Genomic characterization of *Didymosphenia geminata*: current progress and future directions

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Didymosphenia geminata

- Historically distributed among oligotrophic northern boreal and montane streams throughout the northern hemisphere.
- Increasing occurrence of nuisance blooms in regions such as new Zealand and throughout the United States.
- These blooms can extend for several km and cover the entire river/streambed, altering aquatic ecosystems and impacting regional economies.

Why has there been a recent surge in the number of large blooms of Didymo?

- Different hypotheses centering around Phosphorous uptake mechanisms offer explanations of how Didymo can form dense mats in oligotrophic systems.

  …*but* these hypotheses do not necessarily explain the onset of large blooms world-wide.

  –Is there a new genetic variant of Didymo responsible?

  –Has human mediated mixing of Didymo from different stream systems resulted in elevated invasiveness?

  –Are blooms in different locations genetically distinct?

What can molecular genetic analyses tell us about Didymo?

- A recent search of Genbank for Didymo sequence data returned only partial 18S rDNA sequences, illustrating the need for additional genetic information.

- Molecular markers such as microsatellites and SNPs would be informative for delineating population and phylogeographic structure, but need to be developed.

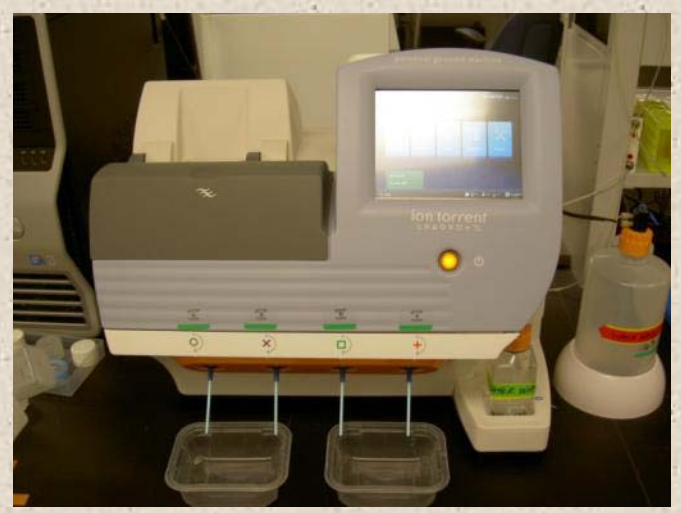
- Comparative analyses of gene expression (RNA-Seq) between blooming and non-blooming Didymo populations could identify differences in gene expression and identification of specific genes responsible for stalk production.

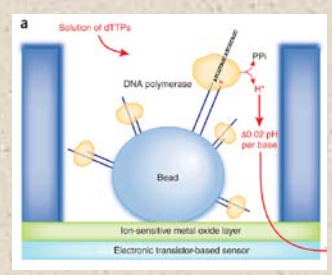
Objectives

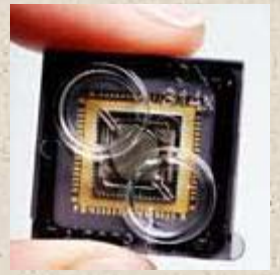
- Develop methods for bacteria free isolation of DNA and RNA from environmental Didymo samples.

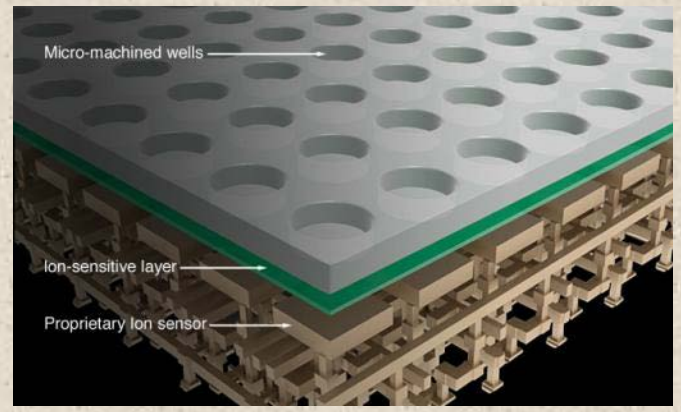
- Use second-generation high throughput sequencing technologies to investigate the genetic structure of Didymo.

  – Develop molecular markers for phylogeographic and population genetic analyses









Objectives continued…

- Begin *de novo* assembly of the *D. geminata* genome.
  - High throughput capability paired with improving de novo assembly algorithms make this a challenging but achievable goal
- Once an RNA extraction method is optimized, perform transcriptome sequencing and *de novo* assembly
  - Identify differential patterns of gene expression between blooming and non-blooming populations

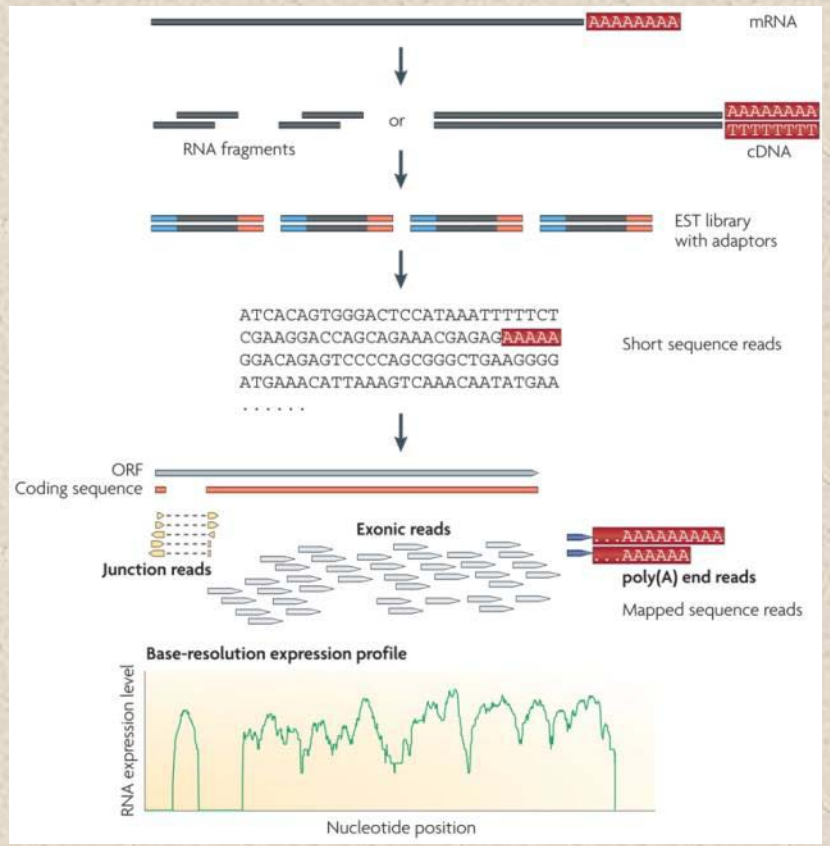
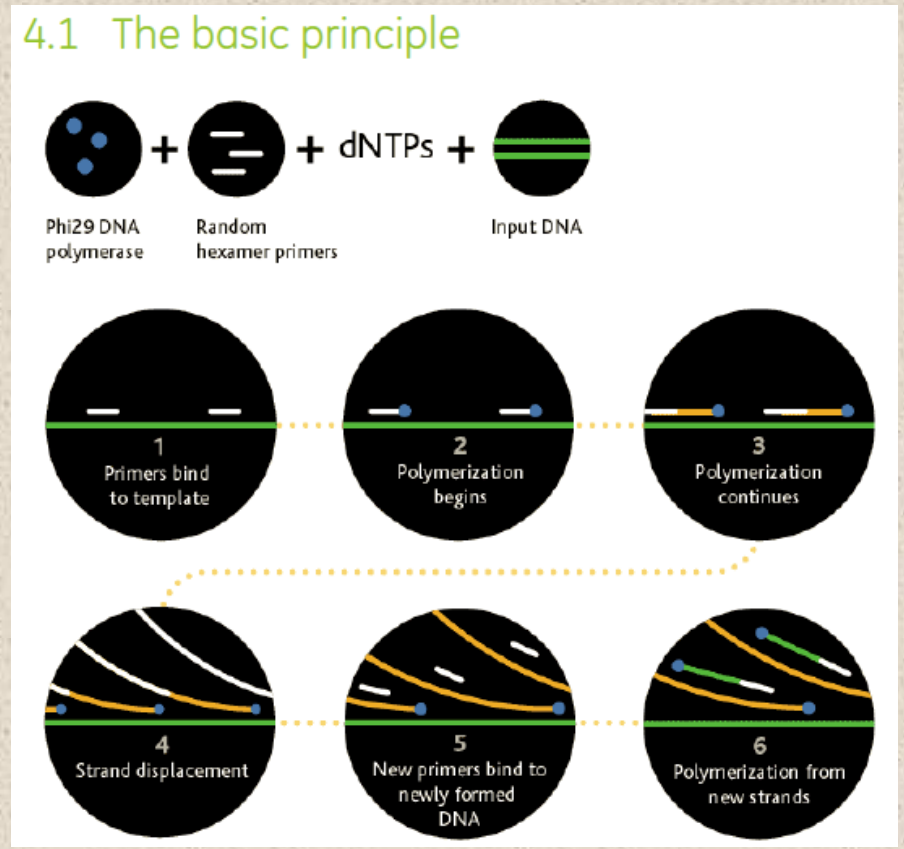


Figure from Wang et al. 2009

Results: Whole genome shotgun sequencing of Didymo

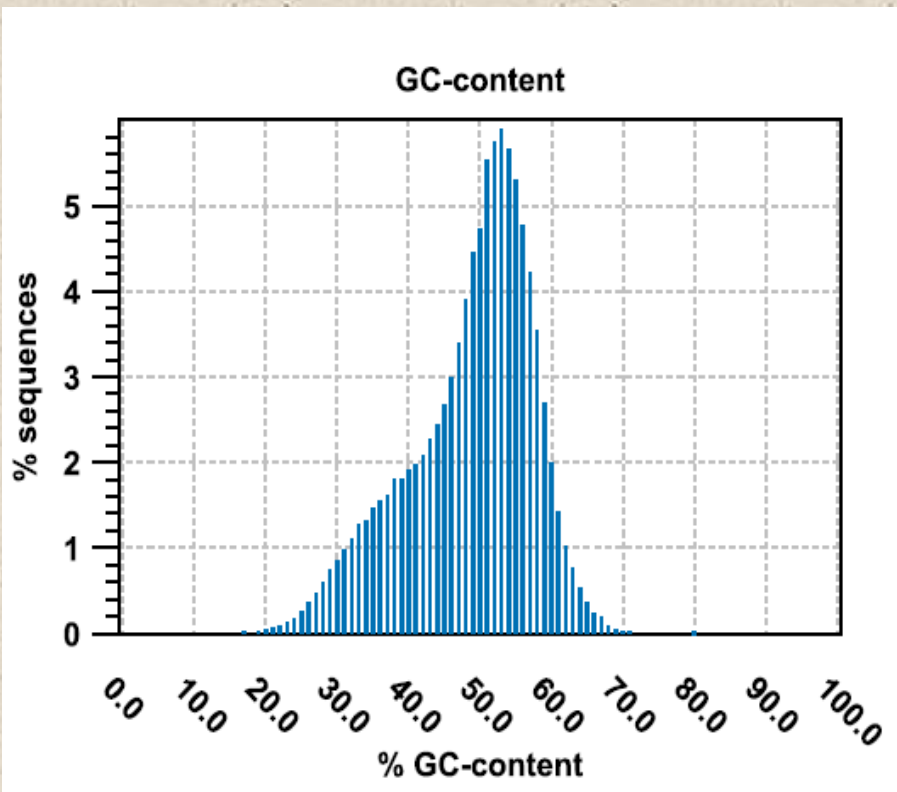
- For next-generation sequencing, need samples of Didymo free from contaminating bacteria, non-target diatoms, etc.
- Presently there is no method for xenic/axenic culturing of Didymo, necessitating isolation of Didymo cells from complex mixtures.
- Initial libraries have been prepared using Genomiphi® whole genome amplification (WGA) of a few cells.



From Genomiphi V2 manual

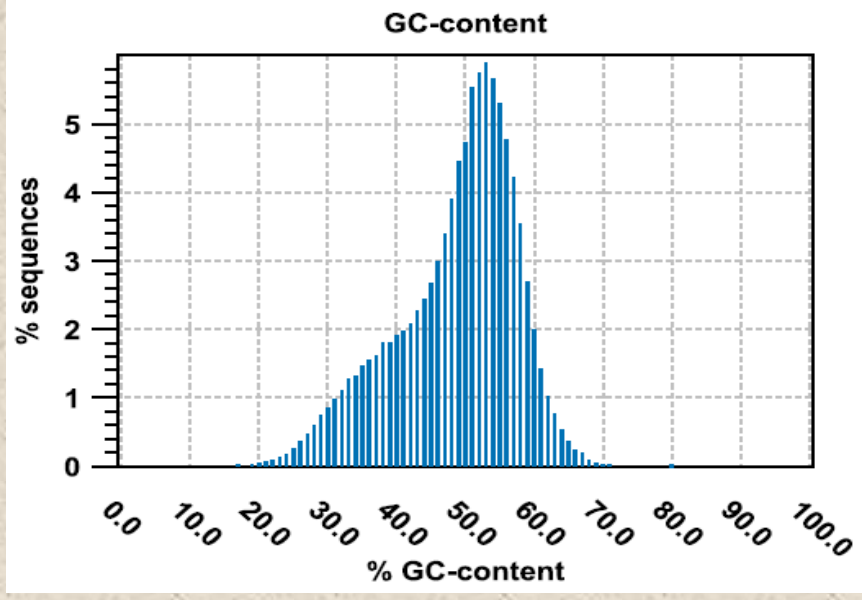
Results of first genomic library

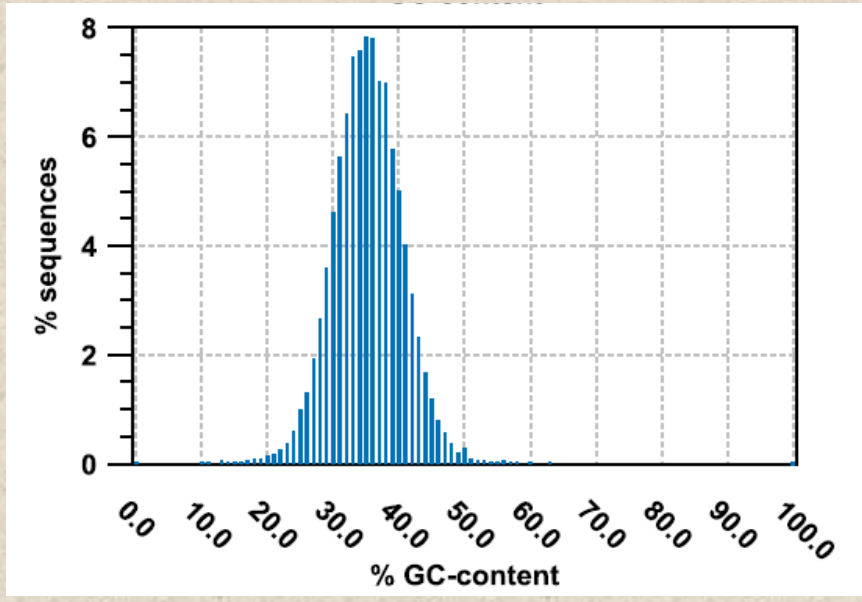
- Obtained >3ug DNA from approximately five Didymo cells using Genomiphi.
- Ion Torrent Run:
  - 2.6 million reads
  - 12,000 contigs generated through de novo assembly of reads
- GC distribution suggests appreciable bacterial contamination, confirmed with BLASTing.
  - Most contigs blasted to E. coli, aquatic bacteria.
  - Some diatom DNA



Results of second genomic library

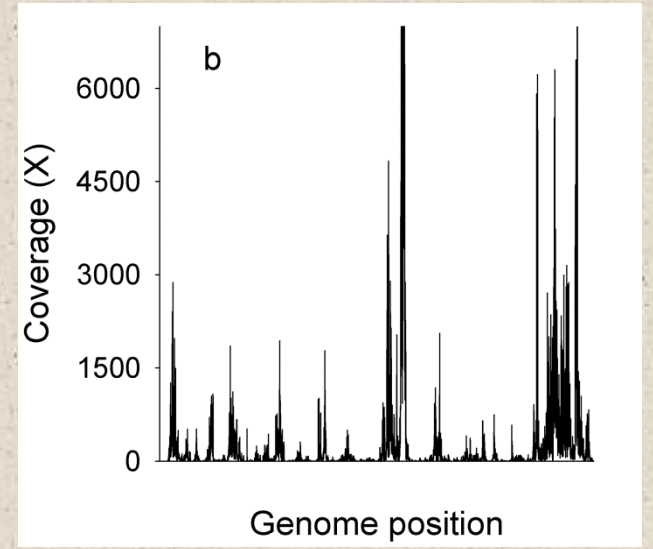
- Isolated Didymo cells were treated with lysozyme and washed over a filter prior to WGA.
- Ion Torrent run:
  - 3.6 million reads
  - ~40,000 contigs generated through de novo assembly of reads
- Significant shift to a more eukaryotic signature in the GC content.
- BLASTing identified more plausible hits to other diatoms, less bacterial contamination, but still a lot of contigs with no hits.





Didymo sorting for DNA and RNA Isolation

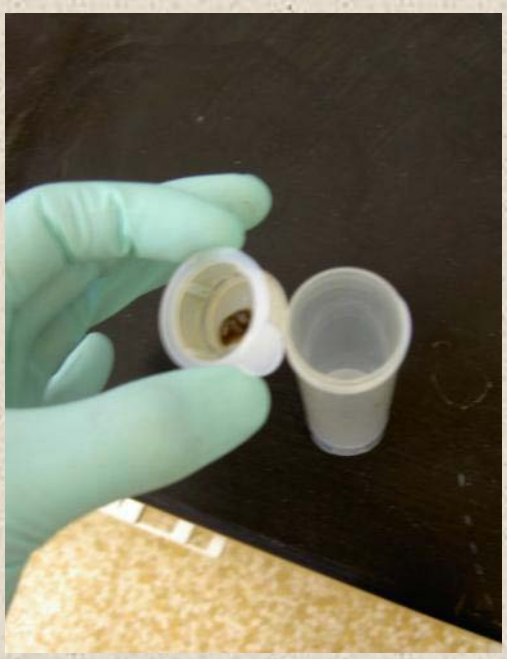
- WGA is useful for investigating the genetic structure of unculturable diatoms, but has biases.
- 1000's of cells needed for ng quantities of DNA for traditional library preparation.
- An alternative to manual picking is needed.

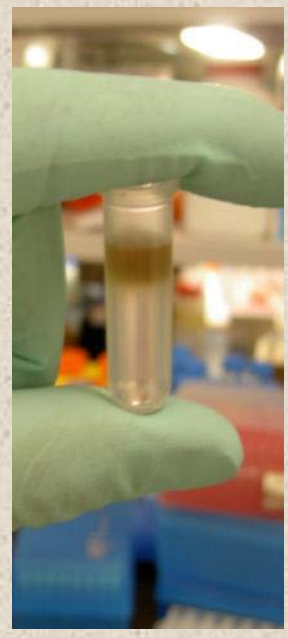


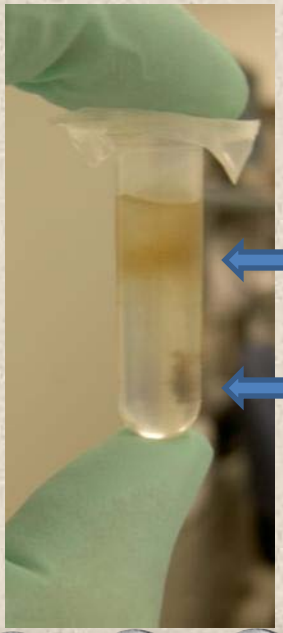
From Rodrigue et al. 2009

Density gradient centrifugation

- If Didymo have densities different from other diatoms and detritus, should be able to concentrate them.
- Studies have demonstrated the utility of media such as Percoll to separate different species of diatoms in a density gradient (Price et al. 1977; van Ierland and Peperzak 1984)
- Generate linear gradients of 1.10 – 1.19 g/ml Percoll



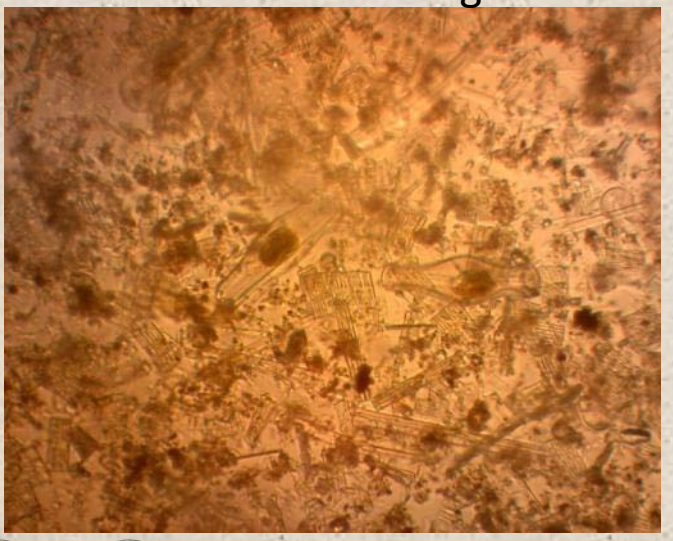




Concentration of Didymo is possible with Percoll

- Appreciable increase in number of Didymo cells separate from other diatoms using a Percoll gradient.
- Still requires manual sorting, but is easier.

Pre-Percoll centrifugation



Post-Percoll centrifugation

Ongoing research

- Runs of non-WGA genomic DNA libraries are in preparation.
  - Looking into reduced representation library methods such as RAD tags for population level comparisons.
- Data mining whole genomic DNA datasets for microsatellite and SNP markers.
- RNA extractions are in progress for gene expression analyses on the Illumina GA2x. Will conduct genome-wide association studies via comparative functional genomic (RNA-Seq) comparisons .

Non-genomic Ongoing research

- Identify and apply a sufficiently large number of nuclear genes for phylogenetic, phylogeographic, and population-level delineation
- Use *culture methods* to identify conditions which will allow the diatom to be brought into the laboratory for extensive physiological research.
- Use *water quality evaluation* to determine the environmental conditions that lead to shifts in demographic and growth (e.g., stalk production) patterns resulting in population explosions (i.e., blooms).

A request for samples!

- We are soliciting a call for didymo samples from throughout their range for sequencing analysis.
- Requires placing a small tuft or unstalked Didymo sample into a microfuge sample tube with RNA-later (provided).
- Please email: auninsaw@vcu.edu for information.

Acknowledgements

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Thank you