

Analysis of Diatom Blooms Using DNA Fingerprints

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LONG-TERM GOAL

My long term goal is to understand those factors - both biological and environmental - that dictate the timing and magnitude of diatom blooms. I am particularly interested in how the extent of genetic and physiological diversity within diatom populations influences their ability to initiate and maintain the dramatic increases in cell number that are seen in coastal waters each spring and fall.

OBJECTIVES

The premise that underlies my research is that bloom dynamics reflect a complex interaction between environmental factors and the extent of diversity present within diatom populations. My first goal, then, is to determine just how diverse – at both a genetic and physiological level - individual species of diatoms are. As I learn more about the characteristics of individual species in particular locales, I will then ask how this diversity is shaped by different environments and on different time scales. Ultimately, this should allow me to determine how population diversity is coupled to future blooming capabilities.

APPROACH

Prior to our work, discussions of the presumed coupling between environmental and biological variability remained largely within the theoretical realm simply because there was no easy way to monitor exactly how different cell “types” within a species behaved since the individuals within a species are morphologically identical to one another. We therefore developed molecular techniques that allow us to distinguish between individuals based on their unique DNA “fingerprints.” By combining DNA fingerprinting techniques with physiological studies we are now able to follow the growth of individual clones within a population and examine in an unprecedented manner the interaction between species responses and environmental conditions.

We base our DNA fingerprints upon highly repetitive regions of DNA known as microsatellites. These repetitive regions are generally considered to be “junk” DNA with no obvious function. The beauty of these “junk” microsatellites for fingerprinting studies is that the length of any given repetitive region can vary dramatically between individuals. We use polymerase chain reaction (PCR) to amplify specific microsatellites from each of our different isolates. The size of multiple microsatellites within a given individual is then determined using an ABI automated DNA sequencer, which is accurate to a single base pair of DNA. When the lengths of enough of these highly variable

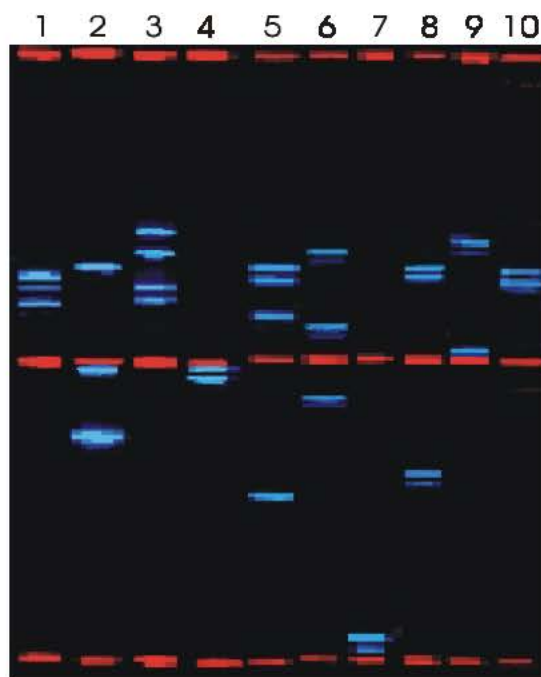
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regions are compared between different individuals, unique DNA “fingerprints” can be generated (Fig. 1). Thus, we can now define genetically distinct clones for use in our physiological studies. But even more importantly, we can target particular cell types within a species and ask how they ‘compete’ with one another during the course of a bloom, under what conditions unique cell types from outside locations are entrained into a bloom, and what happens to the composition of a population as the bloom begins to decline. These are questions that have been impossible to ask before in studies of phytoplankton ecology.



*1. DNA fingerprints of culture collection isolates (lanes 1-4) and Puget Sound field isolates (lanes 5-10) of the centric diatom *Ditylum brightwellii*. Two microsatellites from each individual were amplified by PCR and the products were analyzed on an ABI automated DNA sequencer. Blue bands are fluorescently-labeled microsatellite products and red bands are fluorescently-labeled internal molecular weight standards. The different DNA fingerprint patterns indicate that each of these individuals is genetically distinct.*

WORK COMPLETED

We have participated in three cruises in Puget Sound, WA to collect samples from both Hood Canal, a relatively stable body of water and from Admiralty Inlet, a highly mixed environment.

We now have over 300 frozen, single-cell isolates from Hood Canal and Admiralty Inlet for DNA fingerprinting. The DNA from over half of these isolates has been extracted and about half of these extracts have been analyzed for two different microsatellite loci on our ABI DNA sequencer. We currently maintain about twenty different isolates in culture for physiological studies.

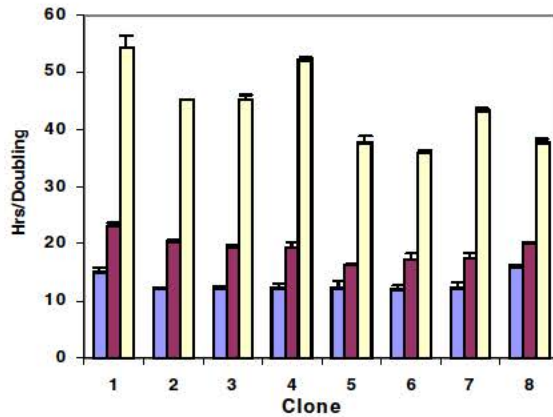
RESULTS

We have made good progress in addressing two of our goals: to determine the extent of diversity within individual populations of diatoms and to determine how the environment shapes diversity. The work is being conducted by myself, my graduate student Tatiana Rynearson and an undergraduate, Chuck Lausche.

Our initial studies began in Hood Canal, a relatively stable body of water in Puget Sound, WA. As a first step towards determining the amount of diversity within field populations, we isolated 24 single cells from a fall population. Each isolate was maintained in culture until we obtained about 300,000 cells; these cells were then frozen until their DNA fingerprints could be analyzed (e.g., Fig. 1). We found that 22 of these 24 original isolates possessed unique DNA fingerprints indicating that a huge amount of genetic diversity is present within this population. This result was the first indication, from any work with phytoplankton, that these populations might contain such an enormous reservoir of diversity. This result suggested to us that more dynamic environments might harbor even more diverse populations. We therefore examined populations from Admiralty Inlet where tidal mixing is intense – over each tidal cycle, as much as 50% of seaward-flowing water is refluxed back into Puget Sound. We monitored the diatom population over the course of 3 months - in August, September, and October. We are part-way through analyzing this data and thus far we have found that in the August population, 27 of 28 clones had unique genotypes; in September, 13 of 18 clones were unique and in the October population, 5 of 5 clones were unique. Again, these results indicate that diatom populations harbor a huge amount of genetic diversity.

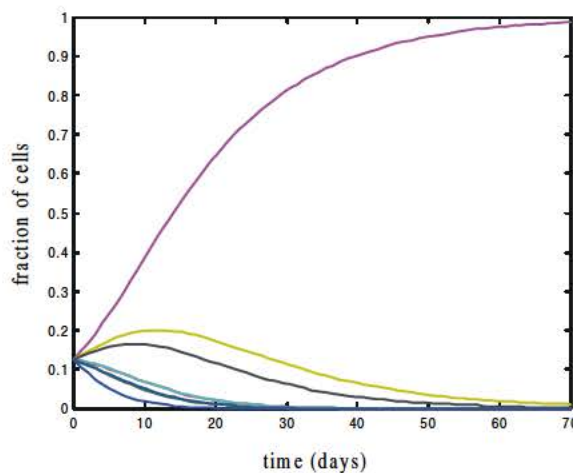
Interestingly, only 3 of the DNA fingerprints we observed in Admiralty Inlet populations were also present in Hood Canal despite the fact that these are contiguous bodies of water. Furthermore, by examining the distribution of fingerprint profiles within a population, we can show that the Hood Canal fall population is genetically distinct from the Admiralty Inlet September population. This result suggests that different selective pressures may act on the populations in these two environments such that certain cell “types” become specific to particular environments. Alternatively, we may have only scratched the surface of the amount of genetic diversity present within these populations and further sampling of these two areas will uncover more overlap in cell types. Either outcome will generate new ways of thinking about diatom population dynamics.

We maintain a number of genetically distinct clones from the two different environments for growth studies to determine if the populations we have studied are as physiologically diverse as they are genetically diverse. For example, we measured the optimum growth rates of 8 isolates from Hood Canal at 14• C under nutrient replete conditions on a 8:16 light:dark cycle of 66, 30 or 2 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$. The doubling times of these 8 genetically distinct clones varied by 24% at 66 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ to as much as 35% at 2 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ (Fig. 2). These results indicate that a huge amount of physiological diversity is also maintained within these populations.



2. Hours/doubling of 8 clones isolated from Hood Canal and maintained exponentially at 66 (blue bars), 30 (red bars), or 2 (yellow bars) $\mu E m^{-2} sec^{-1}$. Error bars indicate the standard deviation of 2-4 independent measurements of growth rates.

Once we realized how much physiological diversity was present in the Hood Canal population, we developed a simple model based on exponential growth to determine the amount of time that clones with such different growth rates could be maintained in a population if constant environmental conditions were assumed (Fig. 3). The growth rates obtained for each of the clones at each of the different light intensities was the input data for the model. At each light intensity, a dramatic shift in the amount of diversity maintained within the population occurs within the first 2-3 days. Within two weeks, a single clone can comprise over fifty percent of the population.



3. Simulation of the change over time in the relative proportion of 8 different clones (growth rate data shown in Fig. 2) maintained under nutrient replete conditions at 30 $\mu E m^{-2} sec^{-1}$

The fact that we observed individuals with widely different growth rates maintained in the same population in Hood Canal indicates that the environmental conditions experienced by this population must be far from constant. Thus, during the fall, subtle environmental changes, even in a relatively stratified environment like Hood Canal must be occurring rapidly enough to prevent small subsets of cell types from gaining dominance.

In summary, then, we have shown that diatom populations are characterized by huge amounts of genetic and physiological diversity. This level of diversity has never been observed before in diatom populations simply because it has never been possible before to distinguish between individuals within a single species. By focusing on how this diversity is shaped by the environment, we will gain new insights into how species respond to environmental changes and thus what factors dictate the formation and maintenance of diatom blooms.

IMPACT/APPLICATION

Diatom bloom dynamics dictate, to a large extent, both the ecology of coastal ecosystems and the optical properties of these waters. Despite years of study, however, oceanographers still have little understanding of what drives particular species of diatoms to bloom at particular times and locales and equally importantly, what factors determine the exact magnitude of the bloom. The approach we have taken combines novel molecular techniques with more traditional physiological studies to allow us to examine in unprecedented detail how diatoms respond to changing environmental conditions. A potential outgrowth of the development of these fingerprinting techniques for phytoplankton is the added ability to track particular populations over space and time. We are currently examining whether populations can be uniquely characterized by their distribution of microsatellite sizes. This would allow us to ask, for example, the source population of toxic phytoplankton blooms.

TRANSITIONS

We are collaborating with Dr. J. Newton from the Washington State Department of Ecology. to incorporate our results into a comprehensive model of Puget Sound known as PRISM (Puget Sound Regional Ecosystem Model).