changes in genetic compositions (Abrams, 2005).

Ecological community can be viewed as a complex network of biological interactions between species. It has been recognized that network topology affects ecological consequences, especially the stability of an ecosystem (Ives and Carpenter, 2007). Many studies assumed that

network topology is static (but see Valdovinos et al., 2010). In this case, network topology determines population dynamics in the system, but not vice versa. However, when rapid adaptation occurs, population dynamics may also affect the network topology. For example, a consumer's adaptive food choice changes trophic links depending on the resource population densities, and this change in trophic links, in turn, influences population densities of resources. Thus, rapid adaptations can alter the strength of the links and the network topology, and the feedback between ecological and adaptation dynamics exists. Since the feedback process continuously influences network links, ecological networks are essentially dynamic. Thus, we need to understand the ecological network as a dynamical system that is influenced by the feedback between ecology and adaptation. Such an understanding has been considered to develop both community ecology and evolutionary biology (Johnson and Stinchcombe, 2007; Kokko and López-Sepulcre, 2007) and has important implications on applied problems in biology (Bell and Gonzalez, 2009; Bell and Gonzalez, 2011; Fukano and Yahara, 2012; Kinnison and Hairston, 2007).

Details of intraspecific diversity

Theoretical studies have predicted the feedback between evolutionary and population dynamics in different systems (Abrams and Matsuda, 1997; Jones and Ellner, 2004; Jones and Ellner, 2007; Jones et al., 2009; Yoshida et al., 2007). These studies suggested that evolution can stabilize or destabilize population dynamics (Abrams, 2000) and that some unknown patterns of population dynamics, such as cryptic cycles (Yoshida et al., 2007), appear in the presence of rapid evolution. Since the theoretical results are quite variable and depend on details of the genetic variation (Jones and Ellner, 2004; Jones and Ellner, 2007; Mougi and Iwasa, 2010; Tien and Ellner, 2012;

Yamamichi et al., 2011), demonstrations that model predictions actually occur in real biological systems are important. Several studies in natural and laboratory systems have shown that evolutionary change can drive ecological dynamics (Bassar et al., 2010; Duffy and Sivars-Becker, 2007; Grant and Grant, 2006; Harmon et al., 2009; Palkovacs and Post, 2009; Palkovacs et al., 2009; Post et al., 2008; terHorst et al., 2010; Turcotte et al., 2011; Whitham et al., 2006). However, previous empirical studies have usually compared ecological consequences between populations with and without a single adaptation mechanism. For instance, Yoshida et al. (2003) revealed that rapid prey evolution alters population dynamics in a predator-prey system by comparing between the absence and presence of intraspecific genetic diversity of the prey. Verschoor et al. (2004) showed that phenotypic plasticity stabilized population dynamics by comparing between the undefended and inducible defended algae. To bridge the gaps between theoretical predictions and empirical tests, we need to consider not only the presence or absence of rapid adaptation but also details of intraspecific diversity.

In this work, I focus on an evolutionary tradeoff as a detail of intraspecific diversity. Existing theory predicts that rapid evolution affects predator-prey dynamics when prey has multiple genotypes and the tradeoff between antipredator defense and reproductive ability exists among genotypes (Jones and Ellner, 2004; Jones and Ellner, 2007). To show the effect of details of intraspecific diversity on ecological dynamics, I explore how different forms of the evolutionary tradeoff between antipredator defense and growth in algal prey (*Chlorella vulgaris*) affect the population dynamics of a predator-prey system and the evolutionary changes in the clonal frequency of the algal prey. Previous experimental studies showing that population dynamics of this predator-prey system were influenced by the rapid adaptation of prey did not directly observe

evolutionary dynamics (Yoshida et al., 2003; Yoshida et al., 2007), which give us important insights of the feedback between ecology and evolution though (Fussmann et al., 2007, Schoener, 2011). Thus, in my experiment, I decided to observe both the ecological and evolutionary dynamics.

I constructed an experimental system using *Chlorella vulgaris*, in which the tradeoff between antipredator defense and resource uptake rate was previously reported (Meyer et al., 2006; Yoshida et al., 2004), in order to reveal how different forms of an evolutionary tradeoff can result in different eco-evolutionary dynamics. I examined the difference of reproductive and defensive ability between two algal clones as the form of the evolutionary tradeoff (see **Figure 2-3**). I used the pairs of algal clones because the method for observing their evolutionary dynamics (clonal frequency change) was available (Meyer et al. 2006). Also, theory suggests that even if there exist multiple clones in the algal population at the beginning, two extreme or a few intermediate clones will be eventually selected in this predator-prey system (Jones et al., 2009). In the general discussion later in this thesis, I discuss the case, in which genotype diversity is expanded into multiple clones and continuous trait variation. I also consider some other details of intraspecific diversity, such as phenotypic plasticity, learning, epigenetics, and individual differences of behavior (i.e., animal personality).

Comparing among adaptation mechanisms

Organisms can adapt to their environment by various mechanisms including rapid evolution, phenotypic plasticity, learning behavior and epigenetics (Shimada et al., 2010), and their adaptation mechanisms can coexist in a single population. However, we know little about how these different

adaptation mechanisms coexist in a population and when one adaptation mechanism is beneficial to the population over the other. Thus, comparing among the adaptation mechanisms under different environmental conditions is worth being explored, and I focused in this thesis on rapid evolution and phenotypic plasticity as adaptation mechanisms. Either of them can affect population dynamics, but their effects on ecological stability can be different in a predator-prey system (Yamamichi et al., 2011). Phenotypic plasticity tends to stabilize ecological dynamics and promote persistence more than rapid evolution (Cortez, 2011; Kovach-Orr and Fussmann, 2013). An empirical study indicated that rapid evolution and phenotypic plasticity have distinct effects on ecological dynamics (Fischer et al., 2014). Their study used the four genetically distinct strains of the prey alga (*Chlamydomonas reinhardtii*) that varied in their growth rate, genetically determined defense, and inducible defense. Although the difference of the effect on population dynamics between rapid evolution and phenotypic plasticity has been revealed, our understanding of dynamics of a population with these different adaptations through ecological and adaptation feedback is still limited.

What factors can affect the relative importance of phenotypic plasticity and rapid evolution as an adaptation mechanism? The timescale of environmental fluctuation is a good candidate because the relative speed of phenotype plasticity compared to the timescale of environmental fluctuations can alter the competition outcome between the plastic generalist and specialists with fixed traits (Stomp et al., 2008). Phenotypic plasticity can produce a faster change of phenotype than rapid evolution does (Olsson and Eklöv, 2005; Svanbäck and Persson, 2004; Svanbäck and Persson, 2009) because plastic organisms can change their traits within a generation while evolution of traits occurs over generations. Thus, the faster timescale of environmental fluctuation seems beneficial for phenotypic plasticity. However, Stomp et al. (2008) experimentally

indicated and theoretically showed that flexible phenotypes are not advantageous when the phenotypic adaptation occurs too slowly compared to the environmental fluctuations. Why is phenotypic plasticity not always advantageous under environmental fluctuations? To answer this question, I explored in this thesis how the timescale of environmental fluctuations influences the intraspecific competition between a generalist genotype with phenotypic plasticity and specialist genotypes with fixed traits.

Chapter contents

In this thesis, I explore the consequences of dynamic change of biological interactions by considering two key concepts: details of intraspecific diversity and comparison of adaptation mechanisms. In **Chapter 2**, to reveal how different forms of the evolutionary tradeoff affect population and evolutionary dynamics, I used rotifer-algal chemostat microcosms that allowed direct observations of population and evolutionary dynamics using algae having different forms of an evolutionary tradeoff. Mathematical model was developed to understand the experimental results, based on a model developed by Jones and Ellner (2007). I showed that different forms of an evolutionary tradeoff produced remarkably divergent eco-evolutionary dynamics. In **Chapter 3**, I explore dynamics of a population with these different adaptations through ecological and adaptation feedback in fluctuating environments using a model based on Lotka-Volterra competition equations. I showed that the dominant adaptation strategy changed depending on the timescale of environmental fluctuation. In **Chapter 4**, I consider how the dynamic change of biological interactions works in the field and where the studies of feedbacks between adaptation and ecology may go in the future based on the results of the preceding chapters. In particular, I discuss the key

topics to develop beyond my studies and illustrate how to approach these topics, including (1) expansion into a large community network, (2) generalization of the intraspecific variations, and (3) comparing among adaptations other than evolution and phenotypic plasticity.

Chapter 2

Impacts of different evolutionary tradeoffs on eco-evolutionary feedback

Introduction

Evolutionary dynamics, changes in intraspecific genotype frequency over generations, can have a time scale similar to that of ecological dynamics (Hairston et al., 2005; Hendry and Kinnison, 1999; Thompson, 1998). 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 (1961) proposed genetic feedback as a mechanism regulating animal populations (e.g., ref. Kokko and López-Sepulcre, 2007; Lennon and Martiny, 2008; Pelletier et al., 2009; Post and Palkovacs, 2009; Saccheri and Hanski, 2006; Sinervo et al., 2000; Stockwell et al., 2003). This integration of evolutionary biology and ecology has important implications in both basic and applied problems in biology (Carroll et al., 2007; Gonzalez et al., 2013; Hughes et al., 2008; Johnson and Agrawal, 2003; Matthews et al., 2011; Schoener, 2011).

Empirical studies have shown that rapid evolution can affect many ecological interactions, including predator-prey (Palkovacs and Post, 2009; Post et al., 2008; terHorst et al., 2010), host-parasite (Duffy and Sivars‐Becker, 2007), herbivore-plant (Turcotte et al., 2011), competitive interactions (Grant and Grant, 2006), and interactions with abiotic environments (Bassar et al., 2010; Harmon et al., 2009; Palkovacs et al., 2009; Whitham et al., 2006). 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 (Abrams, 2000; Abrams and Matsuda, 1997; Cortez and Ellner, 2010) but also the form of the evolutionary tradeoff among genotypes is important in generating

qualitatively different dynamics (Jones et al., 2009; Mougi and Iwasa, 2010; Tien and Ellner, 2012; Yamamichi et al., 2011; Yamamichi et al., 2014). Indeed, the forms of evolutionary tradeoffs within populations are known to be remarkably variable in plants and microbes (Andersson and Hughes, 2010; Gagneux et al., 2006; Koricheva, 2002). Thus, there should be various eco-evolutionary dynamics depending on the form of evolutionary tradeoffs existing in wild populations. Nevertheless, to my 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, **Figure 2-1**), I 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 (Becks et al., 2010; Becks et al., 2012; Fussmann et al., 2003). I 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. I 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 (Meyer et al., 2006). I also developed a mathematical model for the experimental system, based on a model of Jones and Ellner (2007), parameterized the model using data from separate experiments, and compared the model's predictions to the observed population and genotype dynamics.

Materials and Methods

Experimental species

The predator-prey system I used in this study consisted of *B. calyciflorus* (asexually reproducing rotifer predator, **Figure 2-2**) and *C. vulgaris* (asexually reproducing algal prey, **Figure 2-2**), which was the same system used in previous studies (Fussmann et al., 2000; Meyer et al., 2006; Yoshida et al., 2003). Because the original algal strains can be composed of multiple clones (Jones and Ellner, 2004), I isolated a single clone from each strain for the tradeoff and chemostat experiments described below. The all-algal cultures were kept axenic.

Allele-specific Quantitative PCR

To examine the clonal frequency changes in the algal population (i.e., natural selection in the population), I used the AsQ-PCR (Allele-specific Quantitative PCR) technique developed by Meyer et al. (2006), 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.

To test whether the alleles of pairs of candidate UTEX algal strains amplified at the same rate, I mixed strains in a range of known proportions (100:0, 80:20, 50:50, 20:80, 0:100) and checked the amount of PCR products amplified from each strain. I used two pairs of clones, UTEX396 and UTEX265, and UTEX1809 and UTEX1811. The method is as follows. First, I concentrated samples of mixed clones by filtering 2.5×10^6 cells onto a 0.7 μm mesh glass fiber filter, placed the filter in a 1.5-ml micro tube with 200 μl of 5% Chelex solution, and homogenized the filter with a pipette tip. The sample for DNA extraction was frozen in liquid nitrogen and thawed at 55°C. The freeze–thaw step was repeated two times to break the cell walls. The samples were incubated for 4 h at 55°C, boiled for 10 min at 100°C and frozen for more than 15 minutes at -20°C. The frozen samples thawed at room temperature and then centrifuged at 14,000 g in a desktop centrifuge for 3 minitues. Next, the PCR was run with 4μl of supernatant of the Chelex–DNA extraction and 6μl of "master mix" (**Table 2-1**). The reactions were heated to 94°C for 2 min, then run for 35 cycles of 95°C for 50 s, 53°C for 1 min, and 72°C for 1 min, and held at 72°C for one 10-min interval. Last, these samples were read and performed a fragment analysis by a CEQ 8000 sequencer. In consequence, each of which the clonal frequency can be accurately quantified by the AsQ-PCR, as the correlation between known and estimated frequencies was highly significant ($r^2 > 0.97$ for the UTEX396-265 pair, $r^2 > 0.98$ for the UTEX1809-1811 pair).

Measuring a tradeoff between palatability and reproductive ability

I examined the evolutionary tradeoff for each pair of algal clones. First, I measured the reproductive

ability of each clone in the culture medium that was used for a chemostat system (**Figure 2-1**). The medium was the same as in previous studies (Fussmann et al., 2000; Meyer et al., 2006; Yoshida et al., 2003) and had the limiting nutrient (nitrate) at 80 μmol•L^{-1} (**Table 2-2**). I inoculated algal cells of each clone (1×10^4 cells per mL) into 50 mL of fresh medium with nine replicates per clone, and maintained at 24°C in continuous light (120 μE•m^{-2}•sec^{-1}). Algal density was monitored daily until population growth saturated. Algal densities exponentially increased from low but observable density to nearly saturation, and I 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, I inoculated algal cells of each pair of clones (3.5×10^6 cells per mL for each clone) into 50 mL of fresh medium with 100 rotifers. To prevent the 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 I 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

$$d = \frac{\log(C_{\text{end}}) - \log(C_{\text{start}})}{t} \qquad \textbf{(2-1)}$$

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

I ran rotifer-algal chemostat experiments following the methods of previous studies (Fussmann et al., 2000; Meyer et al., 2006; Yoshida et al., 2003; Yoshida et al., 2007). The rotifer population consisted of a strain that reproduced only asexually in the chemostat (Fussmann et al., 2003). For the algal population, I used two pairs of algal clones (UTEX396 and UTEX265; UTEX1809 and UTEX1811) that showed the different forms of tradeoff (see 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 $\mu E \bullet m^{-2} \bullet sec^{-1}$). The rotifer and algal densities were measured at 1- to 2-day intervals using a microscope and a cell counter (CASY Model TTC, Roche), respectively. I 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.

Models and parameters

The mathematical model describes the population and evolutionary dynamics of the rotifer-algal system cultured in a chemostat as in my experiment. According to a model of Jones and Ellner (2007), 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

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

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

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

where UTEX1809 is C_{11}, UTEX1811 is C_{12}, UTEX396 is C_{21}, and UTEX265 is C_{22}. I assume a tradeoff between prey palatability p_{ij} and maximum recruitment rate β_{ij} as in the study of Meyer et al. (2006). Definitions, units, and estimated values are shown in **Table 2-3**. I also assume $m = 0$ because the experimentally estimated value is negligibly smaller than dilution rate δ according to the model of Jones and Ellner (2007). Based on the results of my experiment, $\beta_{11} = 2.96$, $\beta_{12} = 2.36$, $\beta_{21} = 1.77$, and $\beta_{22} = 1.57$ 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.

I try to find out a parameter set of palatability that matches the experimental results for measuring the tradeoff and the predator-prey dynamics. I 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. **(2-2)**, algal dynamics in the experiment to measure palatability is

$$\frac{dC_{ij}}{dt} = \frac{G p_{ij} B C_{ij}}{K_b + \sum_j p_{ij} C_{ij}}, \qquad \textbf{(2-3)}$$

where algae did not grow because the medium lacked nitrate and the culture was kept in darkness.

This can be rewritten as

$$\frac{dC_{ij}}{dt} = -C_{ij}p_{ij}f_i(t), \tag{2-4}$$

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

$$\begin{gathered}\frac{d\log C_{ij}}{dt} = -p_{ij}f_i(t), \\ \log C_{ij}\,(t) = \log C_{ij}\,(0) - p_{ij}\int_0^t f_i(s)s, \\ d \equiv \frac{\log C_{ij}\,(t) - \log C_{ij}\,(0)}{t} = -p_{ij}\left[\frac{1}{t}\int_0^t f_i(s)s\right].\end{gathered} \tag{2-5}$$

Hence, Eq. **(2-5)** 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 due 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 I 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 $f_1(t) \approx f_2(t)$]. I assume the relative relationships of prey palatabilities as

$$\begin{gathered}p_{12} = 0.460p_{11}, \\ p_{22} = 0.688p_{21},\end{gathered} \tag{2-6}$$

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

Results

Both pairs of algal clones that I used showed an evolutionary tradeoff between defense against rotifer predation and reproductive ability (**Figure 2-3**). The pair of UTEX1809 and UTEX1811 clones had a relatively "costly defense" tradeoff: Defense is not very effective in spite of 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, $t = 3.683$, $P < 0.05$; **Figure 2-3**). The pair of UTEX396 and UTEX265 clones, which was already known to have a tradeoff (Meyer et al., 2006), 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, $t = 3.338$, $P < 0.05$; **Figure 2-3**). Meyer et al. (2006) 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 (**Figure 2-4**). 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 (**Figure 2-3**). Meanwhile, rotifer abundance gradually decreased after 30 days, 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 (**Figure 2-4*E*** and ***F***).

In the cheap defense tradeoff pair, I observed two different types of population and evolutionary dynamics (**Figure 2-5**), 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 (**Figure 2-5*B*** and ***D***) and relatively low abundance of rotifers (**Figure 2-5*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 **Figure 2-5*C*** and ***D***, suggesting the influence of algal clonal frequency on rotifer population growth (**Figure 2-6**). The second type of dynamics with the cheap defense pair was characterized by dominance or near fixation of the defended algal clone (**Figure 2-5*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 (**Figure 2-5 *E*-*H***). This type of dynamics with this pair of algal clones was consistent with previous results for the same pair (Meyer et al., 2006), whereas the first type of the dynamics (**Figure 2-5 *A*-*D***) was not observed in the previous study.

In order to understand the experimental results, I analyzed a mathematical model based on a model of Jones and Ellner (2007). I 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. I found a set of palatability parameters (**Figure 2-7**), subject to this constraint, such that the model reproduced the observed population and evolutionary dynamics of both clone pairs (**Figure 2-8**).

First, I consider the condition where the UTEX1809-1811 pair shows a stable equilibrium with predator and undefended prey genotype (as in **Figure 2-4**). For the state to be stable, per-capita growth rate of the defended clone when it is rare should be negative:

$$\frac{1}{C_{12}}\frac{dC_{12}}{dt} = \chi_c \frac{\omega_c}{\varepsilon_c}\frac{\beta_{12}\bar{N}}{K_c + \bar{N}} - \frac{Gp_{12}\bar{B}}{K_b + p_{11}\bar{C}_{11}} - \delta, \qquad \textbf{(2-7)}$$

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. Jones and Ellner, 2007, Yamamichi et al., 2011). I found that defended clone cannot invade the system when undefended clone's defense is effective (solid line in **Figure 2-7*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 **Figure 2-7*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 **Figure 2-7*A***).

Second, I 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 **Figure 2-5**). This kind of dynamics arises when per-capita growth rates of the undefended clone when it is rare is close to zero, thus I calculated

$$\frac{1}{C_{21}}\frac{dC_{21}}{dt} = \chi_c \frac{\omega_c}{\varepsilon_c}\frac{\beta_{21}\widehat{N}}{K_c + \widehat{N}} - \frac{Gp_{21}\widehat{B}}{K_b + p_{22}\widehat{C_{22}}} - \delta, \qquad (2\text{-}8)$$

where $\widehat{N}$, $\widehat{C_{22}}$, and $\widehat{B}$ 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 the relative relationship $p_{21} = 1.86p_{11}$ measured in the experiments, the green zone in **Figure 2-7*A*** corresponds to that in **Figure 2-7*B***, and the observed eco-evolutionary dynamics for the UTEX396-265 pair can be reproduced when p_{21} is within the green zone Therefore, I found that the observed dynamics can arise, for example, when $p_{11} = 0.055$ and $p_{21} = 0.102$ (red lines in **Figure 2-7**) by keeping the relative relationship between the algal clone pairs measured in the experiments (i.e., $p_{21} = 1.86p_{11}$).

With this parameter set, the UTEX1809-1811 pair shows a stable equilibrium with predator and undefended prey genotype (**Figure 2-8*C***) whereas the UTEX 396-265 pair shows a stable equilibrium with predator and defended prey (**Figure 2-8*D***) or a stable equilibrium with coexisting clones (**Figure 2-8*E***) depending on the dilution rate of chemostat, under the condition of Eq. (**2-3)** (see also **Figure 2-8*A*** and ***B***).

To explore how the population dynamics interacts with the evolutionary dynamics, I 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) (**Figure 2-9**). If the algal population consists of only UTEX1811 (defended clone), rotifers cannot persist because of the low food quality. On the other hand, 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 (**Figure 2-9*B*** and ***C***). However, population dynamics are different from when the algal population consists of the two clones (**Figure 2-8*D*** and ***E***). When the algal population consists of either clone, rotifers smoothly reach the equilibrium density at the beginning (**Figure 2-9**), 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 the different ecological dynamics than those when no clonal diversity is assumed in the algal population.

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 (**Figure 2-8*C***). The fixation of the undefended clone allows the rotifer population to persist, which would go extinct only if the defended clone is present (**Figure 2-9*A***). With the cheap defense tradeoff between UTEX396 and UTEX265, the tradeoff parameters were very near the border of two different types of dynamics (**Figure 2-8*D*** and ***E***). 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 (**Figure 2-8*D*** and ***E***). This suggests that an experimental system could display either type of dynamics (as I observed in the 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 (**Figure 2-10**). Also, the model predicts that the system will reach equilibrium irrespective of whether the algal population can evolve or not (**Figure 2-9**). Overall, the model predictions are qualitatively consistent with the experimental data, capturing some quantitative aspects as well (see Discussion).

Discussion

My 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 my predator-prey system (Jones and Ellner, 2007; Yoshida et al., 2007), empirical studies using real organisms have not tested this prediction so far. Here I show, for the first time to my 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 is important to understand eco-evolutionary feedbacks (Bolnick et al., 2011; Fussmann et al., 2007; Hersch-Green et al., 2011). Intraspecific trait variation has been often measured quantitatively, such as the frequency

distribution of trait values (Hughes et al., 2008; Violle et al., 2012; Whitham et al., 2006). 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 this 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 (**Figure 2-4**). In contrast, the defended clone became dominant, or the two clones coexisted with comparable frequencies, for the cheap defense tradeoff pair (**Figure 2-5**). 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 (**Figure 2-8**).

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 **Figure 2-8**. 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 (Fussmann et al., 2000; Jones and Ellner, 2004;

Jones and Ellner, 2007). 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 (**Figure 2-5**) would not be intuitively understandable because rotifer density was lower when palatable, undefended clone was more abundant. The model predicts that the rotifer density is higher when the defended clone is dominant than when the two clones coexist (**Figure 2-8**), 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, my mathematical model did not show the bistability corresponding to the observed dynamics. Stage- or age-structured models often show complex multistability, and my results of the UTEX 396-265 pair may be explained by alternative stable states driven by structured interactions. For example, McCauley et al. (1999, 2008) (McCauley et al., 2008; McCauley et al., 1999) demonstrated that small- and large-amplitude cycles coexisted in *Daphnia*-algal microcosm systems due to resource-dependent mortality and a dynamic development delay in consumers (*Daphnia*). However, in my case, consumers are rotifers that do not have as distinct age structure as daphnids have. Also, previous theoretical studies found that age structure of rotifers (senescence) did not change the dynamics substantially (Jones and Ellner, 2007; Yoshida et al., 2007). 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 (Yamamichi et al., 2011). 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 I had (**Figure 2-11**), 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 (**Figure 2-8*C***). The defended clone itself cannot support the rotifer population (**Figure 2-9*A***). However, for the cheap defense tradeoff, the persistence of rotifer population is independent from the algal evolution (**Figure 2-9*B*** and ***C***).

My 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 (Fussmann et al., 2003; Meyer et al., 2006; Yoshida et al., 2003; Yoshida et al., 2007). This study provides a previously unidentified insight into the importance of the details of genetic diversity. The details are likely to be very variable due to intraspecific variation in evolutionary tradeoffs (Andersson and Hughes, 2010; Gagneux et al., 2006; Koricheva, 2002; Yoshida et al., 2004). Theory predicts that numerous details can greatly affect eco-evolutionary dynamics (Ellner, 2013): tradeoffs between defense cost and resource availability (Mougi and Iwasa, 2010; Tien and Ellner, 2012), interactions between phenotypic plasticity and evolution (Chevin et al., 2010; Cortez, 2011; Kovach-Orr and Fussmann, 2013; Yamamichi et al., 2011), and spatial heterogeneity and gene flow (Leibold et al., 2004; Urban and Skelly, 2006). But empirical studies were lacking. My 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.

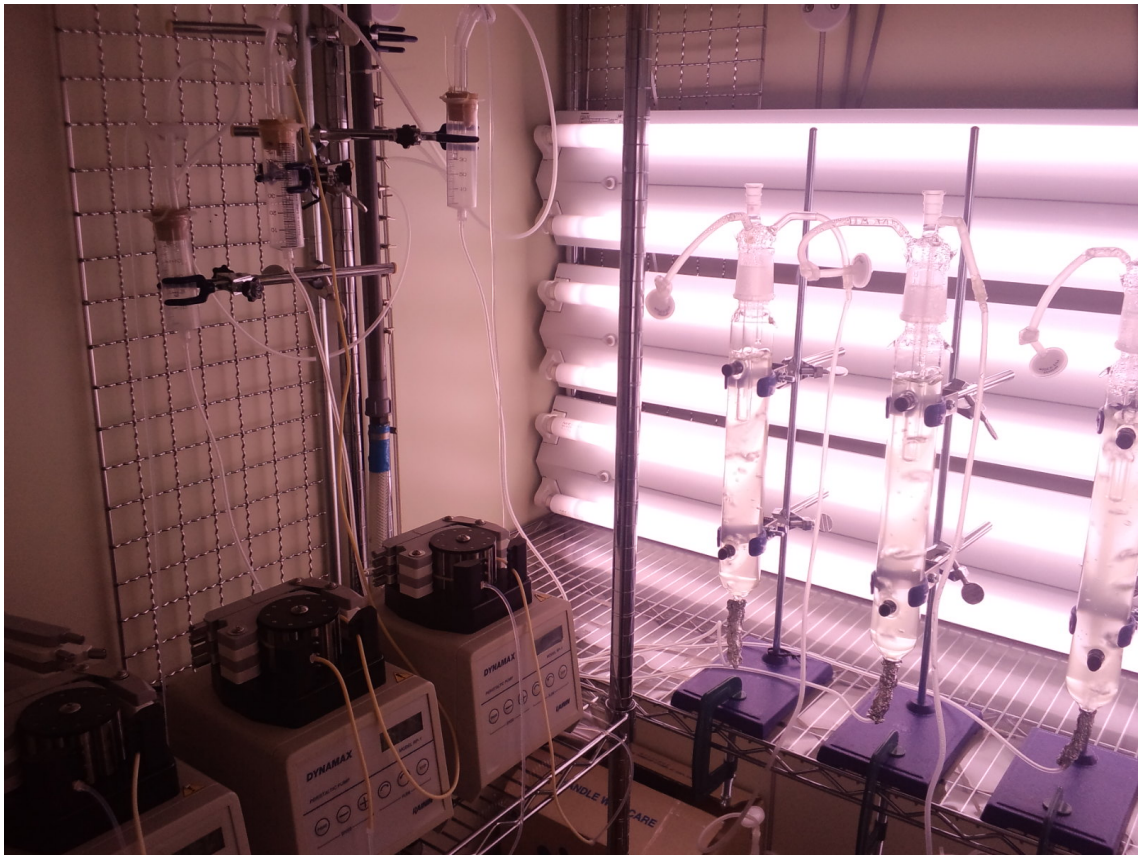

Figure 2-1. Chemostats experiment system. A continuous flow of the medium was pumped through the chemostats; sterile air was bubbled continuously both to prevent CO_2 limitation of the algae and to enhance mixing.

Figure 2-2. Pictured is a rotifer predator (*Brachionus calyciflorus*) eating algal prey (*Chlorella vulgaris*). Although not visually apparent, the algal population has genetic diversity in the defense against rotifer predation and the rate of growth using the limiting nutrient.

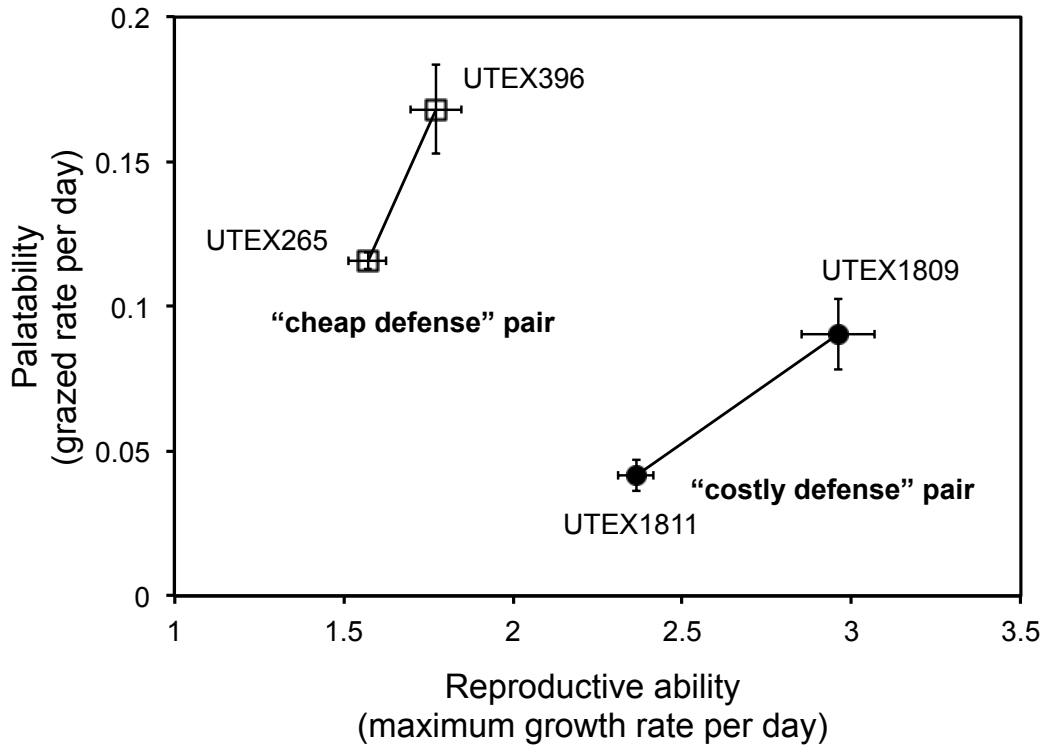

Figure 2-3. Different forms of an evolutionary tradeoff between two pairs of algal clones. The pair of UTEX396 and UTEX265 had a cheap defense tradeoff (better defended clone has only slightly lower maximum growth rate) compared to the pair of UTEX1809 and UTEX1811 showing a costly defense tradeoff. Error bars represent SD (n=3 for palatability, n=9 for maximum growth rate).

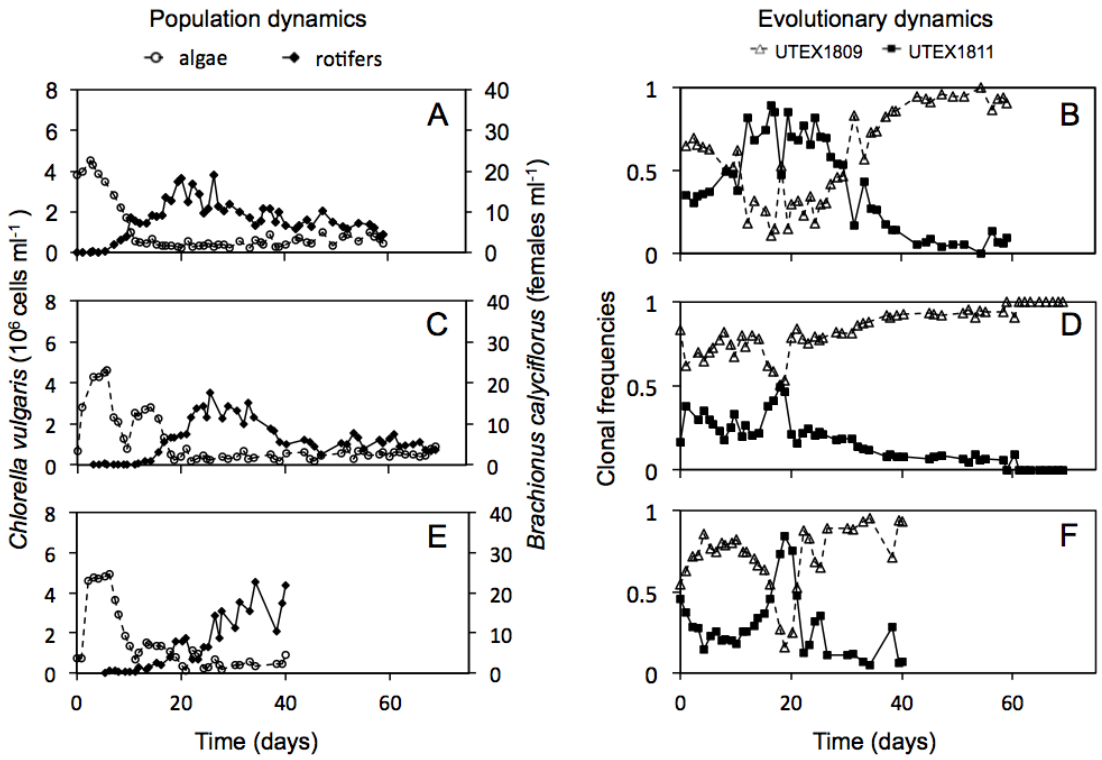

Figure 2-4. Population dynamics and evolutionary dynamics for the pair showing a costly defense tradeoff (panels in the same row are data from the same run of chemostat). *A*, *C* and *E* show rotifer and algal population dynamics, corresponding to *B*, *D* and *F*, respectively, which show the changes 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) day^{-1} (*A* and *B*), 0.52 (0.49-0.55) day^{-1} (*C* and *D*) and 0.52 (0.50-0.54) day^{-1} (*E* and *F*).

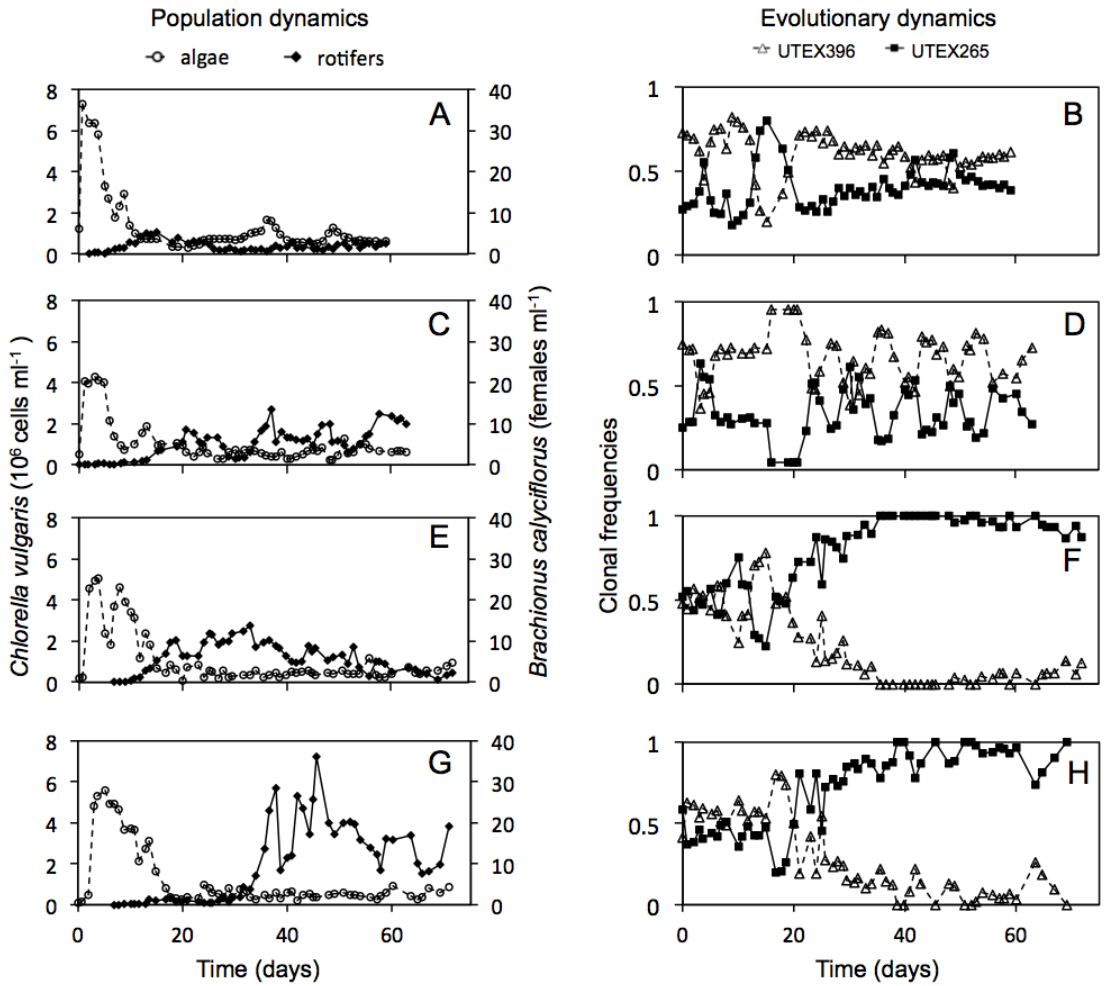

Figure 2-5. Population dynamics and evolutionary dynamics for the pair showing a cheap defense tradeoff (panels in the same row are data from the same run of chemostat). *A*, *C*, *E* and *G* show rotifer and algal population dynamics, corresponding to *B*, *D*, *F* and *H*, respectively, which show 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) day^{-1} (*A* and *B*), 0.49 (0.46-0.52) day^{-1} (*C* and *D*), 0.48 (0.42-0.56) day^{-1} (*E* and *F*), and 0.49 (0.48-0.51) day^{-1} (*G* and *H*).

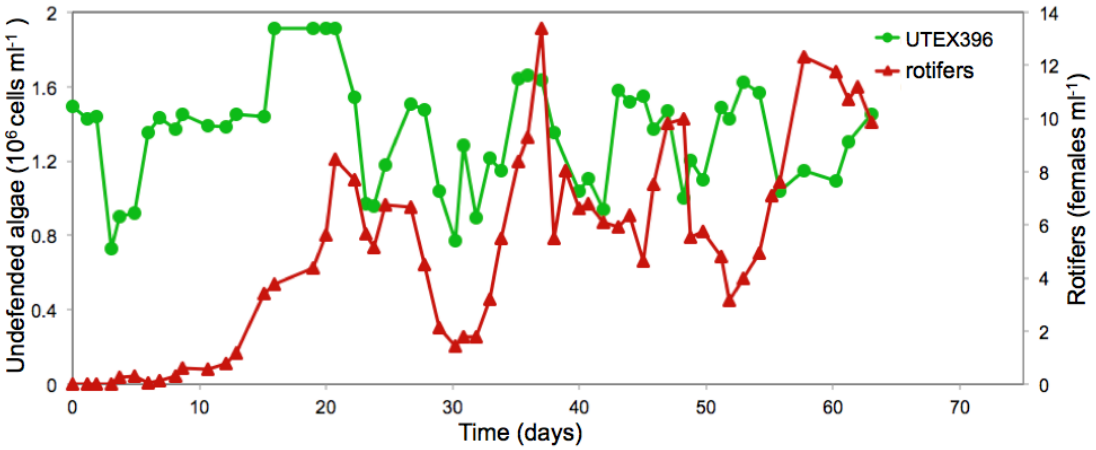

Figure 2-6. Close-up of population dynamics of rotifers and undefended algae (UTEX396) in **Figure 2-5*C*** and ***D***. Red triangles, rotifers (individuals per milliliter); green circles, undefended algae (10^6 cells per milliliter).

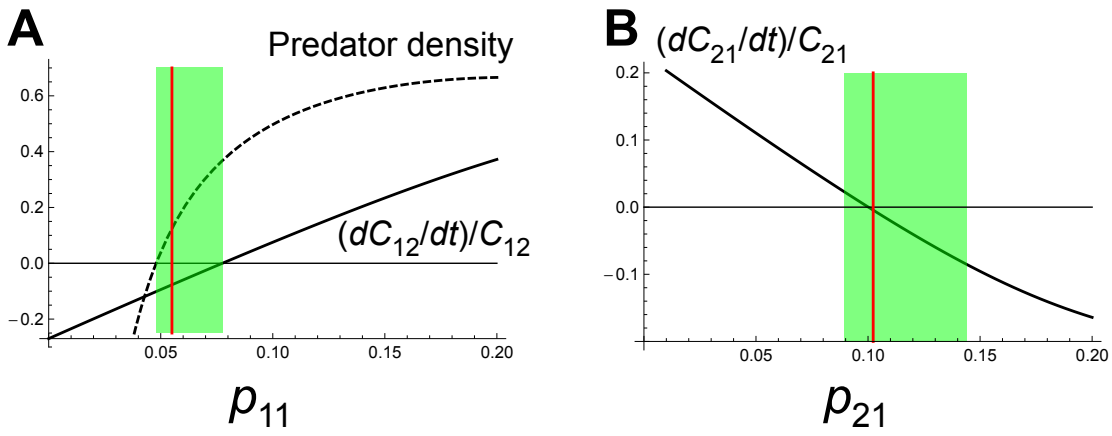

Figure 2-7. (*A*) Per-capita growth rate of defended clone when it is rare (black solid line) and predator equilibrium density (black dashed line) for the UTEX1809-1811 pair. Green zone indicates the condition for the observed chemostat dynamics. (*B*) Per-capita growth rate of undefended clone when it is rare for the UTEX396-265 pair. Green zone indicate the case where $p_{21} = 1.86\ p_{11}$. Red lines indicate the parameter condition for **Figure 2-8** and **Figure 2-9**.

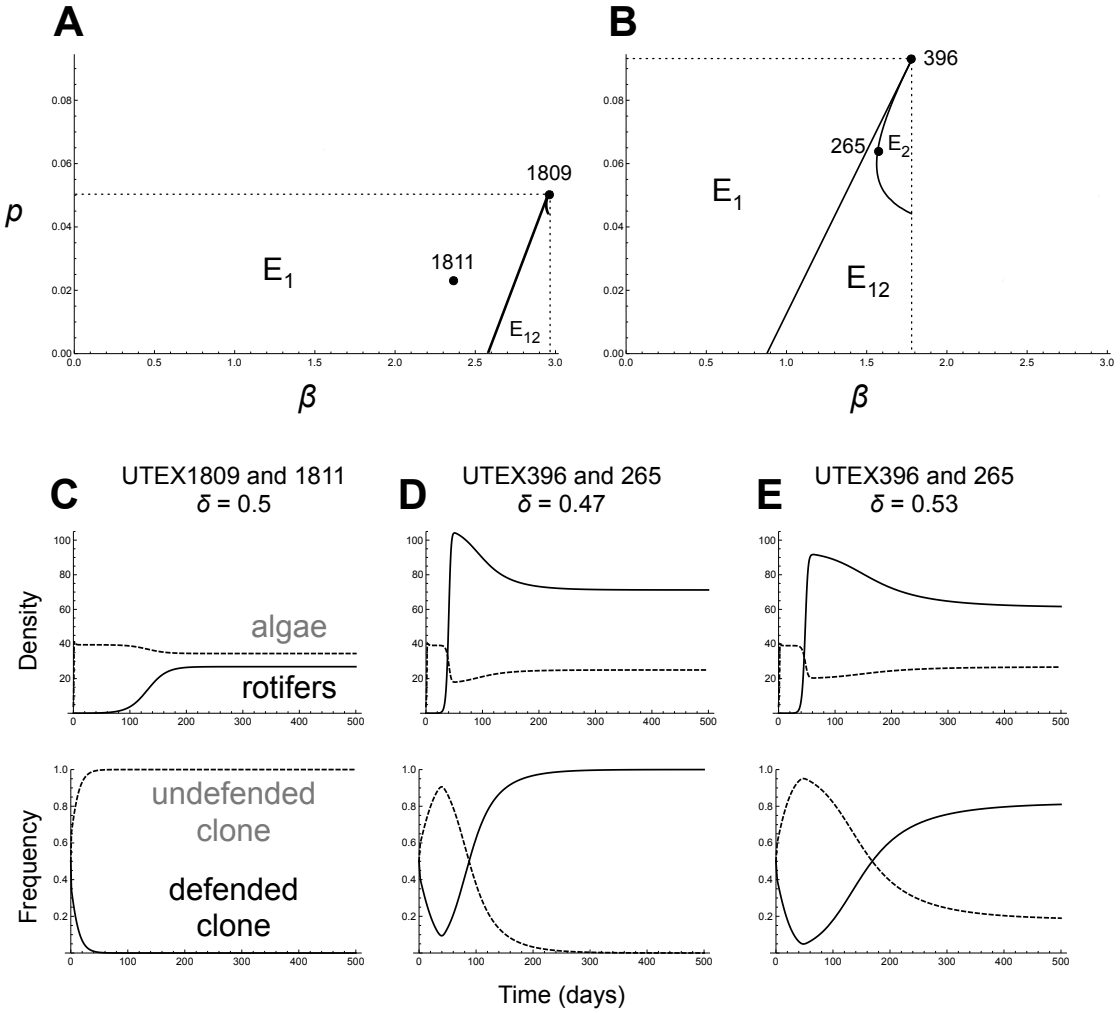

Figure 2-8. (*A* and *B*) Phase diagram for each pair of algal clones showing different eco-evolutionary dynamics. Parameters p and β are palatability and maximum recruitment rate, respectively, in the mathematical model. Black circles represent the estimated parameters based on the experimental results. 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 $p_{11} = 0.055$ for the UTEX1809–1811 pair when $\delta = 0.5$ (*C*) and the UTEX396–265 pair when $\delta = 0.47$ (*D*) and $\delta = 0.53$ (*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.

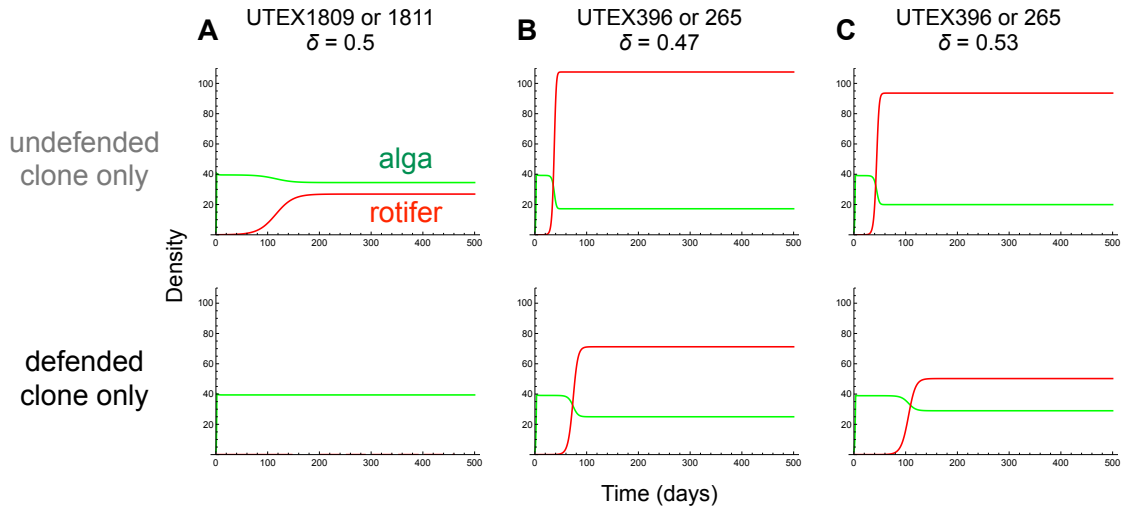

Figure 2-9. Predator-prey dynamics of rotifers and algae consisting of a single clone. (*A*) the UTEX1809 (undefended) or UTEX1811 (defended) when $d = 0.5$. (*B*) the UTEX396 (undefended) or UTEX265 (defended) when $d = 0.47$. (*C*) the UTEX396 or UTEX265 pair when $d = 0.53$. Red lines, rotifers (individuals per milliliter); green lines, total algae (10^5 cells per milliliter).

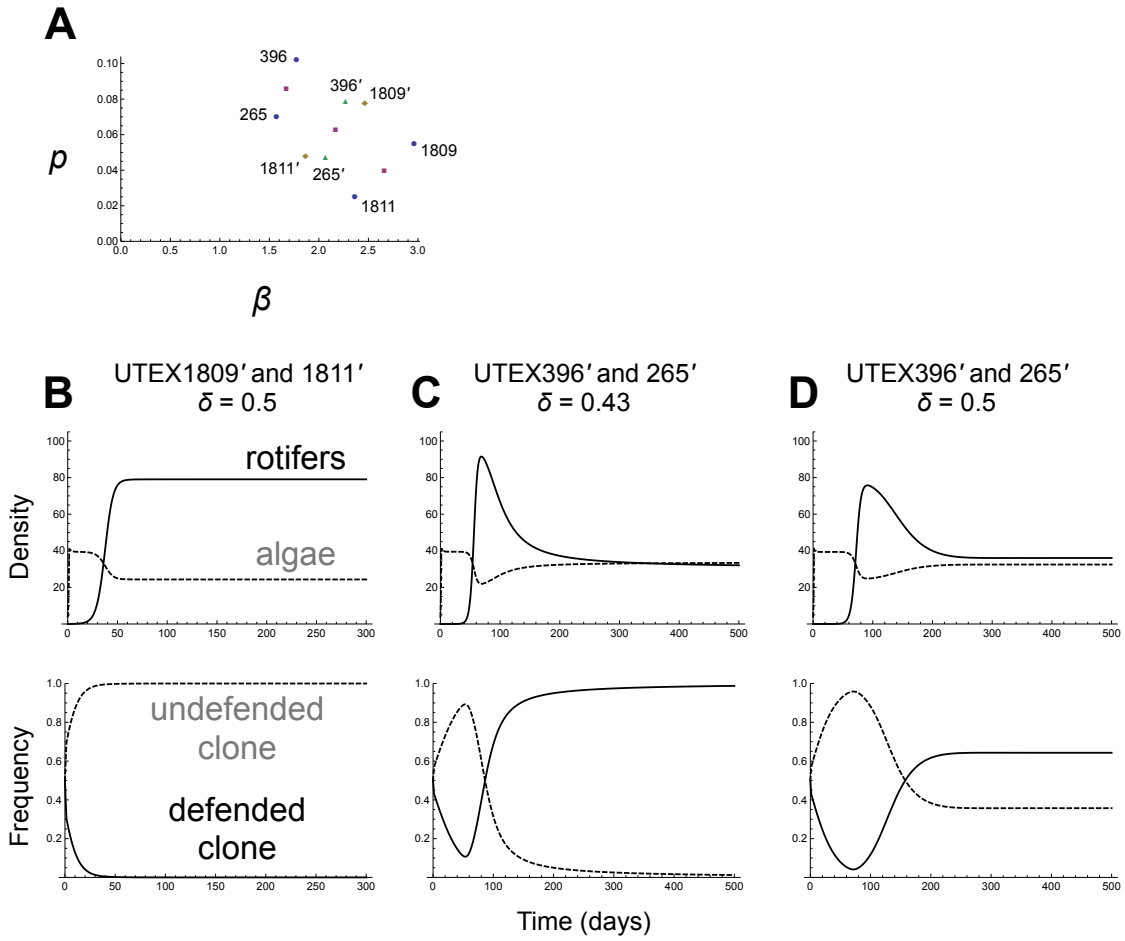

Figure 2-10. Predator-prey and evolutionary dynamics predicted by the model with the scaled tradeoffs of the two pairs of algal clones to have the same mean trait values. (*A*) The scaled tradeoff forms; 1809'-1811' and 396'-265' are scaled from original 1809-1811 and 396-265, respectively. (*B-D*) The eco-evolutionary dynamics that are qualitatively consistent with those predicted with the original tradeoff forms (**Fig. 2-8**), although the dilution rate for *C* should be slightly lower than that in the original model to show the same dynamics.

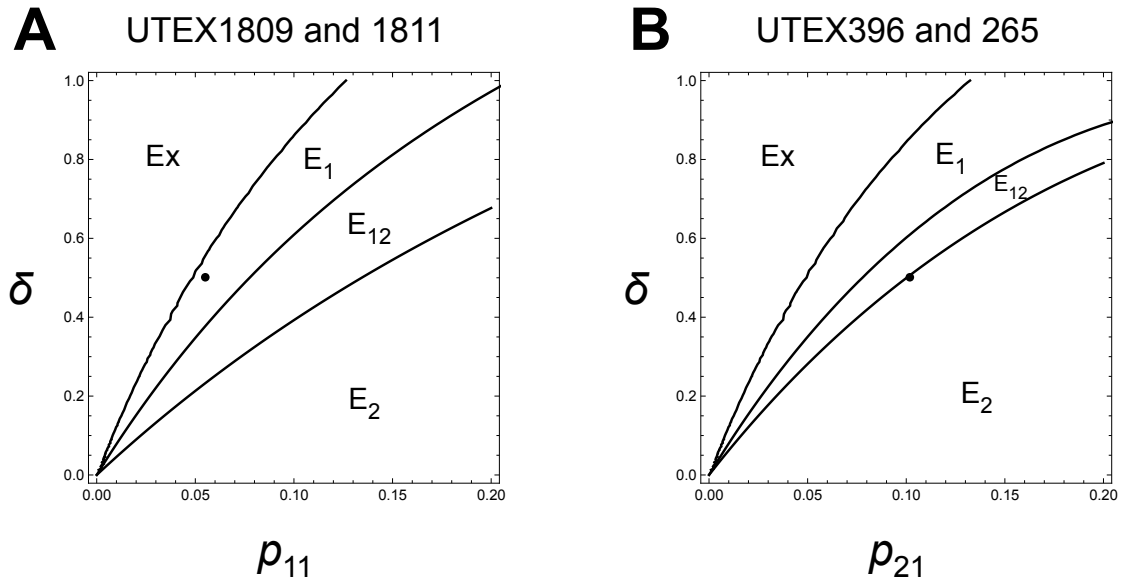

Figure 2-11. Phase diagrams of eco-evolutionary dynamics for dilution rate δ and $p_{\#1}$ (palatability of undefended algal clone). (*A*) UTEX1809-1811 pair. (*B*) UTEX396-265 pair. Ex, predator extinction; E_1, stable equilibrium with undefended algal clone; E_2, stable equilibrium with defended algal 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 day^{-1} for an equilibrium with coexisting clones (E_{12}) or less than 0.23 day^{-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 day^{-1}. This range of dilution rate contrasts to my experimental setting of the dilution rate (0.52-0.55 day^{-1} for UTEX1809-1811 and 0.44-0.50 day^{-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 2-1. Recipe of "master mix".

H_2O	3.8 µL
Buffer	1 µL
10-µM Dyed Labeled 1A Forward Primer [1]	0.15 µL
10-µM 1A Reverse Primer [2]	0.15 µL
2.5mM dNTP	0.8 µL
Taq	0.1 µL

[1] 5’ CAC TAT GCG CCT CCA CTT GAC C 3’

[2] 5’ ATG GAC ATG AGC ATG GAA ACG AC 3’

Table 2-2. Recipe of the culture medium. These materials are dissolved with distilled water.

Materials	Chemical formula	Concentration (μmol/L)
calcium chloride	$CaCl_2 * 2H_2O$	15.0204
potassium nitrate	KNO_3	80.0000
magnesium sulfate	$MgSO_4 * 7H_2O$	81.1359
potassium phosphate dibasic	K_2HPO_4	183.7180
boric acid	H_3BO_3	73.7506
iron-EDTA	$C_{10}H_{12}N_2NaFeO_8$	10.0790
copper sulfate	$CuSO_4 * 5H_2O$	0.0040
zinc sulfate	$ZnSO_4 * 7H_2O$	0.0765
cobalt chloride	$CoCl_2 * 6H_2O$	0.0420
mangane chloride	$MnCl_2 * 4H_2O$	0.9096
sodium molybdate	$Na_2MoO_4 * 2H_2O$	0.0248
lithium chloride	$LiCl$	3.6093
rubidium chloride	$RbCl$	0.2978
strontium	$SrCl_2 * 6H_2O$	0.2851
sodium bromide	$BrNa$	0.0777
potassium iodide	Kl	0.0120
selenious acid	H_2SeO_3	0.0155
sodium orthovanadate	Na_3VO_4	0.0054

Table 2-3. Parameters for the *Chlorella-Brachionus* microcosm model. Set, adjustable parameters set by experimenter.

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]

[1] Fussmann GF, Ellner SP, Shertzer KW, & Hairston Jr NG (2000) Crossing the Hopf bifurcation in a live predator-prey system. *Science* 290(5495):1358-1360.

[2] Jones LE & Ellner SP (2007) Effects of rapid prey evolution on predator-prey cycles. *Journal of Mathematical Biology* 55(4):541-573.

Chapter 3

Comparisons of rapid evolution and phenotypic plasticity in fluctuating environment

I will disclose the contents of this chapter after I have published them in a research paper.

Chapter 4

General discussion

Concluding remarks

To reveal how dynamic change of biological interactions results in ecological consequences, I focused on the details of intraspecific diversity and different adaptation mechanisms. In **Chapter 2**, I re-evaluated the importance of eco-evolutionary feedbacks by observing long-term eco-evolutionary dynamics. Several previous studies have shown time-series of phenotypic traits, but those observations might have been confounded by phenotypic plasticity. Therefore, direct observation of evolution requires quantifying genotype frequencies through generations, which is generally time-consuming. I used an allele-specific quantitative PCR method (Meyer et al. 2006) and succeeded in obtaining time-series of genotype frequencies. Moreover, by comparing the different forms of evolutionary tradeoff I demonstrated that intraspecific variations, especially the qualitative variations, affected the eco-evolutionary dynamics. Previous empirical studies have seldom considered the quality of intraspecific variation. However, my results here suggest that the qualitative aspects (the form of tradeoffs) are important when evolutionary tradeoff exists in the trait variations.

In **Chapter 3**, I showed that the optimal adaptation mechanism depended on the timescale of environmental fluctuations. The results indicate that the mechanisms allowing plastic organisms dominant to evolvable organisms are different depending on the timescales of environmental fluctuations. If the environment fluctuates on short timescales, the speed of plastic adaptation is important. On the middle timescales and the long timescales, the cost of plasticity and the maintenance of genetic diversity in the evolvable organisms are important, respectively. These results indicate that the time scale of environmental fluctuations requires more attention if we are to better understand the feedbacks between ecological and adaptation dynamics.

Overall, it is impossible to understand the dynamical ecological networks without considering the details of intraspecific diversity and the differences among adaptation mechanisms. In this study, I revealed that the form of tradeoff is important as a detail of intraspecific diversity and that the timescale of environmental fluctuations is a significant factor for the relative advantage of different adaptation mechanisms. While it is well known that the quantitative difference of intraspecific variation affects ecological and adaptation feedback (Johnson and Agrawal, 2003; Violle et al., 2012), I revealed that the qualitative difference is also important. Thus, to track ecological and adaptation dynamics, we must know the details of intraspecific diversity.

In this study, I focused on the form of evolutionary tradeoff (**Chapter 2**) and whether organisms have heritable or plastic traits (**Chapter 3**) as the details. These are very simple intraspecific variations. However, we can consider the complicated intraspecific variations to develop more general concepts in evolution, including mating, gene exchange, and mutations. I argue them in the next section.

Future directions

Since my studies were conducted in the laboratory or in silico, many problems remain that prevent an understanding of the dynamic ecological network in real biological systems. Thus, in this section, I will discuss the problems and approaches to solving them. I focus on three important topics: (1) expansion into a large community network, (2) generalization of the intraspecific variations, and (3) comparing among adaptations other than evolution and phenotypic plasticity.

Expansion into a large community network

In this study, I examined ecological systems consisting of a few species. Since real biological systems are larger and more complex than the systems I studied, we need an understanding of a multispecific community network. In the context of adaptive trophic behavior (ATB), research exists on the relationship between a large dynamic food web and stability (Drossel et al., 2001; Guill and Drossel, 2008; Kondoh, 2003; Kondoh, 2007; Quince et al., 2005; Uchida and Drossel, 2007; Valdovinos et al., 2010). This research showed that ATB increases the stability of population dynamics and provides resilience and resistance of networks against perturbations. However, the current ATB models that assume organisms with adaptive behavior can accomplish a perfectly optimal response to every trophic resource or natural enemy (Valdovinos et al., 2010). In contrast, the optimal response is not always ensured because adaptations of organisms to a circumstance lead to a decrease of their intraspecific variations and that makes them difficult to adapt to other circumstances. Indeed, as described in **Chapter 3**, the adaptation of organisms to one environment through rapid evolution decreases their potential to adapt to another environment. Whether an optimal response can be accomplished or not depends on the intraspecific variation. Thus, future studies should consider a large dynamic ecological network with the details of intraspecific variation.

Generalization of the intraspecific variations

I treated the form of tradeoff between two clones as the intraspecific variation in this study, whereas the intraspecific variation is typically more complicated. When a system consists of a few species, we can consider simple intraspecific variation because a few types of prey clones are selected in a system consisting of one prey with multiple clones and one predator (Jones et al., 2009). However,

in a large ecological network, more complicated intraspecific variations can exist because one species interacts with a number of species, which may result in more genetic diversity compared to that in a simple system. Therefore, we need to reveal the complicated intraspecific variations in the case of a multi-species system.

We can express the complicated variation as the frequency distribution of trait values (Violle et al., 2012) or a multiclonal system (Jones et al., 2009). However, consideration of the frequency distribution of trait values makes dealing with population dynamics difficult. Furthermore, the multiclonal models require parameters of growth, death, biological interactions and so on for each clone. Difficulty of analysis and calculations increases with the number of clones.

One powerful tool for resolving such problems is integral projection model (IPM). IPMs can represent how a population structured by any continuous variable describing the state of an individual changes in discrete time (Coulson et al., 2010; Coulson et al., 2011; Easterling et al., 2000; Ellner and Rees, 2006; Ozgul et al., 2009). IPMs can deal with a change of trait distributions as a substitute for population dynamics. Furthermore, IPMs are built on continuous functions that describe the relationships between a trait at time t and survival rate, development of the trait, recruitment, and offspring values at time $t+1$. Thus, IPMs require smaller numbers of parameters than the multiclonal models. Although Smallegange and Coulson (2013) argue for IPMs as a tool to quantify eco-evolutionary change (Smallegange and Coulson, 2013), most IPMs do not assume interspecific interactions. I propose integration of IPMs with multispecies models. The model would be constructed if the biologically adequate shapes of the functions describing the interspecific interactions could be determined.

Observation of the intraspecific variations

Understanding the details of intraspecific variation of organisms in the field and their dynamics is also important. In **Chapter 2**, I observed evolutionary dynamics using AsQ-PCR method, but it cannot apply multiple clones. Methods of observing the details of intraspecific variation are still limited. Tracking a time series of intraspecific genetic variations (evolutionary dynamics) is particularly difficult. Here, I argue three viable approaches to observe evolutionary dynamics. The first is to use organisms linking a genotype directly to a phenotype. This is one of the simplest methods of estimating intraspecific genetic variations. For example, in the presence of high rotifer densities, *Chlamydomonas reinhardtii* forms palmelloid clumps of cells that are highly heritable in a laboratory culture (Becks et al., 2010; Becks et al., 2012). Another candidate are bacteria because they have various observable phenotypes such as antibiotic resistance and biofilm formation (Davey and O'toole, 2000). Changes can be easily tracked over many generations.

The second is fluorescence in situ hybridization (FISH), a cultivation-independent method for the direct observation and identification of single cells by fluorescence microscopy (Amann et al., 1990; DeLong et al., 1989). FISH is usually used to identify single microbial cells where the target of fluorescently labeled oligonucleotides (probes) is ribosomal RNA (Amann and Fuchs, 2008). However, if we create probes to detect the difference among intraspecific variations, we can use it to observe evolutionary dynamics. Note that this method cannot be applied to living organisms because of the necessary fix to stabilize the cells and make the cell membranes permeable. Therefore, this approach is not applicable to rare species in the field.

The last is a metagenomic-based approach enabled by next-generation sequencing technology. Sequencing uncultured microbes sampled directly from their habitats allows us to examine the community structure of the microbes in the field (Wooley et al., 2010). Toju et al. (2014) succeeded in constructing the network, including the relative abundance of plant species or fungal operational taxonomic units (Toju et al., 2014). Although, they focused on taxonomic units, I propose the possibility of using DNA-barcoding-based research to detect the intraspecific genetic variations. If we obtain the time-series data of the network with the intraspecific variations, this approach will contribute to our understanding feedback between ecological consequences and rapid adaptations.

Adaptations other than rapid evolution and phenotypic plasticity

In **Chapter 3**, I focused on rapid evolution and phenotypic plasticity as adaptations, but we can consider some other adaptations as a target for comparison. Especially, we know that learning behavior can have feedbacks from ecological consequences. Ishii and Shimada (2012) demonstrated that choice behavior of a parasitoid wasp depending on learning affects population dynamics and coexistence of two host species. The following features characterize learning behavior. First, it can produce the adaptation within a generation like phenotypic plasticity. Second, it can give frequency-dependent response to other species like rapid evolution, but the response occurs without selection, unlike rapid evolution. Third, change of predation behaviors such as hunting mode and habitat use coming from learning is likely to provoke the non-consumptive effects (Preisser and Bolnick, 2008; Schmitz et al., 2004). These features distinguish ecological consequences caused by

learning behavior from those of other adaptation mechanisms. Comparing between learning and evolution or phenotypic plasticity is worthwhile.

Biological diversities

The importance of conservation and restoration of biological diversity has been established (The Convention on Biological Diversity (CBD) entered into force on 29 December 1993). One of the goals of understanding ecological networks, in which biological interactions are dynamically changeable, is to contribute to the conservation and restoration of biological diversity. The implication of my study is that intraspecific diversity is a significant element of biological diversity and serves as a foundation for how to preserve biological diversity. However, our understanding is insufficient to answer why and how we preserve biological diversity. Further studies will be needed to that end. I believe that the study of ecological networks with dynamic changes in biological interactions, including the present study, helps to preserve biological diversity, leading to a better future for our children and us.

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