Molecular Phylogenetics and Evolution 71 (2014) 36–40

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Short Communication

# Massive difference in synonymous substitution rates among mitochondrial, plastid, and nuclear genes of *Phaeocystis* algae

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ARTICLE INFO

*Article history:*
Received 10 July 2013
Revised 22 October 2013
Accepted 24 October 2013
Available online 8 November 2013

*Keywords:*
Antarctica
Genetic diversity
Haptophyte
*Kleptodinium*
Mutation rate

ABSTRACT

We are just beginning to understand how mutation rates differ among mitochondrial, plastid, and nuclear genomes. In most seed plants the mitochondrial mutation rate is estimated to be lower than those of the plastid and nucleus, whereas in the red alga *Porphyra* the opposite is true, and in certain green algae all three genomes appear to have similar rates of mutation. Relative rate statistics of organelle vs nuclear genes, however, are lacking for lineages that acquired their plastids through secondary endosymbiosis, but recent organelle DNA analyses suggest that they may differ drastically from what is observed in lineages with primary plastids, such as green plants and red algae. Here, by measuring synonymous nucleotide substitutions, we approximate the relative mutation rates within the haptophyte genus *Phaeocystis*, which has a red-algal-derived, secondary plastid. Synonymous-site divergence data indicate that for *Phaeocystis antarctica* and *P. globosa* the mitochondrial mutation rate is 10 and 3 times that of the plastid and nucleus, respectively. This differs drastically from relative rate estimates for primary-plastid-bearing lineages and presents a much more dynamic view of organelle vs nuclear mutation rates across the eukaryotic domain.



## 1. Introduction

Insights into the relative mutation rates among mitochondrial, plastid, and nuclear DNA (mtDNA, ptDNA, nucDNA) can be gained by measuring synonymous substitution rates ($d_S$) in organelle and nuclear genomes between closely related species (Kimura, 1983). Available data from the green algae *Chlamydomonas* and *Mesostigma*, for example, reveal similar rates of synonymous substitution in all three genetic compartments (Popescu and Lee, 2007; Hua et al., 2012). In most seed plants, however, $d_S$ of the mitochondrial genome is around 3 and 10 times lower than that of the plastid and nuclear genomes, respectively (Drouin et al., 2008), which is opposite to trend observed in the red algal genus *Porphyra*, where mitochondrial substitution rates are 3–5 times greater than those of the plastid and nucleus (Smith et al., 2012).

These findings suggest that the relative mutation rates of mtDNA, ptDNA, and nucDNA can differ significantly among major lineages, and have helped foster hypotheses on genome evolution, including the view that low mutation rates contribute to genomic expansion (Lynch et al., 2006; Hua et al., 2012), but see Smith et al. (2012). That said, much of the plastid-containing eukaryotic diversity falls outside the green plant and red algal lineages (Archibald, 2009), within groups for which there are little or no data on relative rates of synonymous substitution (Smith and Keeling, 2012). This includes groups that acquired their plastids through eukaryotic–eukaryotic endosymbiosis, such as haptophytes—globally distributed planktonic algae with plastids that derive from a red alga (Keeling, 2010). Recent analyses suggest that in some species with red-algal-derived plastids, including the haptophyte *Emiliania huxleyi*, the mitochondrial mutation rate greatly exceeds that of the plastid. But there are currently no data on relative rates for *all three* genetic compartments within a lineage with a secondary red plastid. This is largely because it can be difficult to find two distinct species that are closely enough related that the synonymous substitution rate has not reached saturation.

Here, we explore the relative levels of synonymous substitution within the haptophyte genus *Phaeocystis*, the members of which can form massive ocean blooms (Smith et al., 1991; Arrigo et al., 1999), play a pivotal role in global carbon and sulfur cycles (Verity et al., 2007), and are known to donate their plastids to dinoflagellates in what may be an emerging endosymbiosis (Gast et al., 2007). By comparing the genomes of *Phaeocystis* algae collected from opposite ends of the planet, we uncover massive differences in $d_S$ among mtDNA, ptDNA, and nucDNA, which we argue are the consequence of drastically different mutational patterns among these genomes.

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http://dx.doi.org/10.1016/j.ympev.2013.10.018

## 2. Materials and methods

Nucleotide sequence data come from *Phaeocystis antarctica* strain CCMP1374 of the National Center for Marine Algae and Microbiota (NCMA), collected in the Ross Sea, McMurdo Sound, Antarctica, and *Phaeocystis globosa* strain Pg-G(A) of the University of Groningen Culture Collection (RUG), collected from the North Sea, offshore station T135, Terschelling transect. These are the same strains being used for whole nuclear genome sequencing by the United States Department of Energy Joint Genome Institute (DOE JGI).

The *P. antarctica* organelle genome sequences were generated as part of the DOE JGI *Phaeocystis* Genome Project by whole-genome shotgun sequencing using Applied Biosystem's 3730xl DNA Analyzer technology. Sanger sequencing reads were mined from the National Center for Biotechnology Information (NCBI) Trace Archive and mtDNA and ptDNA sequences were assembled with CodonCode Aligner v3.7.1.1 (CodonCode Corporation, Dedham, MA, USA) following the protocols of Smith et al. (2010, 2011). The *P. globosa* mitochondrial and plastid genomes were assembled using Illumina (HiSeq 2000) DNA sequence data (GenBank Sequence Read Archive accessions SRX113061, SRX142966, SRX144179, and SRX135690), generated by the DOE JGI *Phaeocystis* Genome Project. The Illumina data (~185 Gb), which contained both short (250 and 500 nt) and long (4 and 8 kb) insert libraries, were assembled *de novo* with Ray v1.2.1 (Boisvert et al., 2010), using a *k*-mer of 21, and separately with Geneious v6.1.2 (Biomatters Ltd., Auckland, NZ). The resulting Ray- and Geneious-generated contigs were independently scanned for mitochondrial and plastid sequences using BLAST and the *P. antarctica* mtDNA and ptDNA as search queries. Contigs matching to organelle DNA were identified in each dataset and were extended using the paired-end data and the "Map to Reference" program in Geneious, giving, in both cases, complete circular-mapping mitochondrial and plastid genomes. Nuclear genes were assembled using *P. antarctica* and *P. globosa* data from the NCBI Trace and Sequence Read archives, as described above. The *P. antarctica* and *P. globosa* mtDNA and ptDNA sequences are deposited in GenBank (accessions JN117275, JN131834–5, KC967226, and KC900889) and the nucDNA data are in Supplementary Tables S1 and S2.

Organelle and nuclear genes were aligned with MUSCLE (Edgar, 2004), implemented through Geneious using default settings. Synonymous and nonsynonymous substitutions were measured with the CODEML program of PAML v4.3 (Yang, 2007), employing the maximum likelihood method (Goldman and Yang, 1994) and the F3x4 codon model, and making appropriate adjustments for variation in the genetic code. Substitutions in non-protein-coding regions were estimated with BASEML of PAML using maximum likelihood and the HKY85 model.

## 3. Results and discussion

### 3.1. Substitution rates in Phaeocystis: mito high, plastid low

We measured rates of nucleotide substitution between *P. antarctica* and *P. globosa* (Table 1), two distinct unicellular species isolated from the Ross Sea (Antarctica) and North Sea (Netherlands), respectively. Our dataset included complete mitochondrial and plastid genomes and ~35 functionally diverse nuclear genes (Supplementary Tables S1 and S2). The organelle DNA architecture of *P. antarctica* is identical to that of *P. globosa*, save for a single gene duplication and two gene inversion events (Fig. 1). Both species have partially lost the canonical ptDNA quadripartite structure: one inverted repeat has a standard gene organization, whereas the other is missing *trnI* and *trnA* (Fig. 2). This arrangement, to the best of our knowledge, is unique among available plastid genomes and implies that these algae are in the early stages of losing their ptDNA inverted-repeat architecture, as has already happened in other plastid-bearing eukaryotes, including the haptophyte *Pavlova lutheri* (Fig. 2).

Substitution rates between *P. antarctica* and *P. globosa* differed by an order of magnitude across the three genomes, and, for all nucleotide-site categories, were highest in the mtDNA and lowest in the ptDNA (Table 1). The average number of substitutions per synonymous site for protein-coding regions in the mtDNA (2.41 ± 0.97) was ~10 times that of the ptDNA (0.25 ± 0.16) and ~3 times that of the nucDNA (0.85 ± 0.54). Concatenated datasets gave an almost identical trend (Table 1). The variation in $d_S$ among the different protein-coding genes within each genome was 1–4.3 (mtDNA), 0–0.9 (ptDNA), and 0.2–2.3 (nucDNA) (Fig. 1; Supplementary Table S1), again revealing greater levels of synonymous-site substitution in the mitochondrion relative to the plastid and nucleus. The average relative rates of substitution at nonsynonymous sites followed a similar pattern to those at synonymous positions: $d_N$ of the mtDNA (0.09 ± 0.08) was roughly 9 and 2 times that of the ptDNA (0.01 ± 0.02) and nucDNA (0.05 ± 0.04), respectively. The $d_N/d_S$ ratio was low for all three genomes, with averages of 0.05 (mtDNA), 0.06 (ptDNA), and 0.07 (nucDNA) (Table 1), indicating strong purifying selection on nonsynonymous sites. Substitution rates of rRNA genes were 36 times greater for the mtDNA than for the ptDNA and nucDNA (Table 1).

Together, these data suggest that for *Phaeocystis* the mitochondrial mutation rate greatly exceeds those of the plastid and nucleus. When assuming neutrality at synonymous sites, the rate of mutation among ptDNA, nucDNA, and mtDNA appears to be about 1:3:10 (Table 1). This is probably a conservative estimate. The mtDNA synonymous substitution rate between *P. antarctica* and *P. globosa* is close to saturation (>1), meaning that we were likely unable to capture the full extent of silent-site mitochondrial divergence between these two algae, and that the mtDNA mutation rate relative to the ptDNA and nucDNA is even greater than that predicted here.

### 3.2. Relative mutation rates: a plastid perspective

How do the relative mutation rates of *Phaeocystis* compare with those of other taxa? Plastid-containing species represent much of the known eukaryotic diversity, but our understanding of plastid mutation rates is largely shaped by data from a single lineage: seed plants. With exceptions, seed plant ptDNA mutation rate estimates are intermediate (higher and lower, respectively) to their mitochondrial and nuclear counterparts (Wolfe et al., 1987; Drouin et al., 2008) (Table 2; Fig. 3), but in some cases are predicted to be more than 15-times that of the mtDNA (Richardson et al., 2013). Outside seed plants a completely different picture is emerging. In the green algae *Chlamydomonas* and *Mesostigma*, all three genetic compartments are estimated to have similar mutation rates (Hua et al., 2012), and in the red algal genus *Porphyra*, the rates of both the ptDNA and nucDNA are approximately one-fifth that of the mtDNA (Smith et al., 2012) (Table 2; Fig. 3).

The relative mutation rate estimates for *Phaeocystis*, which has a red-algal-derived plastid, are most similar to those of *Porphyra* (Fig. 3). Both lineages have elevated levels of mitochondrial synonymous-site substitution in relation to the plastid and nucleus, contrasting the situation in the green lineage (Fig. 3). Mutation rate statistics on all three genetic compartments are lacking for other species with red algal plastids, but data for only organelle DNA are available and support the trend described here. In the apicomplexan parasite *Babesia bovis*, the heterokont alga *Heterosigma akashiwo*, and the haptophyte alga *E. huxleyi* the mitochondrial mutation rate is predicted to be 5–30 times that of the plastid

**Table 1**
Plastid, mitochondrial, and nuclear DNA substitution rates between *Phaeocystis globosa* and *P. antarctica*.

| | Substitutions per site | | | Substitution rate ratios (pt:mt:nuc) |
|---|---|---|---|---|
| | ptDNA | mtDNA | nucDNA | |
| *Synonymous sites* | | | | |
| Average (SD) | 0.25 (0.16) | 2.41 (0.97) | 0.85 (0.54) | 1:9.6:3.4 |
| Concatenation | 0.26 | 2.06 | 0.73 | 1:7.9:2.8 |
| *Nonsynonymous sites* | | | | |
| Average (SD) | 0.01 (0.02) | 0.09 (0.08) | 0.05 (0.04) | 1:9:5 |
| Concatenation | 0.02 | 0.07 | 0.04 | 1:3.5:2 |
| $d_N/d_S$ (SD) | 0.06 (0.08) | 0.05 (0.06) | 0.07 (0.07) | – |
| rRNAs[a] | 0.005 | 0.18 | 0.006 | 1:36:1.2 |
| tRNAs[b] | 0.007 | 0.09 | – | 1:12.9:– |

SD: standard deviation; $d_N/d_S$: ratio of nonsynonymous to synonymous substitutions per site, based on averages not concatenations. The substitution rate statistics for the individual loci within the organelle and nuclear compartments, including those that were derived from concatenated datasets, are shown in Supplementary Table S1.
[a] For mtDNA and ptDNA includes the concatenation of all rRNA-coding regions. For nucDNA includes *18S* and *28S* rRNA-coding regions.
[b] Based on concatenation of all tRNA-coding regions.

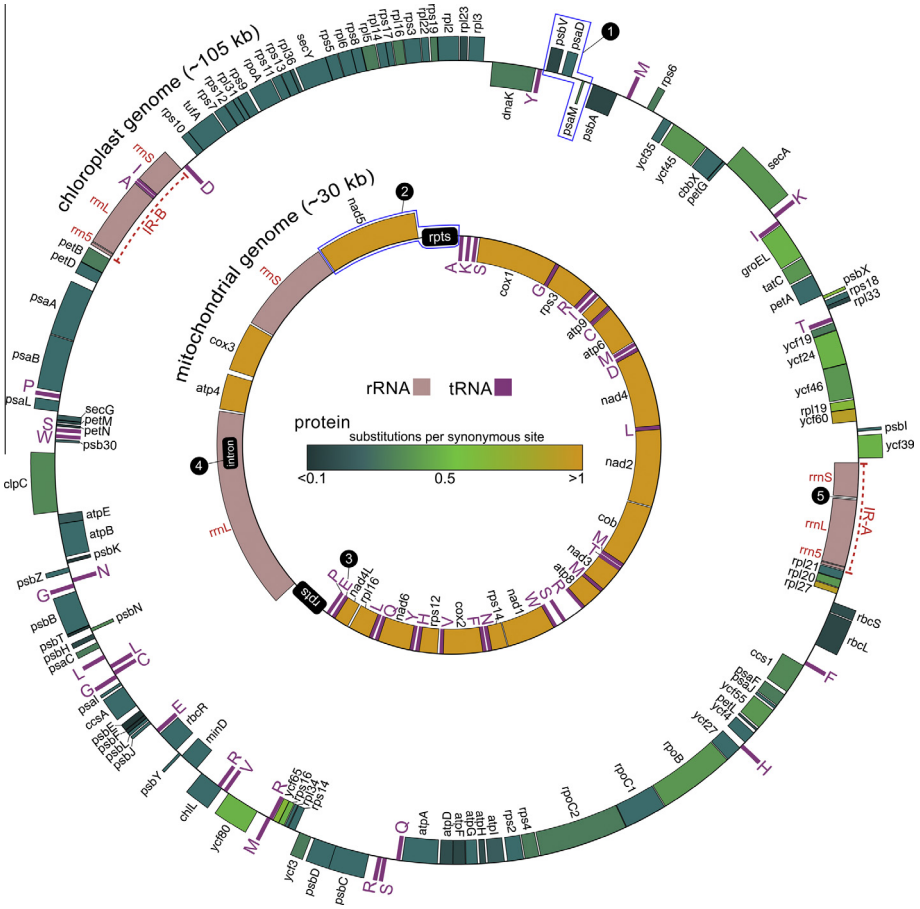


**Fig. 1.** *Phaeocystis* organelle genome architecture. Plastid (outer) and mitochondrial (inner) genome maps for *Phaeocystis globosa* and *P. antarctica*. The average number of synonymous substitutions per synonymous site between the protein-coding genes of *P. globosa* and *P. antarctica* is shown using a blue and orange color spectrum. Structural RNA-coding regions are pink. Single letter amino acid abbreviations specify tRNA species. Plastid inverted repeats (IR) and mitochondrial DNA repeats (rpts) are shown. Transcriptional polarity: clockwise (inside of the circle), counterclockwise (outside of the circle). Black numbered circles: 1 and 2—these regions are flipped and inverted in *P. antarctica* relative to what is shown on maps; 3—tandem duplication of *trnE* in *P. antarctica*; 4— this intron is missing from *P. antarctica*; 5— highlights loss of *trnI* and *trnA* from one copy of the inverted repeat (IR) in both *P. globosa* and *P. antarctica*.

(Smith and Keeling, 2012). Thus, an mtDNA/ptDNA mutation rate ratio of $\gg 1$ is predicted for diverse lineages with red algal plastids, and, given the wide distribution of red plastids across the eukaryotic tree, may represent the norm rather than the exception for plastid-bearing eukaryotes. In *E. huxleyi* strains CCMP373 and CCMP1516 the mtDNA/ptDNA synonymous substitution rate ratio is about 20, suggesting a twofold increase in the mitochondrial vs plastid mutation rates relative to *Phaeocystis* (Smith and Keeling, 2012) and indicating that there can be a large variation in relative substitution rates among haptophyte lineages.

The error associated with calculating the number of generations separating *P. antarctica* and *P. globosa* is too great to accurately predict the absolute rates of mutation within the organelle and nuclear genomes of these algae. However, their mitochondrial and

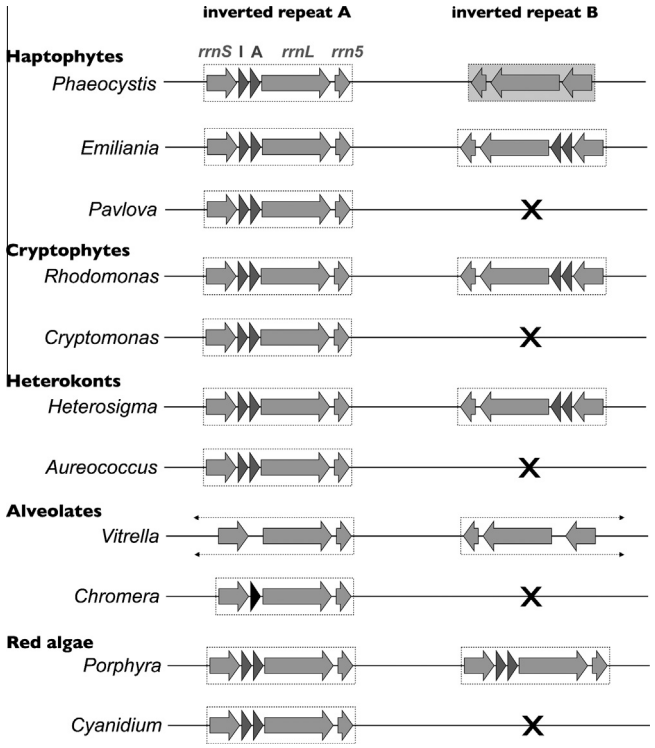


**Fig. 2.** Conservation and loss of canonical inverted repeat structure in red-algal-derived plastid genomes. The *Phaeocystis globosa* and *P. antarctica* plastid genomes have one inverted repeat with a canonical organization (A) and another that is missing the *trnI* and *trnA* genes (B; shaded gray). Other available red-algal and red-algal-derived plastid genomes have either a complete pair of canonical or noncanonical inverted repeats (shown in boxes) or they have lost the inverted-repeat arrangement altogether (shown with "X"). Not to scale. Examples and GenBank accessions: *Emiliania huxleyi* (NC_007288), *Pavolva lutheri* (NC_020371), *Rhodomonas salina* (NC_009573), *Cryptomonas paramecium* (NC_013703), *Heterosigma akashiwo* (NC_010772), *Aureococcus anophagefferens* (NC_012898), *Vitrella brassicaformis* (NC_014345), *Chromera velia* (NC_014340), *Porphyra purpurea* (NC_000925), and *Cyanidium caldarium* (NC_001840).

plastid silent-site substitution rates are high and low, respectively, when compared to those from other plastid-containing lineages (Table 2; Fig. 3), which is consistent with these genomes having high and low absolute mutation rates.

### 3.3. Red-algal-derived plastids: a slow moving target

The large predicted differences in mutation rate among the organelle and nuclear genomes of *Phaeocystis* could help guide future investigations of phytoplankton. *Phaeocystis* and other haptophytes (e.g. coccolithophores) are inordinately important in shaping ocean ecosystems and global biogeochemical cycles (Verity et al., 2007) and, as such, are of interest to scientists from diverse fields, including those studying climate change and ocean acidity. Many scientists rely on the sequencing of genetic markers, like *18S* rDNA, to better understand these algae (Bittner et al., 2013). The data presented here and elsewhere (Smith and Keeling, 2012) indicate that the ptDNA of haptophyte phytoplankton, because of its predicted low mutation rate, is an ideal genetic marker for broad-scale comparative analyses, such as those attempting to resolve relationships between species or lineages. The mtDNA, however, with its potential for high rates of mutation, is likely a better "fine-tune" genetic marker, one that could be used for discerning intra-species or inter-strain relationships.

In addition to having acquired a plastid from a red alga, haptophytes have donated their red algal plastid to certain dinoflagellates (Tengs et al., 2000). In some cases, such as the dinoflagellates *Karenia* and *Karlodinium*, the haptophyte plastid is fully integrated into and a permanent fixture of the host cell. In others instances, the acquired haptophyte plastid is only temporary and needs to be continuously replenished by stealing fresh ones from prey—a process called kleptoplasty. The heterotrophic dinoflagellate *Kleptodinium*, for example, sequesters plastids from its prey *P. antarctica*, and then uses these plastids for photosynthesis (Gast et al., 2007). Remarkably, the *P. antarctica* plastids can function within *Kleptodinium* for several months (Gast et al., 2007). How these stolen *Pheaocystis* plastids remain active for so long is a mystery. One possibility is that *Kleptodinium* acquired photosynthesis-related genes through horizontal gene transfer. *Kleptodinium* is a sister taxon to *Karenia* and *Karlodinium* (Gast et al., 2006), and, therefore, may have descended from an ancestor with a haptophyte plastid.

It is not known if plastid genome mutation rate influences the stability and function of kleptoplasts. The plastid genome of *P. antarctica*, if it does have a low mutation rate, might represent a "slow-moving genetic target" for plastid acquisition. It has been shown that *Kleptodinium* obtains its plastids from a mixture of *P. antarctica* strains and that this mixture of plastids is maintained within its cell (Gast et al., 2007). Hijacking plastids with low rates of mutation, and therefore similar genetic backgrounds, might make it easier for *Kleptodinium* to employ the plastids from different *P. antarctica* strains. Also, if *Kleptodinium* has nuclear-encoded plastid-targeted proteins, it might be easier for these proteins to establish and maintain a functional rapport with a plastid genetic architecture that is consistent among strains and remains relatively stable over time.

**Table 2**
Synonymous substitution rates in plastid, mitochondrial, and nuclear genomes from various eukaryotic lineages.

| | Substitutions per synonymous site | | | Substitution rate ratios (pt:mt:nuc) |
|---|---|---|---|---|
| | ptDNA | mtDNA | nucDNA | |
| *Haptophytes* | | | | |
| Phaeocystis | 0.25 (0.16) | 2.41 (0.97) | 0.85 (0.54) | 1:9.6:3.4 |
| *Red algae*[a] | | | | |
| Porphyra | 0.47 (0.22) | 1.76 (0.58) | 0.43 (0.18) | 1:3.7:0.9 |
| *Green algae*[b] | | | | |
| Chlamydomonas | 0.30 (0.11) | 0.29 (0.05) | 0.37 (0.29) | 1:1:1.2 |
| Mesostigma | 0.11 (0.06) | 0.17 (0.11) | 0.27 (0.18) | 1:1.5:2.5 |
| *Seed plants*[c] | | | | |
| Angiosperms | 0.39 (0.01) | 0.13 (0.01) | 2.11 (0.09) | 1:0.3:5.4 |
| Gymnosperms | 0.61 (0.03) | 0.28 (0.02) | 1.23 (0.09) | 1:0.5:2 |

Synonymous-site substitution rates are based on averages among loci, not concatenations.
[a] Data from Smith et al. (2012).
[b] Data from Popescu and Lee (2007) and Hua et al. (2012).
[c] Data from Drouin et al. (2008).

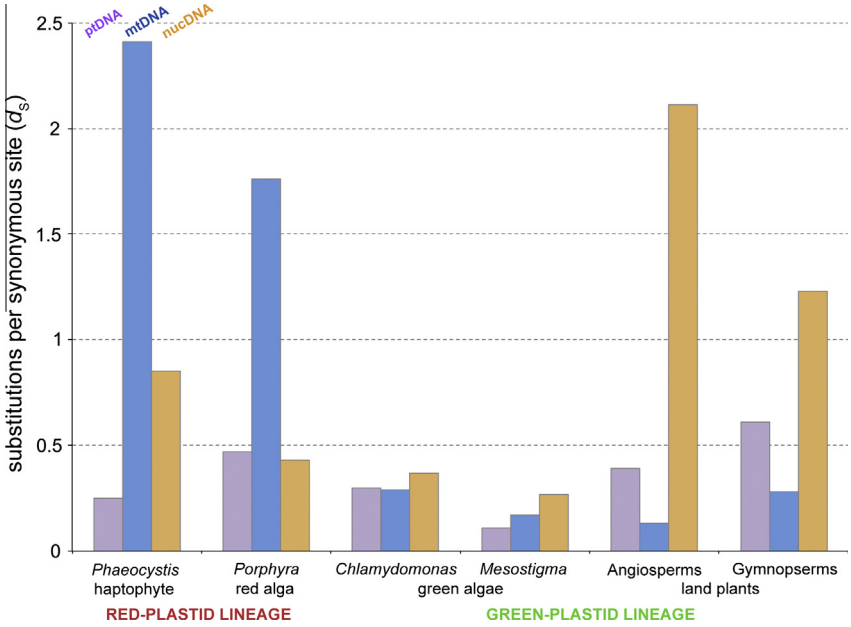


**Fig. 3.** Synonymous substitution rates in the organelle and nuclear genomes from red-plastid and green-plastid lineages. Plastid DNA (ptDNA) is purple, mitochondrial DNA (mtDNA) is blue, and nuclear DNA (nucDNA) is gold. See Table 2 for a complete list of substitution rates and their sources. Synonymous-site substitution rates are based on averages among loci, not concatenations.

Whatever the case, the large predicted differences in mutation rate among the ptDNA, mtDNA, and nucDNA of *P. antarctica* and *P. globosa* will surely impact how we view and interpret the evolution of haptophyte genomes in the years to come. As more data on relative rates emerge from poorly studied plastid-containing lineages, they will likely provide an even more dynamic picture of organelle and nuclear mutation rates across the eukaryotic domain.

## Acknowledgments

This work was supported by a Discovery Grant to DRS from the Natural Sciences and Engineering Research Council (NSERC) of Canada and National Science Foundation (NSF) Grants NSF OCE-1136477 and NSF-ANT 1043671 to AEA.

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.10.018.

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