ALLELOPATHY AS AN EMERGENT, EXPLOITABLE PUBLIC GOOD IN THE BLOOM-FORMING MICROALGA PRYMNESIUM PARVUM

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Many microbes cooperatively secrete extracellular products that favorably modify their environment. Consistent with social evolution theory, structured habitats play a role in maintaining these traits in microbial model systems, by localizing the benefits and separating strains that invest in these products from ‘cheater’ strains that benefit without paying the cost. It is thus surprising that many unicellular, well-mixed microalgal populations invest in extracellular toxins that confer ecological benefits upon the entire population, for example, by eliminating nutrient competitors (allelopathy). Here we test the hypotheses that microalgal exotoxins are (1) exploitable public goods that benefit all cells, regardless of investment, or (2) nonexploitable private goods involved in cell-level functions. We test these hypotheses with high-toxicity (TOX+) and low-toxicity (TOX−) strains of the damaging, mixotrophic microalga Prymnesium parvum and two common competitors: green algae and diatoms. TOX+ actually benefits from dense populations of competing green algae, which can also be prey for P. parvum, yielding a relative fitness advantage over coexisting TOX−. However, with nonprey competitors (diatoms), TOX− increases in frequency over TOX+, despite benefiting from the exclusion of diatoms by TOX+. An evolutionary unstable, ecologically devastating public good may emerge from traits selected at lower levels expressed in novel environments.

KEY WORDS: Cooperation, multilevel selection, Prymnesium parvum, public goods, toxic algal bloom.

Microbes are rapidly becoming a popular tool for experimental tests of social evolution theory. Although isolated examples of microbial sociality have been known for some time (e.g., cellular slime molds [Bonner 1971] and myxobacteria [Kaiser 1979]), recent evidence suggests that cooperative behavior may be very common among microbes. Microbial communities are often marked by high taxonomic and genetic diversity, and would thus appear to be an especially hostile environment for cooperation, given that much social evolution theory emphasizes genetic or phenotypic similarity among interacting individuals. The potential for fast rates of evolution to disrupt cooperation has been illustrated in laboratory experiments, which show the rapid rise of selfish ‘cheater’ strategies in liquid culture populations (Vulic and Kolter 2001; Diggle et al. 2007). Reconciling the ubiquity of microbial cooperation in nature with its expected instability,
based on theory and laboratory experiments is a major, unmet challenge.

Many microbial lineages secrete extracellular toxins that impair or eliminate conspecific and interspecific competitors (Chao and Levin 1981; Greig and Travisano 2008; Brown et al. 2009; Pierson and Pierson 2010). These costly secreted compounds may constitute a “public good,” by improving ecological conditions for cells other than the producer, thus qualifying toxin production as a form of altruism. In this case, individuals that benefit from toxins but do not share in the costs of its production (cheaters) should locally outcompete their altruistically producing counterparts, thus creating a classic social dilemma. The unmitigated success of cheaters will eventually undermine the public good, an outcome known as the “tragedy of the commons” (Hardin 1968). The expected evolutionary instability of such a locally disadvantaged strategy stands in contrast to the ubiquity of exotoxicity among diverse microbial lineages in diverse environments.

One major class of solutions to the public goods problem in general, and microbial exotoxin production specifically, involves structured populations. Hamilton (1964) coined the term ‘viscous populations’ to refer to limited dispersal as a mechanism to facilitate the clustering of relatives. When dispersal is limited and altruism enhances group-level productivity, the benefits of altruism are directed towards other altruists more than cheaters, so that altruists benefit more from the public good (Wilson et al. 1992; Queller 1994; Mitteldorf and Wilson 2000). The most well-studied model system for studying allelopathy in microbes, colicinogenic *Escherichia coli* (Chao and Levin 1981) typically exists in literal viscous populations—matrix-encased, structured biofilm communities. When biofilm-forming bacteria are coerced into unnatural well-mixed liquid cultures, it is not surprising that costly toxin product is destabilized, and cheaters invade the population (Kerr et al. 2002; Le Gac and Doebeli 2010; Driscoll et al. 2011). So far, all theoretical and empirical investigations into costly allelopathy suggest that this trait, like all locally disadvantageous traits, should be profoundly evolutionarily unstable in well-mixed populations (Chao and Levin 1981; Lewis 1986; Jonsson et al. 2009).

Despite this expectation, diverse groups of microalgae invest in extracellular toxins that confer a wide range of ecological benefits, including enhanced access to nutrients through impairing or killing competing algae (Granelli and Hansen 2006). These toxins appear to play major roles in various stages of the development of toxic algal blooms, puzzling and highly destructive phenomena in which a single toxic lineage rises to ecological dominance by annihilating natural enemies (Granelli et al. 2008). Although superficially similar, exotoxin production in unicellular algal lineages differs from allelopathy in biofilm-forming bacteria because many toxic microalgae typically exist in highly unstructured, low viscosity, and genetically heterogeneous populations. These unstructured populations should pose a major challenge for the evolution of altruistic behaviors (Wilson and Dugatkin 1997). However, many current ideas on the adaptive role of microalgal exotoxins continue to invoke higher-level functionality, without considering the potential for cheating to undermine the public good. This and related criticisms have been voiced over the years (Lewis 1986; Thornton 2002; Flynn 2008; Jonsson et al. 2009), but we are unaware of any direct tests of the hypothesis that algal allelopathy is an adaptive, cooperative strategy.

A competing possibility is that microalgal exotoxins are involved in cell-level functions, and the dramatic, large-scale ecological effects associated with toxic blooms are “cross-level byproducts” (Okasha 2012). Cross-level byproducts arise because a trait that is advantageous at a lower level (e.g., cell) also confers benefits at a higher level (e.g., population), and creates the appearance of group-level optimization. There is empirical evidence that toxic cells may escape grazing by zooplankton better than coexisting, nontoxic cells (Selander et al. 2006), and that mixotrophic cells may be able to use secreted toxins to capture and consume prey without the aid of other cells (Blossom et al. 2012). Even though extracellular toxins are released into the environment, these effects may remain localized to a focal producer cell in low-diffusion environments, or in sparse populations (Driscoll and Pepper 2010). In direct contrast to the hypothesis that toxicity is a collective competitive strategy (public good), a cell-level function of toxicity predicts a relative fitness advantage for toxic cells (private good) over their nonproducing counterparts.

Here, we test this prediction using the toxic bloom-forming unicellular alga *Prymnesium parvum*. We have isolated strains that differ dramatically in their hemolytic potential and growth characteristics from the same bloom population. We use a toxin-underproducing strain and two highly hemolytic strains to directly investigate two major ecological benefits of toxicity: assistance in predation, and allelopathy. We then determine whether these benefits confer within-population (relative fitness) advantages to a toxic strain cooccurring with a low-toxicity strain, or are distributed equally among all members of the population, favoring the low-toxicity strain.

**Materials and Methods**

**ISOLATION OF CLONAL P. PARVUM STRAINS**

Samples from *P. parvum* blooms were collected by Greg Southard at the Texas Parks and Wildlife Department from Moss Creek, Texas, on April 5, 2010. Clonal strains were established using the protocol of Lakeman and Cattolico (2007) with modification. We obtained cell counts for *P. parvum*, and then diluted the sample to a density of approximately 100 cells mL−1 using F/2−Si media at a salinity of 30 PSU. Penicillin-G potassium (1000 mg L−1) and kanamycin sulfate (100 mg L−1) were added to the media at this stage to inhibit bacterial growth. These diluted cultures

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**Reference**

were added in a 1:1 ratio for cooling F/2–Si media with 0.24% low melting temperature agarose, yielding a final agarose concentration of 0.12%. This mixture was briefly mixed by swirling the flask, then quickly poured into a petri dish. This dish was then carefully sealed with parafilm and moved into an incubator and maintained at 22°C on a 12:12 h L:D cycle. Plates were periodically checked using an inverted phase contrast microscope to identify isolated colonies of *P. parvum* founded by a single cell. When colonies were sufficiently large (>32 cells total), an agarose plug (~20 µL) containing cells from the colony was removed with a pipette and transferred into a 20 mL test tube containing F/2–Si liquid media. The liquid cultures were grown at 22°C on a 12:12 h L:D cycle. Liquid cultures were grown with antibiotics (Andersen 2005) until the cultures were verified to be free of most bacteria. Isolated strains were gradually acclimated to 6.0 PSU. All experiments described below were conducted at this salinity. We also sequenced the internal transcribed spacer region 1 of each isolated strain to determine genetic relationships among our and other strains of *P. parvum* (see Supporting information).

**MEASUREMENT OF GROWTH RATES IN *P. parvum***

Strains were grown up to late exponential phase in F/2–Si. These cells were then centrifuged and resuspended in F/2 media with phosphorous adjusted to F/50 levels (henceforth denoted P/50; 6.0 PSU). We noticed that cells from one of our strains seemed particularly susceptible to the physical stress of the resuspension process, so we left cells to ‘recuperate’ in fresh P/50 media for a period of 24 h before transferring them into the experimental microcosms. Our data confirmed that this step had the intended effect of ensuring that only viable cells were counted and used to initiate experiments. A volume of ‘recuperated’ cells was then transferred to a fresh tube of P/50 (6.0 PSU) to initiate populations at a density of 10^5 cells mL^-1. Cell counts were taken every ~48 h using a Bright-Line hemacytometer (Hauser Scientific, Horsham, PA).

**HEMOLYSIS ASSAYS**

Monoclonal cultures were assayed for hemolytic potential on a per cell basis, by adjusting the volume of cells to yield a constant density of 10^5 cells mL^-1, according the protocol of Manning (2010) modified after Eschbach et al. (2001). After obtaining cell counts, a sufficient volume of each replicate culture was centrifuged at 14,000 RPMs (Eppendorf centrifuge 5418) for 2 min and resuspended in fresh P/50 media to yield a density of 2 × 10^5 cells mL^-1. Resuspended cells were added to an equal volume (125 µL) of sheep erythrocytes (HemoStat Laboratories, Dixon, CA) diluted to 6.0 × 10^5 cells mL^-1 in ELA media (Eschbach et al. 2001) in a V-bottom 96-well plate. The plate was covered and incubated for 90 min at room temperature in the dark. Following this, the plate was centrifuged at a speed of 1200 RPMs for 5 min at room temperature. The supernatant was then transferred to flat-bottomed 96-well plate. The plate was read by an ELISA plate reader (Spectronic Instruments, Rochester) at 414 and 620 nm. These raw values were converted to a fraction by the formula (experimental (OD414-OD6620) - negative (OD414-OD6620)) / (positive (OD414-OD6620) - negative (OD414-OD6620)).

**SEMICONTINUOUS COMPETITION EXPERIMENTS**

We sought to understand the ecological impacts of variable toxicity at the population level, and to determine the ecological benefits (if any) of toxicity in *P. parvum*. We used *Dunaliella tertiolecta* (CCMP1320) as a generic nontoxic, chlorophyte competitor, to approximate the sort of competitor that *P. parvum* might encounter in freshwater communities. We used two coexisting strains of *P. parvum* isolated from the same bloom sample, one of which was highly toxic (TOX₁) and the other which was largely nontoxic (TOX₂; see Fig. 1 below) in this experiment. Experimental populations were grown in semicontinuous batch cultures in 20 mL glass vials under the same low salinity, phosphorous-limiting conditions described above, and were initiated at low (10^4 cells mL^-1) *P. parvum* density, and moderate and high (10^5 and 10^6 cells mL^-1) *D. tertiolecta* densities. Both *P. parvum* strains were tested under these conditions, in low salinity
(6.0 PSU) and phosphorous limiting conditions (P/50), with a dilution rate of 0.1 day⁻¹. Cells in single species treatments (controls) were fixed and counted using Utermohl's solution, whereas mixed treatments of *P. parvum* and *D. tertiolecta* were fixed using formalin (10%) to facilitate rapid and accurate differentiation between the two species. Cell counts were taken every ~48 h. We counted three biological replicates of each treatment, and replicated each cell count three times per biological replicate (= 9 counts per strain per time point). The experiments reported here were performed at least twice with similar qualitative results.

**MIXED CULTURE BATCH COMPETITION EXPERIMENTS**

We sought to differentiate between TOX— and a highly toxic strain in mixed cultures to determine the relative fitness of each strain in different ecological contexts. The strains used in the experiments described above were morphologically indistinguishable, so we used another, closely related toxic strain isolated from a toxic bloom at E.V. Spence, Texas, that has a substantially larger cell size than TOX— (strain 13A5; TOX₂). This strain has a significantly lower intrinsic rate of growth, but is qualitatively similar to the TOX₁ strain (12A1) in behavior and hemolytic potential (Fig. 1). More importantly, the frequency of TOX₂ is strongly correlated with mean cell size in mixed populations of TOX— and TOX₂ ($R^2 = 0.93$) (see Fig. 6A).

We set up a factorial experiment in triplicate testing of four possible *P. parvum* initial populations (negative control, all TOX₂, half TOX and half TOX—) and four possible competitor communities (negative control, all *D. tertiolecta*, all diatom, half *D. tertiolecta* and half diatom) at two different initial densities ($10^3$ cells mL⁻¹; $10^5$ cells mL⁻¹). These communities were initiated in 2 mL of P/50 media in randomized wells in four 24-well plates, which were sealed with parafilm and periodically rotated under the same growth conditions as described above. After 7 and 11 days, 100 µL samples were removed after vigorous pipette mixing and fixed with Lugol’s solution in 96-well plate for cell size measurements. After cells settled, we acquired five random images per replicate using a Nikon Eclipse TI inverted microscope and NIS-Elements AR 3.10 software and generated cell size measurements using automatic thresholding and the particle analysis tool in ImageJ (Abramoff et al. 2004). After 12 days, we counted cells using the same protocol described in the above section, removing 50 µL samples by vigorous pipette-mixing and using Lugol’s solution and formalin as necessary.

**Results**

We initially focused on two strains that were morphologically indistinguishable, but differed obviously in their growth characteristics in P-limited media (strain 12B1, TOX— and strain 12A1, TOX₂). These strains were isolated from the same 50 mL bloom sample from Moss Creek, Texas. We used another highly toxic strain from a neighboring creek (13A5; TOX₂) for intraspecific competition experiments because it was large enough to be distinguished from TOX— in mixture. This strain was also very closely related to TOX— (Fig. S1). Each strain displayed distinct growth patterns (Fig. 1). When these data were fit to a logistic growth curve, we found that the two coexisting strains had indistinguishable maximal growth rates, which were higher than the larger TOX₂ strain; however, TOX— saturated at approximately twice the final density as TOX₁ and TOX₂ (see Fig. 1 for specific growth parameters). Both toxic strains showed substantial hemolytic potential upon the transition to stationary phase growth, whereas the high-density TOX— strain was only weakly hemolytic (Fig. 1). (Data are deposited in the Dryad repository: doi:10.5061/dryad.dk625.)

The physiological differences between the two coexisting strains profoundly influenced their interactions with a green alga competitor/prey, *D. tertiolecta*. When grown alone in phosphorous-limited semicontinuous cultures, TOX— enjoyed a slight growth advantage over TOX₁ (Fig. 2A). However, this outcome was reversed when each strain was grown in the presence of a dense population of the green alga, with TOX₁ growing rapidly early in growth (Fig. 2B). TOX₁ quickly drove the green alga extinct, whereas the green alga grew undisturbed in the presence of TOX— for ~150 h before gradually diminishing towards eventual extinction after 300 h (Fig. 2C and D).

The presence of a dense ($10^5$ cells mL⁻¹) population of the competitor/prey green alga provided an immediate growth advantage to very sparse ($10^3$ cells mL⁻¹) initial populations of TOX₁ (Figs. 2A and 3A). In contrast, TOX— experienced no beneficial effects from growth with dense green algae, and was inhibited throughout the experiment (Figs. 2B and 3B). The growth advantage of TOX₁ peaked at approximately the same time as the extinction of the green alga, and diminished thereafter (Fig. 3). Consistent with these contrasting effects, filtrate from TOX₁ grown with green algae showed relatively high hemolytic potential early in the experiment, whereas TOX— cells were only hemolytic much later in growth (Fig. 4). Hemolytic potential of filtrate from these cultures was very similar to that of washed cells (data not shown). Notably, we pelleted cells prior to filtering supernatant, so that relatively few cells should have been present in the medium prior to filtration. This step was intended to prevent large-scale lysis of *P. parvum* cells during the filtration step, which may contribute greatly to the toxicity of filtrate (Remmel and Hambright 2011).

The frequency of TOX— and TOX₂ strains dramatically altered the growth of mixed *P. parvum* populations in isolation and in mixture with the green alga competitor. TOX₂ enjoyed a
**Figure 2.** Ecological dynamics of *P. parvum* and competing green algae (g.a.) in semicontinuous, phosphorous-stressed cultures. Asterisks indicate significant differences (*p* = 0.05). A. TOX+ strains enjoy a growth advantage over TOX− in monoculture. B. TOX+ has a highly elevated growth rate compared to TOX− in the presence of a dense initial population of green algae. C. Green algal populations initiated from high initial density (10⁵ cells mL⁻¹) were strongly influenced by both strains of *P. parvum*. Here, double asterisks indicate a significant difference between g.a. +TOX+ and g.a. alone. Green algae were rapidly driven extinct with TOX− (coarse dashed lines), but were only slightly impacted by TOX− over the first ~150 h of the experiment, and remained in the culture until ~300 hours.

significant growth advantage in the presence of dense green algae (consistent with the semicontinuous results using TOX+), whereas TOX− growth was inhibited by increasing densities of this competitor/prey organism (Fig. 5A). In all cases involving growth alone or with green algae, mixed *P. parvum* population densities were statistically indistinguishable from the more successful monomorphic population (TOX− with no or sparse green algae; TOX+ with dense green algae), and significantly higher than the less successful monomorphic population.

The presence of TOX+ shielded *P. parvum* from competitive suppression from a diatom competitor (Fig. 5B), whereas TOX− was strongly inhibited in the presence of a diatom competitor, however there was no significant difference between mixed *P. parvum* populations grown with and without the diatom competitor. The diatom also failed to impact the growth of a monomorphic TOX+ population (Fig. 5B). Diatom growth was significantly suppressed in the presence of all *P. parvum* populations, although diatoms were far more dense with monomorphic

**Figure 3.** The relative effect of growth with a moderate (dashed line; 10⁴ cells mL⁻¹) and dense (solid line; 10⁵ cells mL⁻¹) population of green algae (*Dunaliella tertiolecta*) on two strains of *P. parvum*. The relative effect is reflected in the relative density, the ratio of the density of *P. parvum* grown with green algae (*Pd*) to the same strain of *P. parvum* grown alone (*P0*). The horizontal dashed line indicates a relative density of 1.0, indicating no effect, and asterisks indicate a significant difference between *Pd* and *P0*. Vertical gray bars indicate the first absence of green algae during cell counting. (A) TOX+ growth was enhanced in the presence of green algae, particularly early in growth before the green algae was driven extinct. (B) TOX− growth was very weakly enhanced in the presence of moderate green algae, but was strongly inhibited with dense green algal populations.

**Figure 4.** Time series of hemolytic potential of filtrate from two strains of *P. parvum* grown in mixture with dense green algae, expressed as a fraction of total hemolysis. Asterisks denote significant differences among treatments, and vertical gray bars indicate the absence of green algae from cell counts. Hemolytic potential of cell-free filtrate increased early in growth of cultures containing TOX+. Hemolytic potential of filtrate was undetectable until much later in cultures containing TOX−. Note that extinction of green algae preceded high ambient toxicity in the TOX+ treatment, whereas green algae were only eliminated after the gradual rise in ambient toxicity in the TOX− treatment.
TOX− populations than either mixed or TOX+ populations. In these latter treatments, diatoms were virtually absent after 12 days.

Changes in mean cell size in mixed *P. parvum* populations containing both TOX− and TOX+ reflect changing proportions of each strain (Fig. 6A). Mixed populations grown autotrophically and without competition shifted decisively away from the initial size distribution and toward reduced size after 7 and 12 days, reflecting dominance by TOX− (Fig. 6B). The presence of a dense population of green algae reversed the pattern, resulting in populations that were dominated by TOX+ after 7 and 12 days. Mixed populations grown with diatom competitors also diminished in size over the experimental period, reflecting an increasing frequency of TOX− (Fig. 6), despite the crucial role of TOX+ in reducing competition by suppressing diatom growth (Fig. 5B).

**Discussion**

Toxicity in algae is often assumed to be part of a collective, population-level strategy, because toxicity provides obvious population-level benefits by reducing predation by grazers, improving effectiveness in killing prey and reducing the density of competitors. However, social evolutionary theory predicts that the very toxin-producers responsible for these population-level benefits should be at a local disadvantage in the presence of cheaters that share in these benefits but avoid the costs (Speed et al. 2012). Thus, toxin producers should ultimately be driven to extinction due to their local disadvantage (the paradox of altruism). This outcome can be avoided when toxicity can provide cell-level benefits, a situation perhaps more closely aligned to the snowdrift game (Doebeli and Hauserd 2005) than the public goods game. Notably, our results do not suggest a simple case of negative frequency dependency (as in the snowdrift game), but a more complex dependency of relative fitness on community composition. Our results suggest that, in a constant ecological environment, directional selection should eventually eliminate one of the strategies entirely. However, evolutionary–ecological feedbacks may exist whereby changes in the frequency of toxic lineages feedback to influence community structure, resulting in a sort of frequency dependence. Work using longer-lived communities to explore this possibility is currently underway.

Here, we have demonstrated that two highly toxic strains of *P. parvum* benefit from intraguild predation on coexisting green algae, whereas a less toxic strain suffers from nutrient competition with the same green algae (Figs. 2, 3, and 5A). Many important aspects of *P. parvum* physiology and ecology remain mysterious, including the toxins themselves and their mode of action. Our approach has focused on the measurable, functional differences between the different strains, and thus does not require a mechanistic understanding of the toxins themselves. However, our results suggest a link between toxic mixotrophy and allelopathy, which may arise due to different effects of the same toxins (or sets of toxins), or due to co-regulation of the underlying genes. In either case, it appears that there are indeed two separate benefits of toxicity in *P. parvum*, increased access to nutrients through mixotrophy (Figs. 2, 3, and 5A), and reduced competition for nutrients for autotrophic growth (Fig. 5B).

These two ecological benefits of toxicity in *P. parvum* differ dramatically in the scale over which they are distributed, and this fact bears directly on the evolutionary stability of proposed functions of toxicity in this species. Exotoxins are just one component of a broader mixotrophic phenotype, which includes motility and swarming behavior resulting in cell–cell contact with prey. Recent studies suggest that this contact is likely important for the predatory behavior of *P. parvum* (Remmel and Hambright 2011). Single *P. parvum* cells are able to attack and consume cells of...
similar size, including the green alga *D. tertiolecta*, although these attacks may involve a small swarm of several *P. parvum* cells (Tillmann 1998). This role of toxicity in cell-level behavior is consistent with our finding that highly toxic lineages enjoyed a major benefit from predation, even when initiated at densities two orders of magnitude below those of their prey (Fig. 3A). Thus, the benefits from a single attack are distributed locally, among one or a few cells, which constitute a relatively small trait group. However, even this benefit may spread to nonproducers (e.g., autotrophic TOX−) cells, if they participate in the feeding swarms. Interestingly, we have noticed that TOX− rarely forms swarms, whereas both highly toxic strains studied here readily swarm and attack nearby prey. Any potential linkage between toxin production and propensity to initiate or join feeding swarms (i.e., pleiotropy) may generate assortment between less- and more-toxic strains, creating phenotypic similarity within transient trait groups in which would otherwise be an unstructured population. Whether benefits from attacking the green alga accrue to individual toxic cells or to phenotypically similar swarm mates, in the context of a broader mixotrophic strategy, our data suggest that toxin-mediated mixotrophy is not a universally distributed public good in mixed populations. In the future, direct observation of *P. parvum* in mixed TOX/TOX− populations may definitively determine whether TOX− cells can act as ‘mixotrophic cheaters’ by participating in feeding swarms while toxic cells are present.

In contrast, the ecological benefits of eliminating a nonprey nutrient competitor (allelopathy) are distributed evenly among all members of the *P. parvum* population, regardless of toxicity. In monomorphic populations, TOX2 was unaffected by diatom competitors, whereas their nontoxic counterparts were suppressed by diatoms (Fig. 5B). However, in mixed cultures, TOX− largely replaced TOX2 over the course of the experiment, mirroring the change in mixed *P. parvum* populations growing without competition (Fig. 6). Thus, it appears that the presence of TOX2 released TOX− from competition with diatoms, allowing TOX− to begin replacing its toxic counterpart. Consistent with this interpretation, population-level productivity in mixed populations was significantly higher than either monomorphic strain grown with diatoms, and was not different from TOX− or mixed population growth in isolation (Fig. 5A). These relationships suggest that the anticompetitor function of toxicity (fortuitous though it may be) constitutes a true public good that can be exploited by cheaters. Public good traits may emerge from the interaction between a previously nonsocial (private goods) trait and novel ecological conditions, broadening the range of conditions that may ultimately lead to cooperation. The constructive role of population dynamics in facilitating the evolution of altruism in well-mixed populations has already been explored theoretically (Hauert et al. 2006). Here, we have shown that ecological context may, in principle, transform an inherently nonsocial trait (toxin-mediated mixotrophy) into a true public goods trait (allelopathy) in toxic algae. However, the population itself may drive this ecological change (e.g., during a toxic bloom event), as the local advantage of toxicity may drive toxic strains to threshold densities, thereby creating a niche for exploitative, antisocial strategies. Our findings that the frequency of toxic and nontoxic strains in a *P. parvum* population can shape interspecific interactions (Fig. 5), and that these interspecific interactions can change strain frequencies within *P. parvum* populations (Fig. 6), illustrates the potential for reciprocal interactions between evolutionary and ecological change in a
simple algal community. These results indicate that toxic blooms of *P. parvum* (and other fast-evolving, potentially toxic lineages, such as *Microcystis aeruginosa* [Wilson et al. 2005; Briand et al. 2008; Briand et al. 2009]) may provide ‘natural laboratories’ for the study of ecoevolutionary feedbacks.

The apparent collectivity of toxic bloom-forming microalgae presents a major challenge for evolutionary ecology. Many researchers have rightly pointed out the inherent problems with the popular view of algal toxins as evolved mechanisms of “chemical warfare” among populations, noting that costly niche construction would be highly evolutionarily unstable in unstructured populations (Thornton 2002; Pohnert et al. 2007; Flynn 2008; Jonsson et al. 2009). However, evidence is accumulating that toxic populations do, in fact, often harbor nontoxic varieties (e.g., Meldahl et al. 1994; Briand et al. 2009; Fredrickson et al. 2011), hinting that toxicity may have exploitable social dimensions, after all. The question is then inverted: how can natural selection maintain toxicity in the presence of nontoxic strains in well-mixed populations? Here, we have shown that the social aspect of toxicity depends as much on ecological context as on the trait itself, due to its multiple ecological impacts. The balance between individual-level and higher-level benefits is expected to be dynamic in natural populations, particularly over the course of bloom events. While much work remains, it is possible that the transient, relative fitness advantage of a lineage that is drastically impaired in interspecific interference competition could be used to reduce the raw allelopathic potential of a toxic bloom population.

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**LITERATURE CITED**


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Supporting Information
Additional Supporting information may be found in the online version of this article at the publisher’s web site:

Figure S1. Neighbor-joining tree based on trimmed ITS1 sequences of Prymnesium parvum and related species.