Short Communication

Nucleotide substitution analyses of the glaucophyte Cyanophora suggest an ancestrally lower mutation rate in plastid vs mitochondrial DNA for the Archaeplastida

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Abstract

A lot is known about the evolution and architecture of plastid, mitochondrial, and nuclear genomes, but surprisingly little is known about their relative rates of mutation. Most available relative-rate data come from seed plants, which, with few exceptions, have a mitochondrial mutation rate that is lower than those of the plastid and nucleus. But new findings from diverse plastid-bearing lineages have shown that for some eukaryotes the mitochondrial mutation rate is an order of magnitude greater than those of the plastid and nucleus. Here, we explore for the first time relative rates of mutation within the glaucophyta—one of three main lineages that make up the Archaeplastida (or Plantae sensu lato). Nucleotide substitution analyses from distinct isolates of the unicellular glaucophyte Cyanophora paradoxa reveal 4–5-fold lower rates of mutation in the plastid and nucleus than the mitochondrion, which is similar to the mutational pattern observed in red algae and haptophytes, but opposite to that of seed plants. These data, together with data from previous reports, suggest that for much of the known photosynthetic eukaryotic diversity, plastid DNA mutations occur less frequently than those in mitochondrial DNA.

1. Introduction

Some of the first insights into the relative mutation rates among plastid, mitochondrial, and nuclear DNA (ptDNA, mtDNA, and nucDNA) came from flowering plants (Wolfe et al., 1987). By comparing synonymous-site divergence levels within ptDNA, mtDNA, and nucDNA, Wolfe et al. (1987) showed that the mitochondrial genome mutation rate is about 3 and 10 times lower than that of the plastid and nucleus, respectively. More recent analyses of seed plants have supported these findings (Mower et al., 2007; Sloan et al., 2008, 2012). But we still know very little about the relative mutation rates within plastid-containing protists, which together represent most of the known photosynthetic eukaryotic diversity (Falkowski et al., 2004). This is largely because comparing substitution rates among plastid, mitochondrial, and nuclear genomes requires large amounts of nucleotide sequence data from three different genetic compartments for at least two distinct populations or “species”. Moreover, the two species must be closely enough related that the synonymous-site divergence, which is used as an entrée into the mutation rate (Kimura, 1983), has not reached saturation. These are not trivial requirements when considering that most protist groups are poorly sampled and poorly studied (del Campo et al., 2014).
Relative-rate statistics are lacking for the Glaucophyta, which is a phylum of unicellular freshwater algae. Glaucophytes, red algae, and green plants have plastids that descend directly from the primary endosymbiosis of a cyanobacterium, and together they form the monophyletic supergroup Archaeplastida (Adl et al., 2012). The plastids of glaucophytes (called cyanelles) contain a unique peptidoglycan layer (between the inner and the outer cyanelle membranes) not found in the plastids of other eukaryotes (Pfanzagl et al., 1996; Löffelhardt et al., 1997). Unraveling cyanelle evolution is key to understanding the origin of plastids within the Archaeplastida and whether they derive from a single or multiple primary endosymbiotic events; most data strongly support the former (Price et al., 2012).

The best-studied glaucophyte is the globally distributed, freshwater flagellate Cyanophora paradoxa. All three of its genomes have been sequenced, including the 135.6 kb ptDNA, which has ~180 densely packed genes (Stirewalt et al., 1995); the 51.6 kb mtDNA, which has ~70 genes; and the ~70 Mb nuclear genome, containing ~28,000 genes (Price et al., 2012). By taking advantage of these sequence data and the availability of distinct C. paradoxa geographical isolates from culture collections, we explore, for the first time, relative rates of mutation within the Glaucophyta.

2. Materials and methods

C. paradoxa NIES-763 (obtained from the Microbial Culture Collection at the National Institute for Environmental Studies, Japan) was grown in DY-V medium (Anderssen et al., 1997) at 18 °C (14/10 h light/dark cycle) and harvested at stationary phase. A ~300 μL cellular pellet was submerged in 12 mL of lysis buffer (10 mM Tris–HCl pH 7.6, 10 mM EDTA, 10 mM NaCl), treated with Proteinase K and SDS (final concentrations 30 μg/mL and 0.5%, respectively) for 20 min at 50 °C, vortexed for 1 min with 1.5 mL of 0.5 mm glass beads, then incubated for 1 h at 50 °C. Nucleic acids were extracted once with an equal volume of phenol, followed by phenol/chloroform (1:1), then chloroform, and precipitated using standard ethanol/sodium acetate methods. RNA was removed by the addition of RNase A (25 μg/mL final concentration). Total DNA was sequenced using illumina technology (HiSeq 2000) at the Roy J. Carver Center for Genomics, University of Iowa, giving ~76.5 × 10^6 paired-end reads (length = 100 nt; average insert size = 450 nt).

The C. paradoxa NIES-763 organelle DNA sequences were assembled de novo with Ray v2.2.0 (Boisvert et al., 2010) using k-mers of 21, 27, 31, and 37. The resulting contigs were scanned for mitochondrial and plastid sequences using BLAST and the mitoBASEML of PAML using the HKY85 model.

3. Results and discussion

3.1. Nucleotide substitution rates in Cyanophora paradoxa

Using the maximum-likelihood method (Yang, 2007), we measured nucleotide substitution rates (i.e., pairwise divergence) between two distinct geographical isolates of C. paradoxa: strain NIES-763 (isolated from freshwater in Mitsuakido, Japan, in 1987) and strain CCMP 329 (isolated from freshwater in England, UK, in 1943). The latter strain was previously used to generate the C. paradoxa draft nuclear genome sequence and complete mtDNA sequence (Price et al., 2012), and it is synonymous with strain UTEX LB 555, which was used to sequence the first cyanelle genome (Stirewalt et al., 1995). Our dataset included complete mtDNA and ptDNA gene sets as well as 43 functionally diverse nuclear genes (Supplementary Table S1).

The nucleotide divergence between the two C. paradoxa strains, for all categories of nucleotide site, was lowest for the ptDNA and highest for the mtDNA (Table 1). Within protein-coding regions, the average number of pairwise substitutions per synonymous site (σS) for the mitochondrial genome (5.29 ± 3.2) was ~5.5 times that of the plastid (1.01 ± 1.2). Concatenated protein-coding datasets revealed even greater differences in σS between the mtDNA (~5.0) vs the ptDNA (~0.7) and nucDNA (~0.9) (Table 1).

For the mitochondrial genome, σS differed by more than an order of magnitude (1.5–98.6) among the individual protein-coding genes, with rps4, rps10, and nad6 showing particularly high levels of synonymous substitution (Supplementary Table 1). Synonymous substitution rates for the various plastid- and nuclear-encoded genes were generally much lower than those observed in the mitochondrion, ranging from 0 to 8.1 (ptDNA) and 0.2 to 4.4 (nucDNA) (Supplementary Table S1).

The relative rates of substitution at non-synonymous codon positions (σA) paralleled those observed at synonymous sites. When looking at averages among protein-coding loci, σA for the mtDNA (0.14 ± 0.11) was respectively 5 and 3.5 times that of the ptDNA (0.027 ± 0.04) and nucDNA (0.04 ± 0.03) (Table 1; Supplementary Table S1). The σA/σS ratio, which is often used to gauge the intensity and directionality of natural selection, was similar for the organelle and nuclear genomes, with averages of 0.02 (mtDNA), 0.04 (ptDNA), and 0.06 (nucDNA). This is consistent with strong purifying selection acting on the protein-coding genes in the different genomes (Table 1). The nucleotide substitution rates at tRNA-coding sites were low in all three genetic compartments (<0.1), but were still ~10 times greater for the mtDNA than the ptDNA and nucDNA; a similar trend was also observed for tRNA-coding regions (Table 1).

Together, the data on pairwise divergence between CCMP 329 and NIES-763 suggest that the rate of mutation in the mitochondrial genome of C. paradoxa is respectively 5 and 4 times that of the plastid and nucleus. However, the levels of synonymous substitution for many of the mtDNA-encoded genes, unlike those in the ptDNA and nucDNA, were saturated (Supplementary Table S1). Thus, we may have underestimated σA in the mitochondrial compartment, and the relative mtDNA mutation rate may be even greater than that predicted here.

3.2. Relative rates in the Archaeplastida and beyond

Data on the relative mutation rates among mitochondrial, plastid, and nuclear genomes are slowly accumulating for diverse species. When including the present study, relative-rate statistics now exist for all major archaeplastidal lineages: red algae, green algae, land plants, and glaucophytes (Table 2; Fig. 1). Although
these data are restricted to a small number of taxa, a general trend is emerging: within archaeplastidal species, the mtDNA mutation rate typically exceeds or is similar to that of the ptDNA (Table 2; Fig. 1). The obvious exception to this is seed plants, where the mitochondrial mutation rate is estimated to be approximately a third of that of the plastid (Fig. 1). Recent studies, however, have uncovered some seed plant lineages, including certain Silene species, with exceptionally high mtDNA mutation rates (Mower et al., 2007; Sloan et al., 2008, 2012). Seed plants aside, when looking across the Archaeplastida there is a tendency toward lower rates of mutation between different species or lineages. So the observed exception to this is seed plants, where the mitochondrial mutation rate is estimated to be approximately a third of that of the plastid (Fig. 1). Recent studies, however, have uncovered some seed plant lineages, including certain Silene species, with exceptionally high mtDNA mutation rates (Mower et al., 2007; Sloan et al., 2008, 2012).

### Table 1

<table>
<thead>
<tr>
<th>Substitutions per synonymous site</th>
<th>ptDNA</th>
<th>mtDNA</th>
<th>nucDNA</th>
<th>Substitution rate ratios (pt: mt: nuc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synonymous sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (SD)</td>
<td>1.007 (1.22)</td>
<td>5.288 (3.17)</td>
<td>1.214 (0.83)</td>
<td>1: 5.3: 1.2</td>
</tr>
<tr>
<td>Concatenation</td>
<td>0.667</td>
<td>4.940</td>
<td>0.868</td>
<td>1: 7.4: 1.3</td>
</tr>
<tr>
<td>Nonsynonymous sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (SD)</td>
<td>0.027 (0.04)</td>
<td>0.135 (0.11)</td>
<td>0.040 (0.03)</td>
<td>1: 5: 1.5</td>
</tr>
<tr>
<td>Concatenation</td>
<td>0.024</td>
<td>0.111</td>
<td>0.041</td>
<td>1: 4.6: 1.7</td>
</tr>
<tr>
<td>$dS/dN$ (SD)</td>
<td>0.041 (0.08)</td>
<td>0.022 (0.02)</td>
<td>0.060 (0.03)</td>
<td>–</td>
</tr>
<tr>
<td>rRNAs$^a$</td>
<td>0.007</td>
<td>0.093</td>
<td>0.008</td>
<td>1: 13.3: 1.1</td>
</tr>
<tr>
<td>tRNAs$^a$</td>
<td>0.010</td>
<td>0.076</td>
<td>–</td>
<td>1: 7.6: –</td>
</tr>
</tbody>
</table>

SD: standard deviation; $dS/dN$: ratio of nonsynonymous to synonymous substitutions per site, based on averages of individual loci not concatenated datasets. 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.

$^a$ Average synonymous site substitution rate for mtDNA does not include the following loci, which had extremely high $dS$ values: atp8, nad6, rpl5, rps4, rps7, and rps10. When these loci are included in the analysis, the average $dS$ is ~15.

$^b$ For mtDNA and ptDNA includes the concatenation of all rRNA-coding regions. For nucDNA includes 18S and 26S rRNA-coding regions.

$^c$ Based on concatenation of all rRNA-coding regions. Analysis not performed for nucDNA.

### Table 2

| Synonymous substitution rates in plastid, mitochondrial, and nuclear genomes of various archaeplastidal lineages and the haptophyte genus Phaeocystis. |
|----------------------------------|-------|-------|--------|-----------------------------------|
| Substitutions per synonymous site |       |       |        |                                   |
| Synonymous sites                 |       |       |        |                                   |
| Average (SD)                     | 1.01 (1.22) | 5.29 (3.17) | 1.21 (0.83) | 1: 5.3: 1.2 |
| Concatenation                    | 0.11 (0.06) | 0.17 (0.11) | 0.27 (0.18) | 1: 1.5: 2.5 |
| Nonsynonymous sites              |       |       |        |                                   |
| Average (SD)                     | 0.30 (0.11) | 0.29 (0.05) | 0.37 (0.29) | 1: 1.0: 1.2 |
| Concatenation                    | 0.11 (0.06) | 0.17 (0.11) | 0.27 (0.18) | 1: 1.5: 2.5 |
| $dS/dN$ (SD)                     | 0.041 (0.08) | 0.022 (0.02) | 0.060 (0.03) | – |
| rRNAs$^a$                        | 0.007 | 0.093 | 0.008 | 1: 13.3: 1.1 |
| tRNAs$^a$                        | 0.010 | 0.076 | –     | 1: 7.6: – |

### Data Sources

- **Table 1**: Smith et al. (2012) and earlier results on red algae *Cyanophora* (Table 2; Fig. 1). A similar trend has also been exposed in non-photosynthetic apicomplexan parasites with red-algal plastids (Smith and Keeling, 2012).
- **Table 2**: Data from Popescu and Lee (2007), Hua et al. (2012), Drouin et al. (2008), and Smith et al. (2012).
lineages (Fig. 1). This may suggest that the absolute mutation rates within \textit{C. paradoxa} are high, but this is speculative, as we do not know how long ago CCMP 329 and NIES-763 shared a common ancestor. The number of generations separating these two strains may be much larger than that separating strains (or species) employed in other substitution rate studies, such as \textit{Mesostigma viride} SAG 50-1 vs NIES-296 (Table 2; Fig. 1). Nonetheless, the elevated nucleotide divergence between CCMP 329 and NIES-763, at the very least, implies that these isolates represent distinct populations or “species” of \textit{Cyanophora} (Chong et al., 2014).

### 3.3. Updating our view of organelle genome mutation rates

Next-generation sequencing technologies and improvements in microbial culturing techniques have made it quick and easy to examine the genomes of previously unexplored plastid-bearing lineages. A single run of total DNA (or RNA) from a eukaryotic alga on a high-throughput sequencing platform typically produces enough data to assemble complete mitochondrial and plastid genomes (Smith, 2012, 2013). As a consequence, scientists are now generating, at an unprecedented rate, organelle genome sequences from a wide array of algal groups and are using these data to address a broad range of questions, from understanding the evolution of the malaria parasite (Taylor et al., 2013) to the development of biofuels (Hannon et al., 2010) to tracing the history of ancient Arctic vegetation (Willerslev et al., 2014). Thus, it is crucial that we understand the underlying mutational processes acting on these genomes.

Studies of seed plants have provided a strong foundation for our knowledge of plastid and mitochondrial genetics. But the patterns of organelle genome evolution in seed plants, as we are finding out, do not necessarily reflect those in plastid-bearing protists. If the relative rates of mutation between mitochondrial and plastid genomes for \textit{C. paradoxa} and other plastid-containing protists examined thus far are representative of those in a diversity of microbial eukaryotes then it could have far-reaching implications on how organelle DNA is used for genetic analyses. If it turns out that within most protists the plastid mutation rate is lower than that of the mitochondrion, it would mean that ptDNA is a more suitable genetic marker for broad-scale comparative analyses, such as those attempting to resolve relationships between distantly related species or groups. Conversely, mitochondrial genomes, with their proclivity toward higher rates of mutation, may end up being better “fine-tune” genetic markers, ones that could be used for addressing population-level problems. Whatever the ultimate outcome, there is no denying that the data that are available point toward lower rates of mutation in ptDNA vs mtDNA for a variety algal species.

### Acknowledgements

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### 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.2014.07.001.

### References


