Analysis of intron conservation in diatoms, including newly assembled diatom *Psammoneis japonica*

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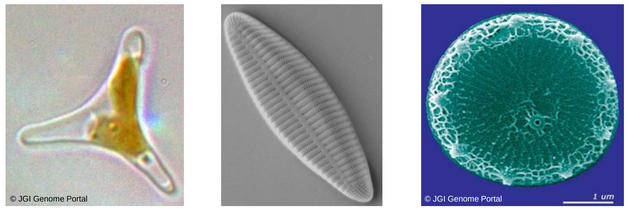
Introduction

Diatoms are a group of heterokont algae thought to be responsible for approximately 20% of the world's primary productivity [1]. Diatoms are very diverse, and there are estimated to be over 100,000 species. To date, only two diatom genomes have been annotated and published *(Thalassiosira pseudonana* and *Phaeodactylum tricornutum).* These genomes represent the two major orders of diatoms, pennate (laterally symmetric) and centric (radially symmetric) [1]; nonetheless, a broader diversity of sequenced and annotated diatom genomes would greatly contribute to understanding evolutionary patterns among this immensely diverse group. In these efforts, sequencing both coding and non-coding regions of the genome can reveal variable yet complementary information about the evolutionary history of organisms. This project focuses specifically on introns, a major class of non-coding DNA in the genome.

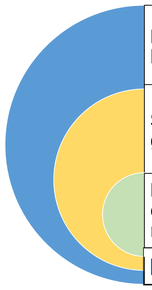
Introns are non-coding regions of genes that are removed by RNA spliceosomes prior to translation. While the frequency of introns varies in every eukaryotic genome, a commonality noted between intron-rich genomes is that a significant number of intron positions are conserved from distant eukaryotic relatives [2]. This implies that a proportion of introns are retained from earlier stages of eukaryotic evolution [2]. Previous work has demonstrated that the diatom *Thalassiosira pseudonana,* although intron rich, does not share a multitude of intron positions with ancient relatives [2].

The genome of the pennate diatom *Psammoneis japonica* has been assembled at the Chicago Botanic Garden, and the first round of annotations have been made. The focus of this project is to use annotations from *Psammoneis, Thalassiosira, Phaeodactylum,* and a non-diatom heterokont out-group (*Nannochloropsis gaditana)* to quantify intron conservation across distantly related heterokont and other eukaryotic lineages.



Diatom taxa *Phaeodactylum tricornutum* (left), *Psammoneis japonica* [3] (center), and *Thalassiosira pseudonana* (right)

Objectives/Hypotheses



Big Picture: Investigate evolutionary relationships between diverse diatom species

Smaller Focus: Sequence and annotate nuclear genomes of more diatom species

My Objective: Compare patterns of intron conservation between three diatom species and more distant eukaryotic relatives

Heterokont Genome Sequencing

Hypothesis 1: Intron density and conservation in *Psammoneis* will be more similar to *Phaeodactylum* because they are both pennate diatoms, as opposed to *Thalassiosira,* a centric diatom.

Hypothesis 2: Intron density and conservation in diatoms will be more similar to each other as opposed to more distant eukaryotes.

Methods

Data Collection:

Genome annotations and nucleotide/protein FASTA files for the newly sequenced *Psammoneis japonica,* published diatoms [1][4] and a heterokont out-group (*Nannochloropsis* [5]) produced in-house or downloaded from NCBI genome database.

Finding and Aligning Orthologous Genes between Species for Comparison:

OrthoFinder [6] software used to find single copy ortholog clusters between diatoms *Thalassiosira, Phaeodactylum,* and *Psammoneis*, and the out-group *Nannochloropsis*. MAFFT [7] used to align orthologous nucleotide sequences including introns.

Intron Position Conservation Analysis:

Custom Python scripts created to analyze intron position conservation in aligned single copy orthologs. Conservation was defined as two species having the same intron position in regions where 75 nucleotides flanking the intron on each side have at least 33% nucleotide identity [2].

Results

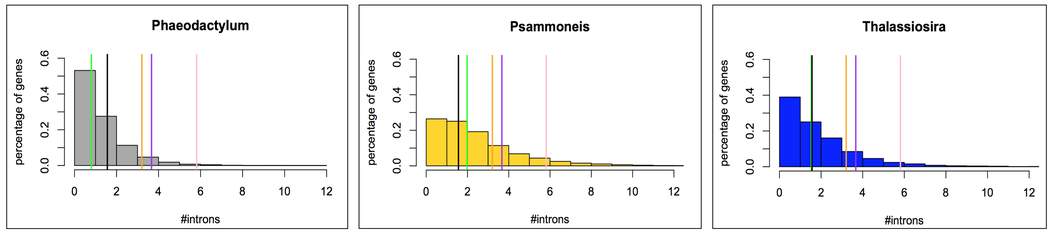


Figure 1: Intron density for all genes of diatom species. Green vertical lines represent the average intron density across the genome (introns / gene) for each diatom species. For comparison, average intron density for *Nannochloropsis* (black), Drosophila(orange) [8], *Arabidopsis*(purple) [9], and *Mus*(pink) [10] are also shown as vertical lines.

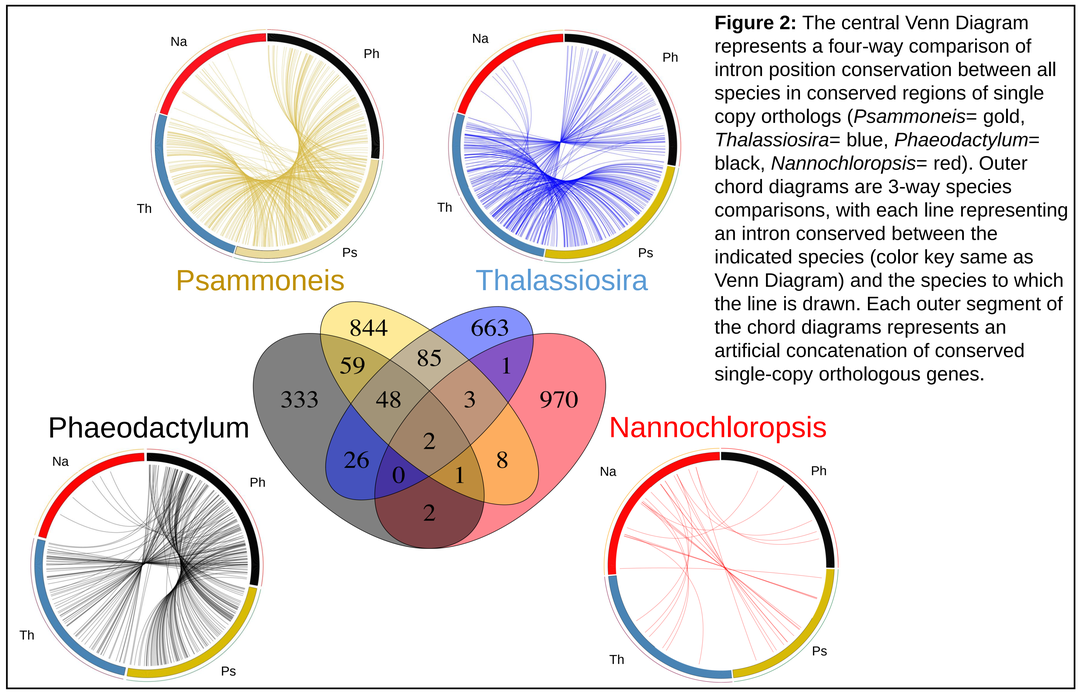


Figure 2: The central Venn Diagram represents a four-way comparison of intron position conservation between all species in conserved regions of single copy orthologs (*Psammoneis*= gold, *Thalassiosira*= blue, *Phaeodactylum*= black, *Nannochloropsis*= red). Outer chord diagrams are 3-way species comparisons, with each line representing an intron conserved between the indicated species (color key same as Venn Diagram) and the species to which the line is drawn. Each outer segment of the chord diagrams represents an artificial concatenation of conserved single-copy orthologous genes.

Conclusions/Discussion

Hypothesis 1:

It was hypothesized that, because *Psammoneis* is in the same diatom order as *Phaeodactylum* (pennate), the introns in this species would show similarities to the *Phaeodactylum* genome. However, while *Phaeodactylum* has an average intron density of < 1 intron per gene, *Psammoneis* has almost 2 introns per gene, which is higher than the intron-rich centric diatom *Thalassiosira* (Figure 1). This implies that the intron patterns of diatoms in general may not be distinguishable simply by the split between the main two orders of diatoms. The between-diatom intron conservation comparisons show *Phaeodactylum* shares more of its introns with *Psammoneis* (23%) than *Thalassiosira* (16%), which was expected (Figure 2). However, both *Psammoneis* and *Thalassiosira* share more of their intron positions with each other (13% and 17% respectively) than with *Phaeodactylum* (10% and 9% respectively), which supports the results shown in Figure 1. Additionally, *Phaeodactylum* has a lower frequency of unique introns which might imply that if the lack of introns in the diatom came from an intron loss event, these lost introns are likely to have had unique positions.

Hypothesis 2:

The average intron densities of all three diatoms are < 2 introns per gene, 0.2-0.5× that of more distant eukaryotic ancestors *Arabidopsis* , *Mus,* and *Drosophila* (Figure 1). The intron density of *Nannochloropsis,* also a photosynthetic heterokont, is similar to the diatoms; however, *Nannochloropsis* shares few intron positions with any of the three diatoms, while between-diatom-species comparisons show higher levels of conservation (Figure 2).

Overall, our analysis has demonstrated that intron conservation decreases with an increase in divergence time. By including *Psammoneis as* a second pennate diatom, we can conclude that the *Phaeodactylum* lineage has likely experienced loss of novel introns. As *Psammoneis* and *Phaeodactylum* are in different sub-orders of pennate diatoms (araphid and raphid, respectively), these results suggest that shallower levels of the phylogeny may be explored to more fully address questions of intron conservation and other patterns of evolution. The centric diatoms can also be divided into radial and polar sub-orders, and investigations within this division should also expose more diversity in diatom traits. Further research including more representation in each order could tell us whether or not our results are generalizable for these major groups.

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REU Site: *Plant Biology & Conservation Research Experiences for Undergraduates - From Genes to Ecosystems*

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