Prey evolution on the time scale of predator–prey dynamics revealed by allele-specific quantitative PCR

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Using rotifer–algal microcosms, we tracked rapid evolution resulting from temporally changing natural selection in ecological predator–prey dynamics. We previously demonstrated that predator–prey oscillations in rotifer–algal laboratory microcosms are qualitatively altered by the presence of genetic variation within the prey. In that study, changes in algal gene frequencies were inferred from their effects on population dynamics but not observed directly. Here, we document rapid prey evolution in this system by directly observing changes in *Chlorella vulgaris* genotype frequencies as the abundances of these algae and their consumer, *Brachionus calyciflorus*, change through time. We isolated a group of algal clones that we could distinguish by using microsatellite-DNA markers, and developed an allele-specific quantitative PCR technique (AsQ-PCR) to quantify the frequencies of pairs of clones in mixed culture. We showed that two of these genotypes exhibited a fitness tradeoff in which one was more resistant to predation (more digestion-resistant), and the other had faster population growth under limiting nitrogen concentrations. A fully specified mathematical model for the rotifer–algal population and evolutionary dynamics predicted that these two clones would undergo a single oscillation in clonal frequencies followed by asymptotic fixation of the more resistant clone, rather than the recurrent oscillations previously observed with other algal clones. We used AsQ-PCR to confirm this prediction: the superior competitor dominated initially, but as rotifer densities increased, the more predator-resistant clone predominated.

Chlorella vulgaris | clonal models | evolutionary tradeoff | grazing resistance | rapid evolution

There has been an increasing appreciation during the past three decades that ecological and evolutionary dynamics can operate on similar time scales and interact in important ways. For example, genetic variation in a prey population can permit evolution that radically alters predator–prey dynamics (1, 2), evolution in environmentally threatened populations can affect population recovery (3), and rapid evolution is now seen as a critical component shaping disease dynamics (e.g., in HIV, refs. 4–7). Each of these discoveries was to some extent unexpected because, despite a growing number of examples of rapid evolution (8–11), the default expectation has often continued to be that ecological and evolutionary dynamics occur on different time scales (12, 13). Although recent discoveries challenge this notion (e.g., refs. 14–16), direct demonstrations of the mechanistic interplay between genetic change and ecological process remain rare. Here, we present a clear demonstration of prey evolution in concert with temporal changes in predator and prey densities, using genetic markers to quantify the evolutionary dynamics.

Laboratory microcosms of rapidly reproducing interacting species have proven to be effective systems for the study of simultaneous ecological and evolutionary dynamics (17), including consumer–victim interactions (e.g., the bacteria-phage study in ref. 18). We have shown for the laboratory predator–prey system we study that, when the algal prey are genetically variable and can evolve in response to temporally varying selection (nutrient availability and predation by rotifers), the period and phase relationships of the predator–prey cycles between rotifers and algae are substantially altered from those when the algae lack genetic variation and cannot evolve (1, 19–21). These dynamics require that there be a genetically based tradeoff in the algae between competitive ability for the limiting nutrient (nitrogen in our system) and vulnerability to predation mortality, and Yoshida *et al.* (22) showed that algal lineages selected either for competitive ability or defense against predation exhibited such an evolutionary tradeoff. Thus, previous studies have indirectly shown the importance of prey evolution in rotifer–algal chemostats. They inferred concurrent changes in algal gene frequencies but did not observe this microevolution directly because Fussmann *et al.* (23) and Yoshida *et al.* (1) used a monospecific mixture of unidentified algal clonal genotypes descended from a single culture strain in their experiments (here a ''strain'' refers to a lineage of cells independently isolated from nature and maintained continuously in culture, usually by a culture collection such as that at the University of Texas, from which we obtained all of our strains). Because the goal of our study was to test the prediction that algal clonal frequencies change during predator-driven population fluctuations, we used a mixture of two genetically and phenotypically distinct clones whose frequencies we could measure at the time scale that the ecological predator–prey dynamics occurred.

The consumer in our system is an obligately asexual lineage of the rotifer *Brachionus calyciflorus* (24). Although these animals are herbivores consuming algae, we refer to them here as ''predators'' because they consume individual prey items (cells of the alga, *C. vulgaris*) whole. We allow their populations to interact in continuous flow-through culture vessels (chemostats) supplied with a nitrogen-limited medium (21, 23, 25). We have shown that under a range of chemostat conditions (dilution rate and nitrogen concentration) the algal population (comprised of an unidentified mixture of algal clones taken from a single strain) and the rotifer population become extinct, reach a stable equilibrium, or oscillate in stable limit cycles (20, 21, 23). Whereas the chemostat conditions that have produced the different population dynamics are well predicted by a mechanistic model of coupled nonlinear differential equations described by Fussmann *et al.* (21, 23), and further refined by Yoshida *et al.* (1) and Jones and Ellner (20), we sought an extension of this model in which the dynamical behavior resulting from a particular combination of clonal traits could be tested against observation. This search required the development of a molecular genetic method for distinguishing algal clones that was practical for daily monitoring of clonal frequencies, and then the isolation of particular clones whose phenotypes lay at different positions

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Abbreviations: UTEX, University of Texas; AsQ-PCR, allele-specific quantitative PCR.

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along the predator–defense versus competitive ability tradeoff relationship.

We found microsatellite DNA markers for our algal prey, *Chlorella vulgaris*, that could be used to distinguish among nine strains obtained from the University of Texas (UTEX) algal collection. We then invented an allele-specific quantitative PCR technique (AsQ-PCR) based on microsatellite differences that allowed us to assay the relative abundance of pairs of *C. vulgaris* strains and thus track evolutionary dynamics in our chemostats. After isolating a single clone from each strain, we showed that one clonal pair possessed a clear tradeoff between relative vulnerability to predation mortality imposed by rotifers and competitive ability based on the growth rates of the clones on limiting concentrations of nitrogen. We predicted simultaneous algal-clone frequency and predator–prey dynamics by using measured tradeoff values of fitness traits in a fully specified two-clone simulation model. Finally, we tested our predictions by using a pair of continuous-flow rotifer–algal chemostat experiments initiated with the two characterized *C. vulgaris* clones in combination with rotifers, and monitored algal density and clonal frequencies and rotifer density. We document an interaction between ecological predator–prey interactions and rapid algal evolution in which each influences the dynamics of the other.

Results

Identification and Quantification of Experimental Clones. Of the nine UTEX strains of *C. vulgaris*, only three pairs showed the required linearity for mixed cultures between known genotype frequencies and those calculated from the AsQ-PCR procedure. One pair of these clones (UTEX 265 and UTEX 396) showed a tradeoff between competitive ability and resistance to predation mortality potentially capable of producing the evolutionarily driven predator–prey cycles we observed between *B. calyciflorus* and *C. vulgaris* (1). The prediction reliability from AsQ-PCR for UTEX 265 and UTEX 396 frequencies was $r^2 = 0.981$ ($P < 0.0001$; $y = 0.0934 + 0.851x$), and further experiments were only carried out by using this clonal pair.

Fitness Tradeoff for Clones: Vulnerability to Predation vs. Competitive Ability. The clone derived from *C. vulgaris* strain UTEX 265 was significantly less susceptible to predator-induced mortality than that from UTEX 396, the former experiencing less than half the mortality rate of the latter (paired t test, $t = 7.344$, $P < 0.001$, Fig. 1). We used the ratio of the population growth rates, r, in the presence and absence of predation to describe relative vulnerabilities, p_i, of the clones, so that by definition $p_{max} = 1$ for the less-defended clone, whereas $p_{\min} = 0.47 \pm 0.02$ (± 1 SEM) for the better-defended clone.

Although the UTEX-265 clone was better defended against predation, it had a significantly lower growth rate at three different resource concentrations than the UTEX-396 clone (paired t tests, $P < 0.035$ in all cases, Fig. 1). When fitted to Monod curves for each clone, the population growth rate, β_c, of UTEX clone 265 was found to be reduced by 20%, making $\gamma = 0.8$ relative to that of UTEX clone 396. As a result, we refer hereafter to UTEX clone 265 as the "defended clone" and UTEX clone 396 as the "competitive clone."

Our short-term feeding and defecation experiments showed that the rotifers did not feed selectively on either clone (t tests, $t = 0.556$, $P > 0.607$, Fig. 2) but that the defended clone survived gut passage significantly more frequently than the competitive clone. This latter result was obtained in two ways: first, AsQ-PCR analysis showed that the genotype of the defended clone was found in significantly higher frequency in feces than that of the competitive clone (paired t tests, $t = 9.446$, $P < 0.0008$, Fig. 2), and second, 18.3 times more colonies grew from the defecated cells of the defended clone than from the defecated cells of the competitive clone.

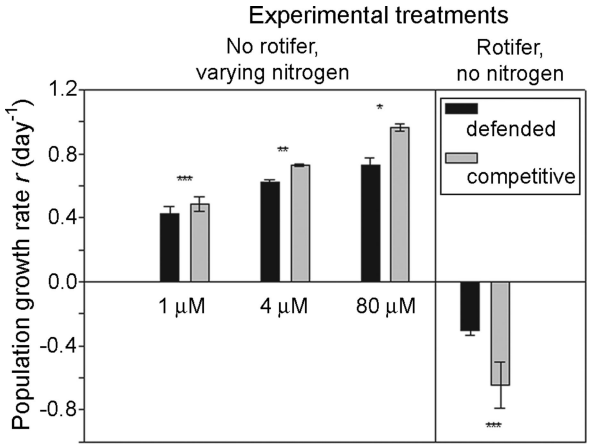

Fig. 1. Algal tradeoff between defense against rotifer predation and algal competitive ability (growth rate over a range of nitrogen concentrations). The defended clone (UTEX 265) showed lower growth rate than the competitive clone (UTEX 396) at three different nutrient levels but suffered lower mortality in the presence of rotifers. Plotted values are clonal means (± 1 SD) for each treatment. However, statistical tests were carried out by using paired t tests: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (paired t tests).

Modeling and Predicting Clonal Dynamics. Yoshida *et al.* (1) presented a mathematical model that successfully described the rotifer–algal dynamics they observed with multiclonal or isoclonal algal cultures. We used this model to predict the predator–prey and algal clonal frequency dynamics of rotifers and algal clones we isolated here, with all parameters unchanged except that we accounted for the measured differences in vulnerability to predation ("palatabilities"), p, and maximum growth rates, β_c, between our two clones. Details of model assumptions and their justification, and a listing of the model equations, can be found in the supporting information, which is published on the PNAS web site.

Whereas Yoshida *et al.* assumed a tradeoff between p and K_c, the data for our clones suggest a tradeoff between p and β_c. Thus,

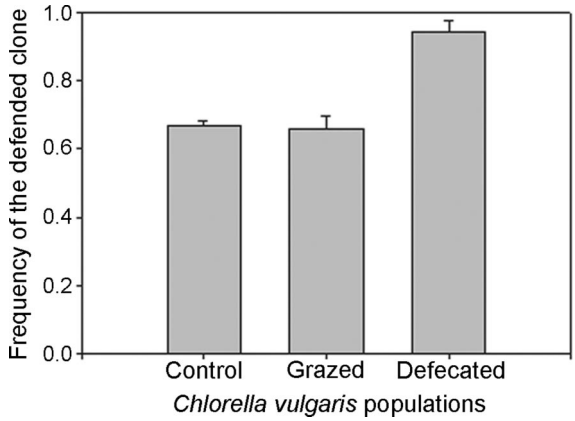

Fig. 2. Comparison of the frequency of the defended clone (UTEX 265) in mixed populations of UTEX 265 and UTEX 396 from the rotifer feeding and defecation experiment. The control and predated populations have the same frequency of the defended clone, indicating that rotifers do not ingest either clone more frequently (t test, $t = 0.556$, $P > 0.60$). The defecated population had a significantly greater percentage of the defended clone than the predated population (t test, $t = 9.446$, $P < 0.0008$), meaning that the rotifers defecated a greater percentage of the defended clone than they consumed.

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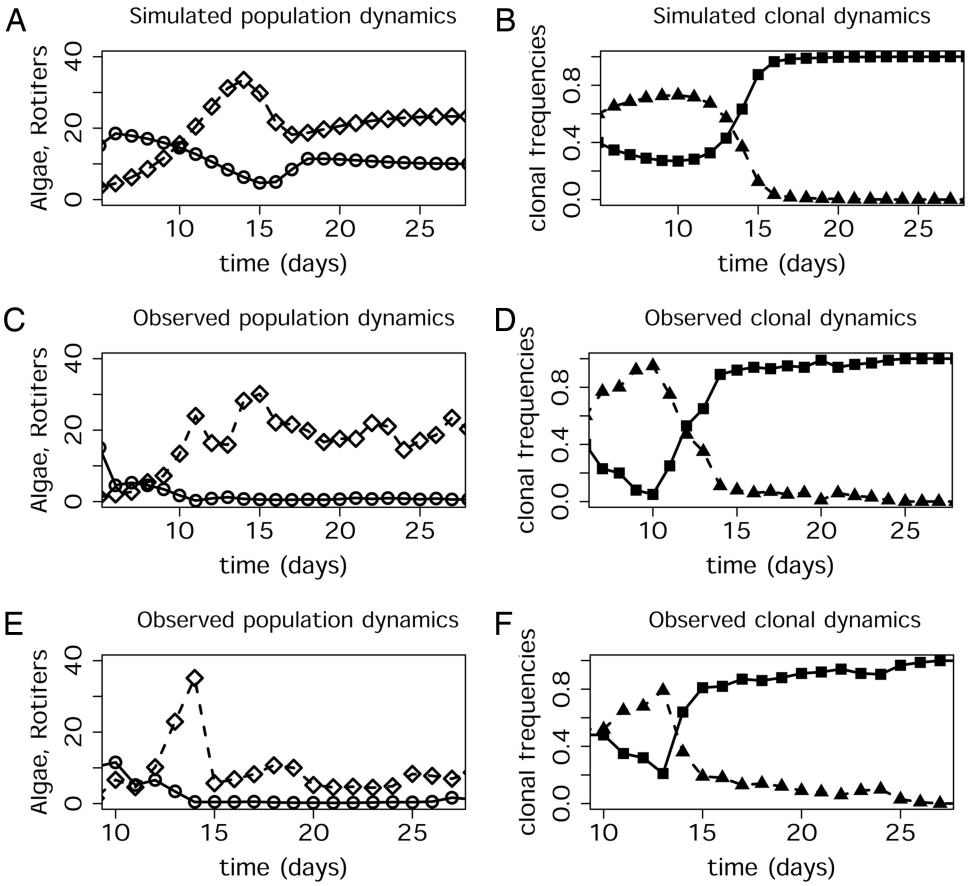

Fig. 3. Comparison between simulated and observed population and clonal dynamics. Simulated (*A* and *B*) and observed (*C–F*) population dynamics of total algal cells (open circles, 2×10^6 cells per liter) and rotifers (open diamonds, females per liter) and clonal frequency dynamics of defended clone (UTEX 265, solid squares) and competitive clone (UTEX 396, solid triangles). (*C* and *D*) Chemostat results using 80 μM nitrogen medium. (*E* and *F*) Chemostat results using 160 μM nitrogen medium. Both chemostats run at a dilution rate, δ, of 0.85.

we assume that reduced vulnerability $p_{min} < 1$ is gained at the cost of a reduction in growth rate, $\beta_c(p_{min}) = \gamma\beta_c$, where $\gamma < 1$. The algal functional response can then be written

$$F_{c,i}(n/V) = \frac{1}{\varepsilon_c}\frac{\omega_c\beta(p)N}{K_c + N}, \qquad [1]$$

where $i = 1, 2$ designates clone, ε_c is the algal assimilation efficiency, ω_c is nitrogen content per 10^9 algal cells, and N is nitrogen concentration of the medium in micromoles per liter. Parameter values β_c and K_c for (1) were estimated by fitting Monod curves to the observed average instantaneous growth rate during the exponential increase phase of the batch culture experiments in which rotifers were not present. Their fitted half-saturation constants were very similar, so we estimated a single shared value by imposing the constraint that the fitted curves have the same half-saturation constant. This gave an estimated $K_c = 0.93$ μmol/liter, which we rounded to 1 for the simulations, whereas the maximum population growth rate of the more defended clone was reduced by 20%, making $\gamma = 0.8$ relative to that of less defended clone.

The batch culture experiments with rotifers present were used to estimate relative vulnerabilities of the two clones to predation mortality. Assuming that consumption by rotifers is the only process affecting algal abundance, we have $C(p, t) = C(p, 0)e^{-pGt}$, where C is the abundance of a clone with vulnerability p, and G is the rotifer clearance rate expressed as the fraction of medium processed per unit time. The average instantaneous rate of population change is then

$$r(p) = [\log C(p, t) - \log C(p, 0)]/t = -pG. \qquad [2]$$

The ratio of P values for two clones can therefore be estimated as the ratio between their estimated r values.

Comparing Model Predictions with Clonal Dynamics in Chemostats. Our model predicts a rapid increase in the frequency of the competitive clone at the start of the experiment when algal densities are high but starting to decline and rotifer densities are low and beginning to increase (Fig. 3 *A* and *B*). When rotifer density has increased to roughly half of its maximum value, the frequency of the competitive clone begins to decline, whereas that of the defended clone begins to increase. Eventually, rotifer density overshoots and then stabilizes at a high value, algal density declines, undershoots and then stabilizes at a low value, and the defended clone goes to fixation. Thus, given the measured competitive abilities and vulnerabilities to predation mortality for the particular clones that we were able to isolate and quantify using our AsQ-PCR technique, our model predicted a period of transitory evolutionary dynamics with clonal frequencies fluctuating and then stabilizing at the fixation of a single type (Fig. 3 *A* and *B*), rather than the stable predator–prey oscillations that we had previously observed and the stable clonal frequency oscillations that we inferred must have occurred (1).

The dynamics we observed in our chemostat microcosms (Fig.

3 *C–F*) are strikingly similar to those predicted by our mechanistic model. There was a transitory oscillation in algal and rotifer densities leading to a slow approach to equilibrium, although algal densities observed ended up rather lower than predicted (compare *A* and *B* with *C–F* in Fig. 3). Linked to these ecological dynamics, and indeed influencing their patterns, was the predicted oscillation in the frequencies of the algal clones. We observed similar dynamics in chemostats run using both 80 μM nitrogen (Fig. 3 *C* and *D*) 160 μM nitrogen (Fig. 3 *E* and *F*) concentrations in the medium, i.e., doubling the level of enrichment did not qualitatively alter the dynamics.

Discussion

We have observed rapid evolutionary changes in algal clonal genotypes occurring on the time scale of days and interacting with what has traditionally been considered to be a purely ecological process: predator–prey oscillatory dynamics. This result is fully consistent with previous observations (1) showing that the presence of multiple algal clones can radically alter the period and the phase relationships in an oscillating predator–prey system. However, in the case of the particular clonal phenotypes that we were able to quantify for the current study using our AsQ-PCR method, the expected and observed dynamics under our experimental conditions were a transitory oscillation to a stable equilibrium. Using the same model, Yoshida *et al.* (1) posited and Jones and Ellner (20) found theoretically that an algal population comprised of multiple clonal phenotypes arrayed along a tradeoff curve between high competitive ability and high defense against predation, would evolve rapidly under rotifer predation to exclude all but two phenotypes located at opposite extremes of the tradeoff such that predator–prey cycles would persist but with markedly distinct periods and phase relationships. Other theoretical results not presented here demonstrate that at a single set of chemostat conditions (nitrogen concentration and dilution rate) three completely different predator–prey dynamics can occur based on the initial clonal composition of the algal population: a genetically uniform population of undefended *C. vulgaris* promotes normal short stable-limit cycles; a clonally diverse population with at least two phenotypes, one extremely defended ($P < 0.22$) and one undefended ($P = 1.0$), has extended predator–prey oscillations; stable dynamics are maintained if a *C. vulgaris* clone of intermediate defense ($0.22 < P < 0.56$) is driven to fixation. It is this last condition observed in the system presented here. The supporting information provides an illustration of the range of dynamics predicted for our clonal types, given the intermediate defense trait of our defended clone, and shows that the evolutionary change we observed in the frequencies of our two ecologically distinct clones produced predator–prey dynamics quite distinct from what would have occurred with either algal clone alone.

To carry out our study, we developed an allele-specific quantitative PCR method that allowed us to measure particular *C. vulgaris* clonal frequencies quickly and accurately. Using this method, we have shown that one of the clonal phenotypes we isolated was better than the other at surviving predation by *B. calyciflorus*. *Brachionus* is a generalist consumer with only limited capacity for size-selective feeding (26, 27) and it is not surprising that it did not ingest either clone selectively (Fig. 2). Instead, the defended clone was defecated in a viable state by *B. calyciflorus* significantly more frequently than our other clonal phenotype. The defended clone had a reduced growth-rate over a wide range of nutrient concentrations, (Fig. 1) consistent with having a classic evolutionary tradeoff. We do not know the mechanistic basis of this tradeoff between defense and competitive ability, but it seems likely that it lies in some property of the algal cell wall that allows cells to survive digestion but at a cost in nutrient uptake capacity. Evolutionary tradeoffs such as these are fundamental to the maintenance of diverse genotypes by natural selection and yet are rarely observed (28).

Bohannan and Lenski (29) used a microcosm system similar to ours to study the effect of heterogeneity in prey vulnerability on top-down versus bottom-up control of trophic dynamics. Their system, like ours, involved a single consumer (T4 phage) and two genotypes within an asexual species (*Escherichia coli*), one more vulnerable to predation than the other. Because the algal and bacterial prey are asexual for our system and theirs, one might ask whether the process observed is evolution within a single species, as we have interpreted it, or community dynamics within a food web of two prey species and a single predator. Indeed, the dynamics and ecological impacts of species replacement are often very similar theoretically to that of evolutionary change (2). We argue that it is algal microevolution that we have observed. There is a long and distinguished history of theoretical and empirical studies of evolution in asexual species, particularly in comparison with sexual species (30), in which clonal replacement of the kind we have observed is the mechanism for response to natural selection. The dynamics reported here do not cover the full allele-substitution process, starting with a novel mutation, continuing through intermediate changes in genotype frequencies, and ending with fixation as a result of selection, drift, or both. Rather, we introduced the genetic variation ourselves so that all prey genotypes could be identified by AsQ-PCR. However the prey variants in our experiments all arose naturally, and the phenotypic differences between them, a negative correlation between competitive ability and defense against predation, were of the same type that we previously observed to arise as spontaneous mutants in our laboratory system (22). Regardless of how the variation arose, its presence led to natural selection and response, the fundamental component of adaptive evolution. It is noteworthy in this regard that Bohannan and Lenski (29) observed the same dynamics in their phage–bacteria system both when they introduced prey variation themselves and when it arose by mutation during an experimental run. Adding an understanding of the origin of prey genetic variation (mutation rate, distribution of fitness effects, etc.) would complete our understanding of the evolutionary dynamics in our system and will be pursued in future work.

If we accept that our clones are genotypes within a single species (rather than abandoning the concept of asexual species), then what we have observed is an ecologically driven reversal in the direction of natural selection, from selection favoring a competitively superior genotype to selection favoring a digestion-resistant genotype, resulting in gene-frequency changes having a direct effect on population dynamics. However, the implications of our experiments are not limited to asexual species, because the kinds of predator–prey dynamics seen in a model for our system with clonal selection (19) are also observed in a model based on a quantitative genetics description of selection response (2), which is appropriate for sexually reproducing species.

Rapid evolution occurring at time scales comparable to those at which ecological process take place have been the topic of much recent research and discussion (reviewed in refs. 8–10), and has raised the possibility that evolution might occur sufficiently fast to impact ecological dynamics directly. Our study of rotifer–algal predator–prey dynamics provides one clear laboratory example. Analysis of field data for systems in which evolutionary rates are particularly rapid has confirmed that evolution can, and at least sometimes does, take place at a rate similar to ecological processes (11). However, the question remains whether the effects of rapid evolution on algal-consumer dynamics that we have documented here are important in nature. For example, are temporal changes in the densities of lake phytoplankton and zooplankton, such as the well-known clear-water phase dynamics observed in many tem-

perate lakes (31, 32), and the wide variation in algal food quality to grazers within and among lakes (33, 34), influenced importantly by the level of algal genetic diversity available when acted upon by the natural selection imposed by grazers?

Materials and Methods

Identification of Experimental Clones. Fussmann *et al.* (23) and Yoshida *et al.* (1, 22) used a single strain of *C. vulgaris* (UTEX 26). For our study, we purchased nine different UTEX strains of *C. vulgaris* (UTEX 26, 30, 265, 395, 396, 1803, 1809, 1911, and 2714). For each strain, we developed *de novo* 10 microsatellite loci for *C. vulgaris* using the procedure reported by Hamilton *et al.* (35). For three of these loci, we obtained dye-labeled primers that proved to be sufficient to distinguish among the nine algal strains. This then gave us 36 unique pairs of strains that we could distinguish based upon microsatellite-DNA genotypes.

Allele-Specific Quantitative PCR (AsQ-PCR). We next identified pairs of UTEX algal strains whose relative abundances could be quickly and accurately estimated by using a method we invented called AsQ-PCR. For PCR to quantify allele frequency accurately, both alleles must amplify at identical rates. Ideally, all alleles should amplify equally during the PCR; however, in practice, PCR often favors the amplification of one allele over another. To test whether the alleles of pairs of candidate UTEX algal strains amplified at the same rate, we mixed strains in a range of known proportions and carried out the extraction and PCRs described below. We then regressed the strain frequencies, calculated from the relative peak heights on the electropherogram (see below), against the known frequencies in the sample. For each analysis, we assayed four replicate DNA samples at each of five different strain proportions ranging between 10:90 and 90:10. Pairs of strains with high r^2 values were judged suitable for use in tracking strain frequencies.

We determined frequencies by comparing the amount of PCR product amplified from each allele read by an ABI 3100 sequencer and reported by GENEMAPPER (version 3.5; Applied Biosystems). Allele (clonal) frequency was estimated as its peak height on the electropherogram divided by the total peak height of both alleles. We concentrated samples of mixed clones by filtering 2.5×10^6 cells onto a 0.7-μm glass fiber filter, placed the filter in a 1.5-ml Eppendorf 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 room temperature. The freeze–thaw step was repeated three times to break the cell walls. The samples were incubated for 4 h at 55°C, boiled for 10 min at 100°C, and then centrifuged at $10{,}000 \times g$ in a desktop centrifuge.

The PCR was run with 4 μl of supernatant of the Chelex–DNA extraction, 4.52 μl of H_2O, 1 μl of New England Biolabs ThermoPol Buffer, 0.15 μl of NED-labeled forward primer (5′-CAC TAT GCG CCT CCA CTT GAC C-3′), 0.15 μl of reverse primer (5′-ATG GAC ATG AGC ATG GAA ACG AC-3′), 0.08 μl of 25 mM dNTP, and 0.1 μl of 5,000 units/liter New England Biolabs TaqDNA polymerase. 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. These samples were then genotyped on an ABI 3100 (Applied Biosystems).

Three pairs of UTEX algal strains showed the required linearity ($r^2 \geq 0.95$, $P \leq 0.05$) between known and calculated genotype frequencies in mixed culture. At this point, each of our UTEX *C. vulgaris* strains (each strain with a unique microsatellite genotype) was a mixture of an unknown number of distinct clones. We isolated a single clone from each strain by spreading a sample of a strain thinly on an agar plate, allowing colonies of cells to grow up and then lifting a single colony (representing growth from a single cell) with a sterile loop to inoculate a new culture.

Determining Fitness Tradeoff for Clones. The predation-vulnerability experiments were performed by using 3.5×10^6 algal cells per ml of each clone and four rotifers per ml in six replicate tubes with 25 ml of medium. To inhibit algal growth, predation experiments were held in darkness and used a medium lacking nitrogen. The cultures were continuously mixed on an orbital shaker and aerated twice daily for the 3- to 4-day duration of each experiment. The algal populations were monitored daily, and an experiment was terminated when total *C. vulgaris* density declined to $\leq 5 \times 10^5$ cells per ml from rotifer predation. Clonal frequencies were determined in each experimental replicate by using our AsQ-PCR method at the start and end of the predation period. Triplicate experimental treatments (rotifers present) and two controls (identical to the experimental treatments but lacking rotifers) were sampled for clonal frequencies at times corresponding to the rotifer treatments. Population decrease (day^{-1}) for each clone in each treatment was calculated to compare predation vulnerabilities between pairs of clones for each of the three distinguishable pairs.

To quantify clonal competitive abilities, pairs of clones were grown under a range of nitrogen concentrations (1, 4, and 80 μM), and the population growth rate (day^{-1}) of each clone was calculated. We inoculated 50 ml of medium with the *C. vulgaris* clones to be tested at 5×10^3 cells per ml per clone, with three replicates run at each nutrient treatment. These cultures were grown at 25°C in continuous light, and the population densities of *C. vulgaris* were sampled daily for 3–7 days of exponential growth. Clonal frequencies were determined in each flask at the end of the experiment by using our AsQ-PCR method.

Because only the clonal pair UTEX 265 and UTEX 396 possessed a tradeoff between competitive ability and resistance to predation mortality of the kind potentially capable of producing the evolutionarily driven predator–prey cycles we previously observed between *B. calyciflorus* and *C. vulgaris* (1, 19), further experiments were only carried out by using this clonal pair. Our AsQ-PCR method also allowed us to explore the mechanism by which algal clones differed in their vulnerability to predation. We can envision two alternative possibilities: either the rotifers can discriminate between algal cell types during capture and ingestion, retaining some cells and rejecting others uneaten, or the rotifers collect and ingest the two types equally, but the cells differ in digestibility, with the less vulnerable type surviving gut passage more frequently than the other. To determine which, if either, was the case for the clones we selected, 2.5×10^3 rotifers, whose guts had been cleared by 18 h of starvation, were allowed to feed on a mixture of the two clones at a total *C. vulgaris* density of 5×10^5 cells per ml in 25 ml of medium for 10 min, enough time for them to eat but not defecate (36). The rotifers were then removed from the feeding suspension, rinsed in fresh medium, placed in another flask of medium, and allowed to defecate for 15 min (sufficient time for them to substantially clear their guts; ref. 36). The rotifers were then returned to the initial feeding flask for another 10 min and to a new flask to defecate for another 15 min. This process was repeated nine times to obtain sufficient predation and defecation to assess changes in clonal frequencies in the feeding suspension and the feces. Thus, clonal frequencies were measured from algae sampled from the *C. vulgaris* population exposed to rotifers before and after feeding and from the combined fecal samples. Control treatments using heat-killed rotifers were run in parallel with the experimental treatments to check for algae adhering to the outside of the rotifers. We ran five replicate experimental treatments and three replicate control treatments, each containing either live or dead rotifers. The frequencies of the two algal clones were measured in each

treatment in the initial algal suspension before predation, the algal suspension after predation, and in the rotifer feces by using our AsQ-PCR method.

Finally, we tested whether the defecated cells whose microsatellite DNA sequences we were able to amplify were in fact viable. We repeated the above feeding and defecation experiment but with the rotifers only allowed to feed on each clone separately (i.e., in isoclonal feeding suspensions). Two replicate feeding trials were carried out on each clonal type, as were two replicate dead-rotifer controls. Following the sequence of feeding and defecation, two 100-μl samples of the algal-free medium, into which the rotifers had defecated, were spread onto nutrient-rich agar plates, and after 14 days we counted the colonies, each of which had grown from a single cell. Four colonies from each plate (24 colonies total for each clonal type) were genotyped to ensure that they were of the expected genotype and not a contaminant.

Observing Clonal Dynamics in Predator–Prey Chemostats. We ran a single-stage rotifer–algal chemostat under the conditions at which Fussmann *et al.* (23) and Yoshida *et al.* (1) observed stable predator–prey oscillations: 80 μM nitrogen with a dilution rate, δ, of 0.85 day^{-1}. The chemostat was sampled daily for rotifer and algal densities as described by Fussmann *et al.* (23). Because the initial algal assemblage we used to inoculate the chemostat was made up of the two *C. vulgaris* clones we isolated (above), we were able also to monitor daily changes in clonal frequencies while the predator–prey dynamics played out. To assay clonal frequencies, each day two samples of 2.5×10^6 *C. vulgaris* cells were removed from the chemostat, rotifers were removed by sieving (30-μm mesh), the algae were filtered on 0.7-μm GF-F filters, and clonal frequencies determined by using our AsQ-PCR method. We ran a second replicate chemostat initiated and sampled in the same way but using a medium of 160 μM nitrogen with the thought that a higher level of enrichment might force the system into prolonged predator–prey oscillations (37).

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1. Yoshida, T., Jones, L. E., Ellner, S. P., Fussmann, G. F. & Hairston, N. G., Jr. (2003) *Nature* **424,** 303–306.
2. Abrams, P. & Matsuda, H. (1997) *Evolution (Lawrence, Kans.)* **51,** 1740–1748.
3. Coltman, D. W., O'Donoghue, P., Jorgenson, J. T., Hogg, J. T., Stobeck C. & Festa-Blanchet, M. (2003) *Nature* **426,** 655–658.
4. Coffin, J. M. (1995) *Science* **267,** 483–489.
5. Korber, B., Theiler, J. & Wolinsky, S. (1998) *Science* **280,** 1868–1871.
6. Korber, B., Gaschen, B., Yusim, K., Thakallapally, R., Kesmir, C. & Detours, V. (2001) *Br. Med. Bull.* **58,** 19–42.
7. Burton, D. R., Stanfield, R. L. & Wilson, J. A. (2005) *Proc. Natl. Acad. Sci. USA* **102,** 14943–14948.
8. Thompson, J. N. (1998) *Trends. Ecol. Evol.* **13,** 329–332.
9. Hendry, A. P. & Kinnison, M. T. (1999) *Evolution (Lawrence, Kans.)* **53,** 1637–1653.
10. Palumbi, S. R. (2001) *The Evolution Explosion: How Humans Cause Rapid Evolutionary Change* (Norton, New York).
11. Hairston, N. G., Jr., Ellner, S. P., Geber, M. A., Yoshida, T. & Fox, J. A. (2005) *Eco. Lett.* **8,** 1114–1127.
12. Waxman, D., Gavrilets, S. (2005) *J. Evol. Biol.* **18,** 1139–1154.
13. Brown, J. S., Vincent, T. L. (2006) *Comp. Oper. Res.* **33,** 479–499.
14. Stockwell, C. A., Hendry, A. P. & Kinnison, M. T. (2003) *Trends Ecol. Evol.* **18,** 94–101.
15. Ashley, M. V., Wilson, M. F., Pergams, O. R. W., O'Dowd, D. J., Gende, S. M. & Brown, J. S. (2003) *Biol. Conserv.* **111,** 115–123.
16. Johnson, M. T. J. & Agrawal, A. (2003) *Trends Ecol. Evol.* **18,** 549–551.
17. Bohannan, B. J. M. & Lenski, R. E. (2000) *Ecol. Lett.* **3,** 362–377.
18. Lenski, R. E. & Levin, B. R. (1985) *Am. Nat.* **125,** 585–602.
19. Shertzer, K. W., Ellner, S. P., Fussmann, G. F. & Hairston, N. G., Jr. (2002) *J. Anim. Ecol.* **71,** 802–815.
20. Jones, L. E. & Ellner, S. P. (2004) *Bull. Math. Biol.* **66,** 1547–1573.
21. Fussmann, G. F., Ellner, S. P. & Hairston, N. G., Jr., Jones, L. E., Shertzer, K. W. & Yoshida, T. (2005) *Adv. Ecol. Res.* **37,** 221–243.
22. Yoshida, T., Hairston, N. G., Jr., & Ellner, S. P. (2004) *Proc. R. Soc. London B.* **271,** 1947–1953.
23. Fussmann, G. F., Ellner, S. P., Shertzer, K. W. & Hairston, N. G., Jr. (2000) *Science* **290,** 1358–1360.
24. Fussmann, G. F., Ellner, S. P. & Hairston, N. G., Jr. (2003) *Proc. R. Soc. London B* **270,** 1015–1022.
25. Boraas, M. E. (1983) *Limnol. Oceanogr.* **28,** 546–563.
26. Ruttner-Kolisko, A. (1974) *Binnengewässer* **26,** Suppl. 1, 1–146.
27. Starkweather, P. L. (1980) in *Evolution and Ecology of Zooplankton Communities*, ed. Kerfoot, W. C. (Univ. Press of New England, Hanover, NH), pp. 151–157.
28. Rainey, P. B., Buckling, A., Kassen, R. & Travisano M. (2000) *Trends Ecol. Evol.* **15,** 243–247.
29. Bohannan, B. J. M., Lenski, R. E. (1999) *Am. Nat.* **153,** 73–82.
30. Kondrashov, A. S. (1997) *Annu. Rev. Ecol. Syst.* **28,** 391–435.
31. Lampert, W., Fleckner, W., Rai, H. & Taylor, B. E. (1986) *Limnol. Oceanogr.* **31,** 478–490.
32. Hairston N. G., Jr., Kearns, C. M., Demma, L. P. & Effler, S. W. (2005) *Ecology* **86,** 1669–1678.
33. Muller Navarra, D. & Lampert, W. (1996) *J. Plankton Res.* **18,** 1137–1157.
34. Tessier, A. J. & Woodruff, P. (2002) *Ecology* **83,** 1263–1270.
35. Hamilton, M. B., Pincus, E. L., Di Fiore, A. & Fleischer, R. C. (1999) *BioTechniques* **27,** 500–507.
36. Starkweather, P. L. & Gilbert, J. J. (1977) *Arch. Hydrobiol. Spec. Issues Adv. Limnol.* **8,** 261–263.
37. Rosenzweig, M. L. (1971) *Science* **171,** 385–387.