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Allelopathy as an emergent, exploitable public good in the bloom-forming microalga *Prymnesium parvum*

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Abstract

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) non-exploitable 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 non-prey 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.

Keywords

Multilevel selection; *Prymnesium parvum*; toxic algal bloom; cooperation; public goods

Introduction

Microbes are rapidly becoming a popular tool for experimental tests of social evolution theory. While 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

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culture populations (Vulic and Kolter 2001; Diggle, Griffin 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, Inglis 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 disadvantageous 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, Pollock 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, Riley et al. 2002; Le Gac and Doebeli 2010; Driscoll, Pepper et al. 2011). Thus 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, Pavia 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 (Graneli 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 (Graneli, Salomon 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, Pavia 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, Thor 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, Daugbjerg 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 non-producing 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 co-occurring 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 were added in a 1:1 ratio to 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 22C on a 12:12 hr L:D cycle. Plates were periodically checked using an inverted phase contrast microscope in order 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 22C on a 12:12 hr 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 in order to determine genetic relationships among our and other strains of *P. parvum* (see Supplementary Material A).

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 hours 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 10^4 cells mL^{-1}. Cell counts were taken every ~48 hours using a Bright-Line hemacytometer (Hausser 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 minutes 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 6.0×10^6 cells mL^{-1} in ELA media (Eschbach, Scharsack et al. 2001) in a V-bottom 96-well plate. The plate was covered and incubated for 90 minutes at room temperature in the dark. Following this, the plate was centrifuged at a speed of 1200 RPMs for 5 minutes 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 nm and 620 nm. These raw values were converted to a fraction by the formula (experimental (OD414-OD620) – negative(OD414-OD620))/(positive (OD414-OD620) – negative (OD414-OD620)).

Semi-continuous 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 of 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 (TOX_1) and the other which was largely nontoxic (TOX-; see Figure 1 below) in this experiment.

Experimental populations were grown in semi-continuous batch cultures in 20 mL glass vials under the same low salinity, phosphorous-limiting conditions described above, and were initiated at low (10^3 cells mL^{-1}) *P. parvum* density, and moderate and high (10^4 and 10^5 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 hours. 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 in order 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; TOX_2). This strain has a significantly lower intrinsic rate of growth, but is qualitatively similarly to the TOX_1 strain (12A1) in behavior and hemolytic potential (Figure 1). More importantly, the frequency of

TOX_2 is strongly correlated with mean cell size in mixed populations of TOX- and TOX_2 ($R^2 = 0.93$) (see Figure 6A).

We set up a factorial experiment in triplicate testing four possible *P. parvum* initial populations (negative control, all TOX_2, all TOX-, half TOX_2 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 miscroscope and NIS-Elements AR 3.10 software and generated cell size measurements using automatic thresholding and the particle analysis tool in ImageJ (Abramoff, Magalhaes 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, TOX_1). 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; TOX_2) 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- (Figure S1). Each strain displayed distinct growth patterns (Figure 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 TOX_2 strain; however, TOX- saturated at approximately twice the final density as TOX_1 and TOX_2. Both toxic strains showed substantial hemolytic potential upon the transition to stationary phase growth, whereas the high-density TOX- strain was only weakly hemolytic (Figure 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 semi-continuous cultures, TOX- enjoyed a slight growth advantage over TOX_1 (Figure 2A). However, this outcome was reversed when each strain was grown in the presence of a dense population of the green alga, with TOX_1 growing rapidly early in growth (Figure 2B). TOX_1 quickly drove the green alga extinct, whereas the green alga grew undisturbed in the presence of TOX- for ~150 hours before gradually diminishing towards eventual extinction after 300 hours (Figure 2C and 2D).

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 TOX_1 (Figures 2A and 3A). In contrast, TOX-experienced no beneficial effects from growth with dense green algae, and was inhibited throughout the experiment (Figures 2B and 3B). The growth advantage of TOX_1 peaked at approximately the same time as the extinction of the green alga, and diminished thereafter (Figure 3). Consistent with these contrasting effects, filtrate from TOX_1 grown with green algae showed relatively high hemolytic potential early in the experiment, whereas TOX- cells were only hemolytic much later in growth (Figure 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 TOX_2 strains dramatically altered the growth of mixed *P. parvum* populations in isolation and in mixture with the green alga competitor. TOX_2 enjoyed a significant growth advantage in the presence of dense green algae (consistent with the semi-continuous results using TOX_1), whereas TOX- growth was inhibited by increasing densities of this competitor/prey organism (Figure 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_2 shielded *P. parvum* from competitive suppression from a diatom competitor (Figure 5B.) whereas TOX- was strongly inhibited in the presence of a diatom competitor, while 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_2 population (Figure 5B). Diatom growth was significantly suppressed in the presence of all *P. parvum* populations, although diatoms were far more dense with monomorphic TOX- populations than either mixed or TOX_2 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_2 reflect changing proportions of each strain (Figure 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- (Figure 6B). The presence of a dense population of green algae reversed the pattern, resulting in populations that were dominated by TOX_2 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- (Figure 6), despite the crucial role of TOX_2 in reducing competition by suppressing diatom growth (Figure 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. 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 Hauert 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 occur 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, while a less toxic strain suffers from nutrient competition with the same green algae (Figures 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 (Figures 2, 3 and 5A), and reduced competition for nutrients for autotrophic growth (Figure 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 (Figure 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 non-producers (e.g. autotrophic TOX-) cells, if they participate in the feeding swarms. Interestingly, we have noticed that TOX- rarely forms swarms, while 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 what 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 suggests 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 non-prey nutrient competitor (allelopathy) are distributed evenly among all members of the *P. parvum* population, regardless of toxicity. In monomorphic populations, TOX_2 was unaffected by diatom competitors, while their nontoxic counterparts were suppressed by diatoms (Figure 5B). However, in mixed cultures, TOX- largely replaced TOX_2 over the course of the experiment, mirroring the change in mixed *P. parvum* populations growing without competition (Figure 6). Thus, it appears that the presence of TOX_2 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 (Figure 5A). These relationships suggest that the anti-competitor function of toxicity (fortuitous though it may be) constitutes a true public good that can be exploited by cheaters. Public goods 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, Holmes 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, anti-social strategies. Our findings that the frequency of toxic and nontoxic strains in a *P. parvum* population can shape interspecific interactions (Figure 5), and that these interspecific interactions can change strain frequencies within *P. parvum* populations (Figure 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, Sarnelle et al. 2005; Briand, Gugger et al. 2008; Briand, Escoffier et al. 2009)) may provide 'natural laboratories' for the study of eco-evolutionary 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, Steinke et al. 2007; Flynn 2008; Jonsson, Pavia et al. 2009). However, evidence is accumulating that toxic populations do, in fact, often harbor non-toxic varieties (e.g. Meldahl, Edvardsen et al. 1994; Briand, Escoffier et al. 2009; Fredrickson, Strom 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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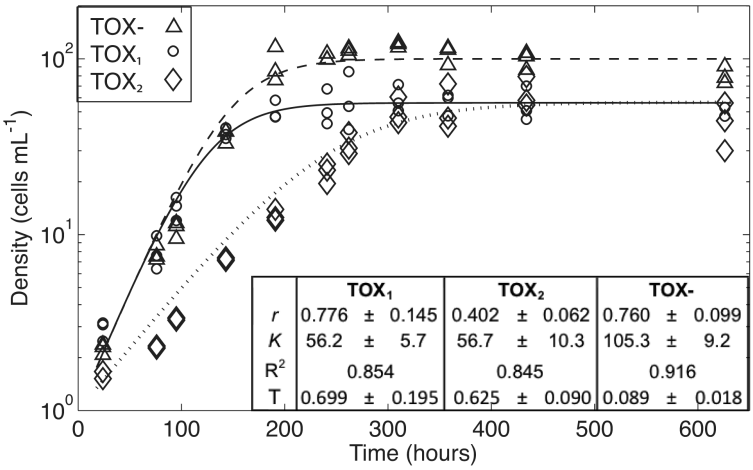

Figure 1.

Growth data for three genetically similar, coexisting strains of *P. parvum* fit to logistic growth curves on a log scale. The coarse dashed line is a logistic curve fit to strain TOX- data (triangles), the solid line is the fit to strain TOX_1 data (circles), and the fine dashed line is the fit to TOX_2 data (diamonds). While TOX- and TOX_1 increase at a similar maximal rate early in growth, they differ in the timing of the transition to stationary phase, so that TOX- grows to approximately twice the final density of TOX_1. Despite different maximal rates of growth, both toxic strains saturate at the same density. Inset: Parameters from logistic curve fits to growth data, with mean values across three independent cultures ± 99% confidence interval. Here, r is maximal rate of growth, K is carrying capacity, and T is hemolytic potential in early stationary phase, expressed as a fraction of total erythrocyte lysis.

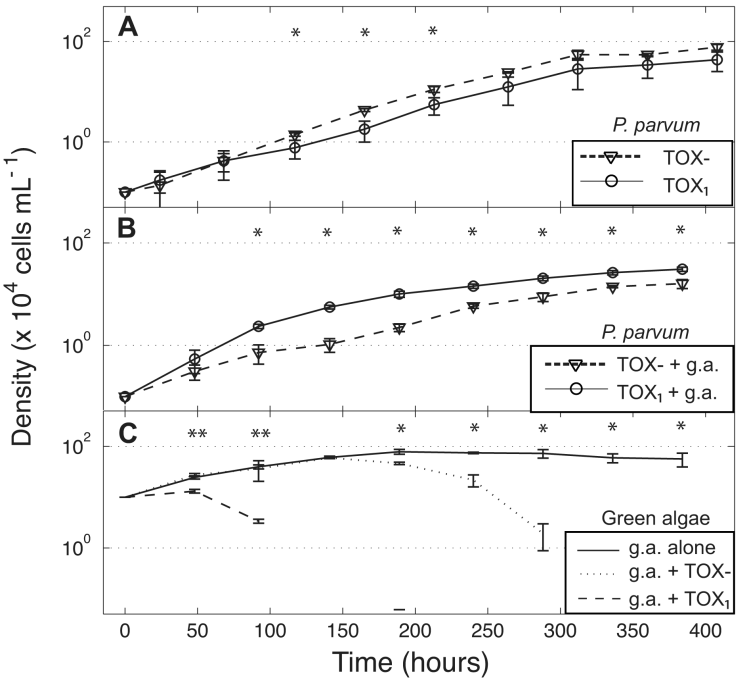

Figure 2.

Ecological dynamics of *P. parvum* and competing green algae (g.a.) in semi-continuous, phosphorous-stressed cultures. Asterisks indicate significant differences (p = 0.05). A. TOX- strains enjoy a growth advantage over TOX_1 in monoculture. B. TOX_1 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 (10^5 cells mL^{-1}) were strongly influenced by both strains of *P. parvum*. Here, double asterisks indicate a significant difference between g.a.+TOX_1, but no difference between g.a.+TOX- and g.a. alone. Green algae were rapidly driven extinct with TOX_1 (coarse dashed lines), but were only slightly impacted by TOX- over the first ~150 hours of the experiment, and remained in the culture until ~300 hours.

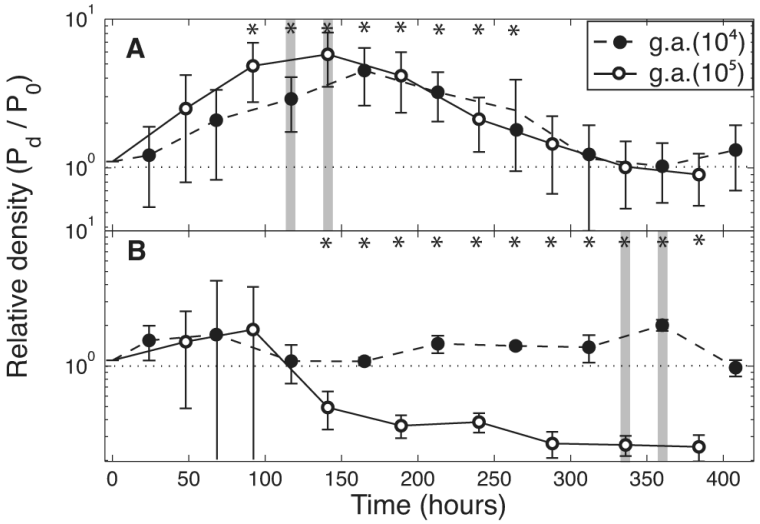

Figure 3.

The relative effect of growth with a moderate (dashed line; 10^4 cells mL^{-1}) and dense (solid line; 10^5 cells mL^{-1}) population of green algae (*D. 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 (P_d) to the same strain of *P. parvum* grown alone (P_0). The horizontal dashed line indicates a relative density of 1.0, indicating to no effect, and asterisks indicate a significant difference between P_d and P_0. Vertical gray bars indicate the first absence of green algae during cell counting. A. TOX_1 growth was enhanced in the presence of green algae, particularly early in growth before the green alga 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.

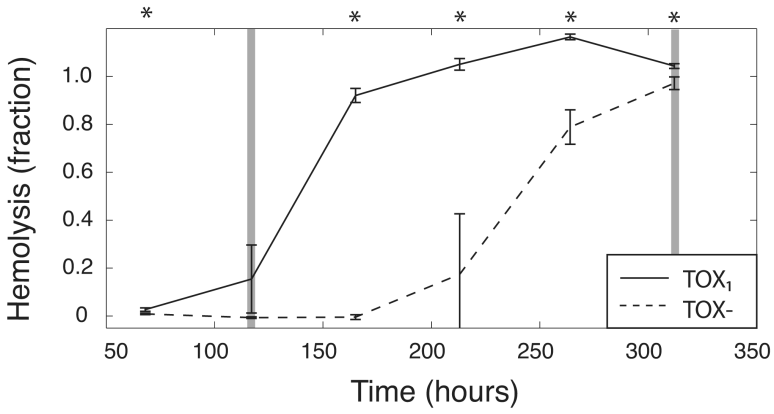

Figure 4.

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_1. 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_1 treatment, whereas green algae were only eliminated after the gradual rise in ambient toxicity in the TOX- treatment.

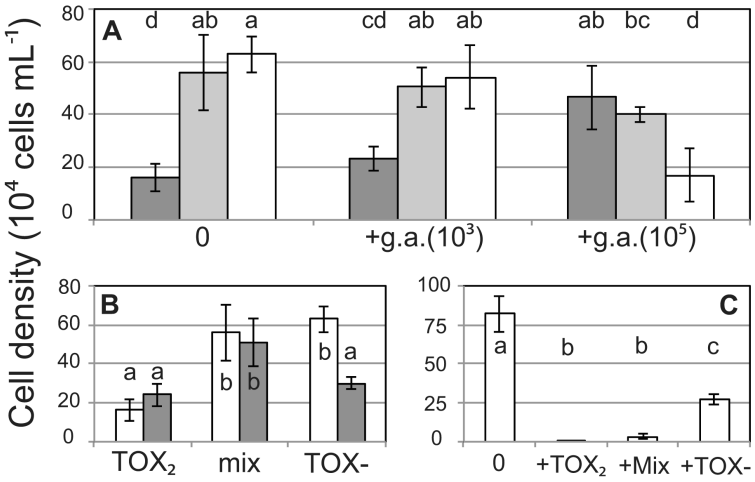

Figure 5.

Frequencies of TOX- and TOX_2 influence interactions between *P. parvum* populations and competing algae. Error bars indicate standard deviation and letters denote statistical groups (p = 0.05, Tukey's test). A. Densities of *P. parvum* populations with varying toxic potential with a green alga competitor/prey after 11 days. Filled bars indicate monomorphic TOX_2 populations, open bars are TOX- populations, and gray bars are 1:1 mixtures of the two. TOX_2 growth is enhanced in the presence of dense populations of green algal prey, while TOX- is suppressed. For all treatments, mixed populations were indistinguishable from the more successful monomorphic population, and significantly higher than the less successful population in all three contexts. B. *P. parvum* densities in isolation (open bars) and in competition with diatoms (gray bars) after 11 days. Both TOX+ and mixed populations were uninhibited by competition with diatoms, while TOX-growth was significantly suppressed. Mixed populations had significantly higher productivity in mixture with diatoms than either strain alone. C. Diatom densities in isolation and in competition with different *P. parvum* populations after 11 days. Diatoms were virtually absent from both communities with TOX +, but persisted in competition with TOX-.

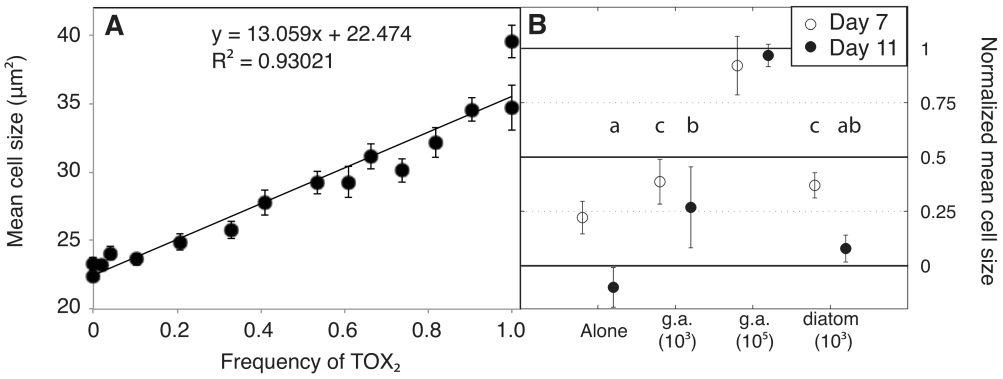

Figure 6.

A. Mean cell size is well predicted by known frequency of TOX_2 cells in a mixed population with TOX-. Error bars indicate standard error. B. Changes in mean cell size in mixed populations beginning from equal frequencies of TOX- and TOX_2 reflect changes in strain frequencies over time. Error bars show standard deviation across replicate populations, and letters denote statistical groups ($p = 0.05$, Tukey's test). Note that statistical comparisons were only made within each time point. The smaller TOX- strain increases in frequency in populations grown in the absence of competitors, with only sparse green algae, and with non-prey diatom competitors, indicated by diminished mean cell size in these populations. The larger TOX_2 strain only increases in frequency in populations grown with dense green algal prey. Cell sizes in mixed populations are normalized as (mean cell size of mixed populations – mean cell size of TOX- population) / (mean cell size of TOX_2 population – mean cell size of TOX- population), so that deviations from 0.5 reflect changes from initial populations with equal frequencies of TOX- and TOX_2.