Molecular Phylogenetics and Evolution 65 (2012) 339–344

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

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

Short Communication

Relative rates of evolution among the three genetic compartments of the red alga *Porphyra* differ from those of green plants and do not correlate with genome architecture

David R. Smith [a,*], Jimeng Hua [b], Robert W. Lee [b], Patrick J. Keeling [a]

[a] Canadian Institute for Advanced Research, Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4
[b] Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4R2

ARTICLE INFO

Article history:
Received 8 March 2012
Revised 8 June 2012
Accepted 18 June 2012
Available online 1 July 2012

Keywords:
Genome architecture
Mitochondrial DNA
Mutation rate
Plastid DNA
Substitution rate

ABSTRACT

In photosynthetic eukaryotes, relative silent-site nucleotide substitution rates (which can be used to approximate relative mutation rates) among mitochondrial, plastid, and nuclear genomes (mtDNAs, ptDNAs, and nucDNAs) are estimated to be 1:3:10 respectively for seed plants and roughly equal for green algae. These estimates correlate with certain genome characteristics, such as size and coding density, and have therefore been taken to support a relationship between mutation rate and genome architecture. Plants and green algae, however, represent a small fraction of the major eukaryotic plastid-bearing lineages. Here, we investigate relative rates of mutation within the model red algal genus *Porphyra*. In contrast to plants, we find that the levels of silent-site divergence between the *Porphyra purpurea* and *Porphyra umbilicalis* mtDNAs are three times that of their ptDNAs and five times that of their nucDNAs. Moreover, relative mutation rates do not correlate with genome architecture: despite an estimated three-fold difference in their mutation rate, the mitochondrial and plastid genome coding densities are equivalent – an observation that extends to organisms with secondary red algal plastids. These findings are supported by within-species silent-site polymorphism data from *P. purpurea*.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Knowing the mutation rate is important for understanding evolution, but it is a difficult parameter to estimate (Kondrashov and Kondrashov, 2010). Nonetheless, if synonymous and noncoding nucleotide positions (hereafter called silent sites) are assumed to be neutrally evolving, then the silent-site divergence between species can provide an entrée into mutation rate (Kimura, 1983). This approach has some drawbacks: silent sites can be under selective constraints (Andolfatto, 2005; Hershberg and Petrov, 2008), the silent-site divergence can be saturated (Li, 1997), and the number of generations separating two species can be hard to calculate. Most of these problems can, however, be avoided by looking at more than one type of silent site (e.g., synonymous and intergenic sites), comparing only closely related species, and focusing on relative (as opposed to absolute) rates of silent-site substitution, which removes the need for generation- and divergence-time data.

Studies on relative synonymous-site substitution rates (d_S) of mitochondrial, plastid, and nuclear DNAs (mtDNAs, ptDNAs, and nucDNAs) from green plants have provided insights into mutation rate and genome evolution (Palmer and Herbon, 1988; Lynch, 2007; Leliaert et al., 2012). For most seed plants, the average d_S ratios of mtDNA vs. ptDNA vs. nucDNA are 1:3:10, going up to 1:3:20 in basal angiosperms (Wolfe et al., 1987; Drouin et al., 2008). This contrasts with data from the green algae *Chlamydomonas reinhardtii* and *Chlamydomonas incerta* where all three genetic compartments have similar rates of synonymous-site substitution (Popescu and Lee, 2007) and the same is true for *Mesostigma viride* strains NIES 296 and SAG 50-1 (Hua et al., 2012). These findings, which suggest that seed plants have drastically different mutational patterns than certain green algae, have helped forge the hypothesis that low organelle DNA mutation rates contribute to organelle genome expansion and high rates promote genome contraction (Lynch et al., 2006). Green algae and land plants represent only a small proportion of the major lineages of plastid-bearing organisms, and outside the green lineage little is known about the relative rates of mutation among mitochondrial, plastid, and nuclear genomes, and how they impact organelle genome complexity. This is because the data needed for these types of analyses were, until recently, difficult to generate, requiring nucleotide sequences from three genetic compartments of two closely related species or strains.

Here, we explore relative rates among the organelle and nuclear genomes of *Porphyra* – an ancient red algal lineage, comprising

* Corresponding author. Address: Canadian Institute for Advanced Research, Department of Botany, University of British Columbia, 3529-6270 University Blvd., Vancouver, British Columbia, Canada V6T 1Z4.
E-mail address: smithdr@dal.ca (D.R. Smith).

1055-7903/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2012.06.017

multicellular marine species, some of which are models for studying the evolution of sex and multicellularity (Mumford and Miura, 1988; Blouin et al., 2011). *Porphyra* species, and red algae as a whole, have the most gene-rich and architecturally ancestral ptDNAs from all eukaryotes, and both their plastid and mitochondrial genomes are renowned for containing very little noncoding DNA. Moreover, the lateral spread of plastids between distantly related groups means that many diverse eukaryotes harbour red-algal-derived plastids (Teich et al., 2007; Keeling, 2010; Burki et al., 2012). Relative rate data from *Porphyra* will, therefore, provide a valuable point of comparison with those from other eukaryotic lineages and be useful for addressing hypotheses on organelle genome evolution.

2. Materials and methods

2.1.1. Specimens

Porphyra umbilicalis (P.um.1, UTEX LB 2951) and *P. purpurea* were isolated for the US Department of Energy Joint Genome Institute (DOE JGI) *Porphyra* Genome Project from Schoodic Point, Maine (USA) and Rye, NY (USA), respectively. We also used nucleotide sequence data from an isolate of *P. purpurea* from Avonport, NS (Canada) (Reith and Munholland, 1995; Burger et al., 1999). Northwestern Atlantic *P. umbilicalis* reproduces asexually via neutral spores (Blouin et al., 2007) whereas *P. purpurea* from the Northwest Atlantic normally reproduces via a sexual pathway (Mitman and van der Meer, 1994).

2.2. Organelle and nuclear DNA data

The *P. umbilicalis* mitochondrial and plastid genomes were assembled using Roche 454 (GS FLX Titanium) and Illumina (Genome Analyzer II) DNA sequence data (GenBank Sequence Read Archive accessions SRX030665-78 and SRX030432-36, respectively), generated by the DOE JGI *P. umbilicalis* Genome Project and the National Science Foundation *Porphyra* Genomics Research Collaboration Network (RCN). The Illumina data contained both short (0.3 kilobase; kb) and long (5 kb) insert libraries, allowing us to orient the organellar DNA reads over large genomic distances (e.g., across intergenic regions). The 454 and Illumina reads were mapped onto the *P. purpurea* organelle genomes (GenBank accessions NC_002007 and NC_000925) (Reith and Munholland, 1995; Burger et al., 1999) with the "Assemble to Reference" program from the Geneious v5.5.6 software suite (Biomatters Ltd., Auckland, New Zealand), using a sensitivity setting of "medium" and a fine tuning setting of "some". Approximately 2×10^7 *P. umbilicalis* reads assembled to the *P. purpurea* mtDNA and ptDNA, giving complete coverage with >300-fold redundancy. The "mapped" *P. umbilicalis* reads were reassembled *de novo* (i.e., without the reference) using the Geneious Assembler (medium sensitivity), giving complete *P. umbilicalis* mitochondrial and plastid genome sequences (GenBank accessions JQ388471 and JQ408795) (Fig. 1; Supplementary Fig. 1; Supplementary Table S1). To verify that the mtDNA of *P. umbilicalis*, unlike its *P. purpurea* counterpart, does indeed lack an inverted repeat, we blasted the *P. purpurea* mitochondrial inverted repeat element (which also contains the fragment of a pseudo gene) against all of *P. umbilicalis* sequencing reads. No hits were found. Also, many of the 454 reads for *P. umbilicalis* are >0.3 kb, which is longer than the inverted repeat in question, and should therefore have spanned the entire element, preventing assembly errors. *P. umbilicalis* 454 (GS FLX Titanium) cDNA sequences (GenBank accessions SRX100206-09) confirmed intron locations and the absence of RNA editing in the organelle genomes. Organelle DNA polymorphism data were generated by

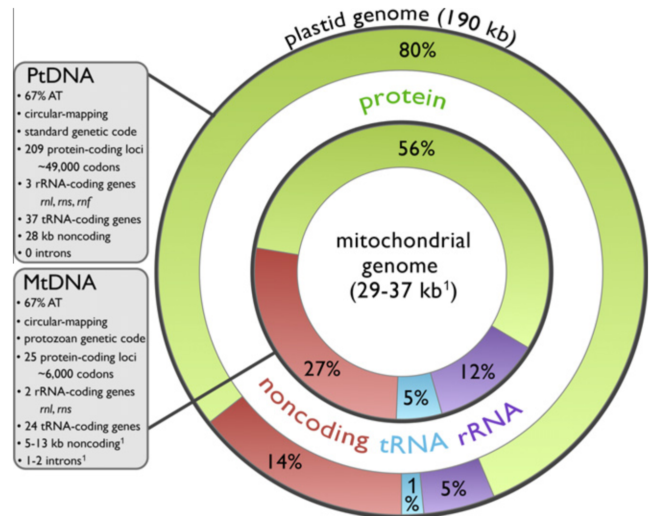

Fig. 1. Organelle genome architecture in *Porphyra*. The coding and noncoding compositions of the *Porphyra* plastid (outer ring) and mitochondrial (inner ring) genomes are shown on the right, and architectural features are boxed in gray on the left. Genome statistics represent averages of the *P. purpurea* and *P. umbilicalis* mtDNAs (GenBank accessions NC_002007 and JQ388471) and ptDNAs (GenBank accession NC_000925 and JQ408795). The different loci within the *Porphyra* organelle genomes are listed in Supplementary Table S1.
[1]Genome size and noncoding content vary because the *P. purpurea* mtDNA has one more intron and ~5 kb more intergenic DNA than the *P. umbilicalis* mtDNA (Supplementary Fig. S1).

mapping *P. purpurea* 454 cDNA sequencing reads (GenBank accessions SRX100229-30) to the *P. purpurea* mitochondrial and plastid genomes, using the same protocols as above. Nuclear transcripts from *P. umbilicalis* and *P. purpurea* were collected from the NoriBLAST databank (Supplementary Table S2) (http://dbdata.rutgers.edu/nori/) – this databank contains filtered, assembled, and annotated EST sequences from *P. purpurea* and *P. umbilicalis*, most of which were generated using the 454 cDNA sequences listed above.

2.3. Nucleotide divergence analyses

DNA loci 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 F3 × 4 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. Intergenic regions <20 nt were ignored. Nucleotide diversity was calculated with DnaSP v5.10.01 (Librado and Rozas, 2009), using the Jukes and Cantor correction.

3. Results and discussion

3.1.1. Nucleotide substitution rates between P. purpurea and P. umbilicalis

We measured substitution rates in the organelle and nuclear genomes between *Porphyra purpurea* and *Porphyra umbilicalis* (Table 1), two closely related species, distributed primarily throughout the North Atlantic (Brodie et al., 2008; Blouin et al., 2011; Sutherland et al., 2011). Our analyses included complete mitochondrial and plastid genomes and ~65 functionally diverse nuclear loci (Fig. 1; Supplementary Tables S1 and S2). *P. purpurea* and *P. umbilicalis* have identical ptDNA gene contents and

Table 1
Nucleotide substitution rates in the mitochondrial, plastid, and nuclear genomes between *Porphyra purpurea* and *Porphyra umbilicalis*.

	Substitutions per site			Substitution rate ratios (mt:pt:nuc)
	mtDNA	ptDNA	nucDNA	
Synonymous sites				
Average (SD)	1.76 (0.58)	0.47 (0.22)	0.43 (0.18)	1:0.27:0.24
Concatenation	1.45	0.46	0.33	1:0.32:0.23
Nonsynonymous sites				
Average (SD)	0.09 (0.08)	0.03 (0.03)	0.01 (0.04)	1:0.30:0.15
Concatenation	0.08	0.03	0.01	1:0.42:0.22
d_N/d_S (SD)	0.05 (0.05)	0.06 (0.08)	0.04 (0.05)	–
Functional RNAs[a]	0.06	0.01	0.07	1:0.23:1.17
Noncoding sites[b]	0.25	0.17	0.15	1:0.68:0.60

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- and tRNA-coding regions. For nucDNA includes *18S* and *28S* rRNA-coding regions.
[b] For mtDNA and ptDNA includes only intergenic sites. For nucDNA includes only 5′ and 3′ untranslated regions.

arrangements, including a 5 kb direct repeat, which contains the rRNA-coding genes, but their mitochondrial genomes, although having the same genes, differ in compactness, with the *P. purpurea* mtDNA containing ~8 kb of noncoding DNA, including five pseudogenes, one intron, and a pair of inverted repeats, that are absent from the *P. umbilicalis* mtDNA (Supplementary Fig. 1). Recombination between the inverted repeats within the *P. purpurea* mtDNA is known to promote major genome rearrangements, and because of this the *P. purpurea* mtDNA is unique among those from red algae for assuming two isomeric conformations (Burger et al., 1999). Our assembly and sequence analyses of the *P. umbilicalis* mtDNA suggest that it has only one conformation, a finding supported by the lack of inverted repeats in this genome.

Nucleotide substitution rates in *Porphyra* are higher in the mitochondrial genome than in the plastid and nuclear genomes. The average number of substitutions per synonymous site between the *P. purpurea* and *P. umbilicalis* genomes was 1.7–9.4 times greater for the mtDNA (1.76 ± 0.58) relative to the ptDNA (0.47 ± 0.22) and nucDNA (0.43 ± 0.18). An almost identical trend was observed when substitution rates were calculated using concatenated datasets (Table 1). The variation in d_S among the different protein-coding loci within each compartment was 0.79–2.76 (mtDNA), 0.09–2.09 (ptDNA), and 0.03–0.86 (nucDNA) (Supplementary Table S1), which again supports a larger overall silent-site substitution rate in the mitochondrial genome. The rates of substitution at nonsynonymous sites (d_N) were low in all three compartments (<0.1), but were, on average, three and nine times greater for the mtDNA relative to the ptDNA and nucDNA. The d_N/d_S ratio, which can be used to gauge the intensity and directionality of selection, was similar across the three genomes, with averages ranging from 0.04 to 0.06 (Table 1; Supplementary Table S1), which is consistent with purifying selection acting on the nonsynonymous sites. Substitution rates at noncoding sites were also highest in the mtDNA, but for both of the organelle DNAs and the nucDNA they were lower than the respective rates at synonymous sites (Table 1), suggesting that the noncoding regions we employed may be under selective constraints. Overall, these results imply that for *P. purpurea* and *P. umbilicalis* the mutation rate of the mitochondrial genome is 3–5 times greater than that of the plastid and nuclear genomes.

3.2.1. Polymorphisms within the P. purpurea organelle genomes

To complement the substitution rate data, we measured the organelle nucleotide diversity between two distinct isolates of *P. purpurea*, one from Nova Scotia, Canada, and the other from Maine, USA (Table 2). Analyses of complete mitochondrial and almost-complete plastid genome sequences revealed four times more silent-site nucleotide diversity (π_{silent}) in the mtDNA than the ptDNA (3.6×10^{-3} vs. 0.9×10^{-3}). A previous report on the *P. purpurea* mitochondrial genome noted similar levels of silent-site mtDNA diversity (~3.5×10^{-3}) (Burger et al., 1999). Nucleotide diversity at nonsynonymous sites was also four times greater in the mitochondrial compartment relative to that of the plastid (1.0×10^{-3} vs. 0.24×10^{-3}).

Although influenced by many forces, π_{silent} can provide a proxy for $2N_g\mu$: twice the effective number of gene copies at a locus times the per-generation, per-site mutation rate (Nei, 1987; Lynch, 2007). Given that both the mtDNA and ptDNA of *Porphyra* are uniparentally inherited – with rare occurrences of biparental inheritance for both mtDNA and ptDNA) (Choi et al., 2008; Niwa et al., 2010) – one may expect them to have a similar N_g. If true, the difference in π_{silent} between the *P. purpurea* organelle genomes could be explained by a 4-fold higher mutation rate in the mtDNA relative to the ptDNA, thus, supporting the findings of the substitution rate analyses described above.

3.3.1. Relative mutation rates in Porphyra: the opposite of land plants

The substitution rate and nucleotide diversity data indicate that for *P. purpurea* and *P. umbilicalis* the mutation rate of the mitochondrial genome exceeds that of its plastid and nuclear counterparts. To the best of our knowledge, this is the first investigation on relative mutation rates among mtDNA, ptDNA, and nucDNA within the red algae. There has been a large and impressive effort to measure nucleotide sequence variation within and between red algal lineages (Saunders and Hommersand, 2004), including *Porphyra* (Lindstrom and Fredericq, 2003; Milstein et al., 2008; Teasdale and Klein, 2010). These studies have excelled at resolving phylogenetic relationships among red algae (Verbruggen et al., 2010; Sutherland et al., 2011), but because most use a single locus from only one or two genetic compartments they could not address the relative rates of mutation between compartments. Nevertheless, most of the studies employing both mitochondrial and plastid loci observed more inter- and intra-specific nucleotide divergence in the mtDNA than the ptDNA, particularly for lineages in the Bangiales, like *Porphyra* (Robba et al., 2006; Milstein et al., 2008), which is in line with the results presented here. Some, however, have found approximately equal or slightly greater divergence in nucDNA than mtDNA or ptDNA: Teasdale and Klein (2010) showed that for various *P. umbilicalis* isolates there was no variation in a 0.3 kb stretch of noncoding mtDNA, but that there was 0–3.7% variation for a 0.7 kb noncoding nucDNA region. Klein et al. (2003) found the interspecific divergence among various species of *Porphyra*,

Table 2
Polymorphisms within the *Porphyra purpurea* mitochondrial and plastid genomes.

	mtDNA				ptDNA			
	N	*S*	Indels	π	*N*	*S*	Indels	π
Ribosomal DNA[a]	3995	5	1	0.00125	4384	4	0	0.00091
Synonymous sites[b]	4944	19	–	0.00385	12,687	18	–	0.00142
Nonsynonymous sites[b]	16,521	17	2	0.00103	41,289	10	2	0.00024
Introns	1581	3	0	0.00190	n/a	n/a	n/a	n/a
Intergenic sites	7183	27	5	0.00376	7551	0	3	0
Silent sites[c]	13,708	49	5	0.00358	20,238	18	3	0.00089

N, number of sites (includes all sites in the nucleotide alignment, including those with indels); *S*, number of polymorphic sites; Indels, insertion–deletion events (indels involving more than one nucleotide are counted as a single event); π, nucleotide diversity; n/a, not applicable.

[a] Includes large- and small-subunit ribosomal RNA-coding regions.

[b] Includes the following genes. MtDNA: *atp6*, *atp8*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *orf176*, *orf544* (intronic ORF), *orf546* (intronic ORF), *rpl16*, *rpl20*, *rps11*, *rps12*, *rps3*, *sdh2*, *sdh3*, *sdh4*, *ymf16*, *ymf39*. PtDNA: *accD*, *apcA*, *apcB*, *apcD*, *apcE*, *apcF*, *atpA*, *atpB*, *atpD*, *atpD*, *atpE*, *atpF*, *atpF*, *atpG*, *atpH*, *atpI*, *chlB*, *chlL*, *chlN*, *clpC*, *cpcB*, *cpeA*, *cpeB*, *dnaK*, *fabH*, *ftsH*, hypothetical protein, *orf199*, *orf203*, *orf75*, *petB*, *petD*, *psaA*, *psaB*, *psaF*, *psaK*, *psbA*, *psbB*, *psbC*, *psbC*, *psbD*, *psbD*, *psbE*, *psbF*, *psbJ*, *psbL*, *psbN*, *psbT*, *psbV*, *psbX*, *rbcL*, *rbcS*, *rpl11*, *rpl13*, *rpl14*, *rpl16*, *rpl18*, *rpl2*, *rpl2*, *rpl21*, *rpl22*, *rpl23*, *rpl23*, *rpl27*, *rpl28*, *rpl29*, *rpl3*, *rpl31*, *rpl36*, *rpl4*, *rpl5*, *rpl6*, *rpoA*, *rpoC2*, *rps11*, *rps12*, *rps13*, *rps17*, *rps19*, *rps19*, *rps2*, *rps3*, *rps5*, *rps8*, *trxA*, *tsf*, *ycf22*, *ycf3*, *ycf32*, *ycf37*, *ycf46*.

[c] Includes synonymous, intergenic and (when applicable) intronic sites.

including *P. purpurea* and *P. umbilicalis*, was 4–14% for the nuclear SSU rRNA gene and 3–11% for the ptDNA-encoded *rbcL* gene.

If, indeed, the relative mutation rates of *Porphyra* are greater in the mtDNA as compared to the ptDNA and nucDNA, it would represent a marked difference from what is observed in green plants (Table 3). For both *Chlamydomonas* and *Mesostigma*, which together represent the two main green algal lineages (the chlorophytes and charophytes, respectively), the rates of silent-site substitution are almost constant (within a factor of two) across the three genetic compartments (Table 3). And for seed plants, with few exceptions (Mower et al., 2007; Sloan et al., 2008), the silent-site substitution rates of mtDNA are, on average, 3- and 10-fold lower than those of the ptDNA and nucDNA (Table 3) – the opposite trend to that of *Porphyra*. There is reason to believe, however, that the relative rates of *Porphyra* organelle genomes may be similar to those of species with secondary plastids derived from red algae. In a comparison of a haptophyte, a stramenopile, and an apicomplexan, the rates of silent-site mtDNA divergence greatly exceeded those of the ptDNA (Smith and Keeling, 2012), paralleling the trend for *Porphyra*, which has a primary red plastid. Altogether, these data suggest that the relative rates of mutation for mtDNA, ptDNA, and nucDNA differ widely across the eukaryotic domain, and that a mtDNA/ptDNA mutation rate ratio of $\geqslant 1$ may be the norm among plastid-bearing protists, with a ratio of <1 being restricted to seed plants.

3.4. Mutation rate and organelle genome architecture

Certain studies have found a positive relationship between mutation rate and coding density within organelle genomes (Lynch et al., 2006; Smith and Lee, 2010) (but see Sloan et al. (2012), and references therein, which note the opposite trend). One explanation for these observations is the mutational burden (or hazard) hypothesis (MBH) (Lynch, 2007), which argues that a higher mutation rate makes for a less permissive environment for the proliferation of noncoding nucleotides, such as repeats and introns. Data on the relative mutation rates of organelle genomes allow for a reasonable test of this hypothesis, at least in these systems. The MBH would predict that the relative silent-site substitution rates of mtDNA and ptDNA should reflect the relative proportion of coding and noncoding nucleotides within these genomes, where a lower relative rate is associated with a larger relative noncoding DNA density (i.e., smaller d_S = more bloated genome) and vice versa. When looking at average values within major groups (Table 3; Supplementary Table S3), the data from green plants support the MBH but those from *Porphyra* and species with secondary red plastids do not (Fig. 2). For seed plants, the mtDNA has on average a smaller d_S and a larger noncoding DNA density than the ptDNA, and in green algae the mtDNA and ptDNA have similar average d_S values and noncoding densities (Fig. 2). Conversely, for *Porphyra* and certain species with secondary red algal-derived plastids, a much larger d_S in the mtDNA is associated with either a slight increase or no change in noncoding density relative to the ptDNA (Fig. 2), which is inconsistent with the MBH. That said, more than half of the noncoding nucleotides within the *Porphyra purpurea* mtDNA appear to have been acquired through a recent lateral DNA transfer event, potentially involving a mitochondrial plasmid (Burger et al., 1999). Moreover, the *P. umbilicalis* mtDNA, which does not harbour plasmid-like sequences (Fig. 1; Supplementary Fig. S1), *is* more compact than the ptDNA (89% vs. 86% coding).

Table 3
Relative synonymous substitution rates among mitochondrial, plastid, and nuclear genomes within various eukaryotic lineages.

	Substitutions per synonymous site			Substitution rate ratios (mt:pt:nuc)
	mtDNA	ptDNA	nucDNA	
Red alga				
Porphyra	1.76 (0.58)	0.47 (0.22)	0.43 (0.18)	1:0.27:0.24
Green algae[a]				
Chlamydomonas	0.29 (0.05)	0.30 (0.11)	0.37 (0.29)	1:1.03:1.28
Mesostigma	0.17 (0.11)	0.11 (0.06)	0.27 (0.18)	1:0.65:1.59
Seed plants[b]				
Angiosperms	0.13 (0.01)	0.39 (0.01)	2.11 (0.09)	1:3:16.23
Gymnosperms	0.28 (0.02)	0.61 (0.03)	1.23 (0.09)	1:2.18:4.39

Synonymous-site substitution rates are based on averages among loci, not concatenations.

[a] Data from Popescu and Lee (2007) and Hua et al. (2012).

[b] Data from Drouin et al. (2008).

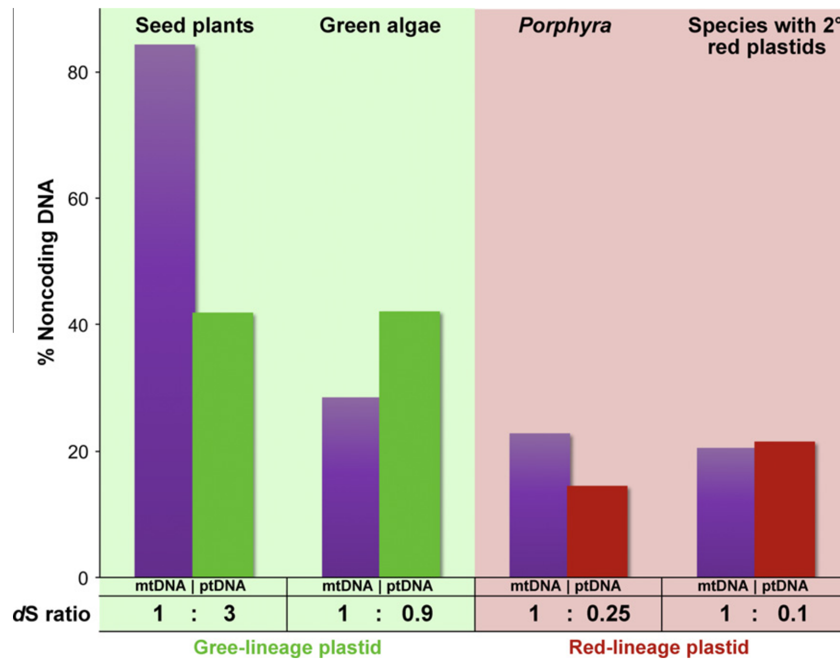

Fig. 2. Relationship between the relative rates of synonymous-site substitution and the relative noncoding DNA density in mitochondrial and plastid genomes from various eukaryotic lineages with green plastids (left, shaded green) and red plastids (right, shaded red). For noncoding DNA content, mitochondrial data are shaded purple and plastid data are shaded green (green plastids) and red (red plastids). The relative rates of synonymous-site substitution (d_S) are shown for each group beneath the bar graph. According to the MBH, mutation rate and noncoding DNA density should be negatively correlated (i.e., a lower relative d_S should result in a larger relative noncoding DNA content), as is seen in the green lineage (shaded light green), but in red algae and species with red-algal derived plastids (shaded light red) the correlation breaks down. The plot was constructed using the genome content data in Supplementary Table S3 and the relative rate data from Table 3, except for those from species with secondary red algal-derived plastids, which come from Smith and Keeling (2012).

When ignoring noncoding density, some features of mtDNA architecture within *Porphyra* and taxa with secondary red plastids are compatible with genome evolution under the MBH: an alternative genetic code, reduced gene content, and smaller genome size in the mtDNA relative to the ptDNA are all consistent with a higher relative rate in the mitochondrial compartment (Lynch et al., 2006; Lynch, 2007).

As more data on relative rates of evolution become available, our understanding of the mutational patterns among mtDNAs, ptDNAs, and nucDNAs across major groups will improve, which will allow us to test how much variation in eukaryotic genome evolution can be explained by the MBH, and how much is derived through other forces. In regards to the data presented here, it will be particularly interesting to see how the relative substitution rates across the genetic compartments of *Porphyra* (and other red algae) compare to those of cryptophyte algae, which have four different genomes, including a red-algal-derived plastid and a highly reduced and compacted nucleomorph, derived from a red algal nucleus.

Acknowledgments

We are grateful to the National Science Foundation (NSF) for their support of the *Porphyra* Research Collaboration Network (David Smith, RCN Member) and for access to the *P. umbilicalis* and *P. purpurea* genome and transcriptome sequencing data, which were generated with the help of the following grants (and PIs): NSF 0741907 (Susan Brawley, Elisabeth Gantt, Arthur Grossman, John Stiller), National Oceanic and Atmospheric Administration (NOAA) #NA06OAR4170108 (S. Brawley), NOAA R/A-38 (Charles Yarish), NSF EF-0626678 (Senjie Lin), DOE JGI Contract DE-AC02-05CH11231 (S. Brawley, E. Gantt, A. Grossman, J. Stiller), NSF 0732440 and Division of Environmental Biology 1004213 (Debashish Bhattacharya). Jimeng Hua is supported by a grant to Robert Lee from the Natural Sciences and Engineering Research Council of Canada (NSERC). Patrick Keeling is funded by a grant from NSERC (227301) and is a Fellow of the Canadian Institute for Advanced Research and a Senior Scholar of the Michael Smith Foundation for Health Research. D. Smith is supported by postdoctoral fellowships from NSERC and the Izaak Walton Killam Trusts.

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.2012.06.017.

References

Andolfatto, P., 2005. Adaptive evolution of non-coding DNA in Drosophila. Nature 437, 1149–1152.
Blouin, N.A., Brodie, J.A., Grossman, A.C., Xu, P., Brawley, S.H., 2011. *Porphyra*: a marine crop shaped by stress. Trends Plant Sci. 16, 29–37.
Blouin, N.A., Xiugeng, F., Peng, J., Yarish, C., Brawley, S.H., 2007. Seeding nets with neutral spores of the red alga *Porphyra umbilicalis* (L.) Kützing for use in integrated multi-trophic aquaculture (IMTA). Aquaculture 270, 77–91.
Brodie, J., Irvine, L., Neefus, C.D., Russell, S., 2008. *Ulva umbilicalis* L. and *Porphyra umbilicalis* Kütz. (Rhodophyta, Bangiaceae): a molecular and morphological redescription of the species, with a typification update. Taxon 57, 1328–1331.
Burger, G., Saint-Louis, D., Gray, M.W., Lang, B.F., 1999. Complete sequence of the mitochondrial DNA of the red alga *Porphyra purpurea*: cyanobacterial introns and shared ancestry of red and green algae. Plant Cell 11, 1675–1694.
Burki, F., Okamoto, N., Pombert, J.F., Keeling, P.J., 2012. The evolutionary history of haptophytes and cryptophytes: phylogenomic evidence for separate origins. Proc. Roy. Soc. B. 279, 2246–2254.
Choi, S.J., Park, E.J., Endo, H., Kitade, Y., Saga, N., 2008. Inheritance pattern of chloroplast and mitochondrial genomes in artificial hybrids of *Porphyra yezoensis* (Rhodophyta). Fisheries Sci. 74, 822–829.
Drouin, G., Daoud, H., Xia, J., 2008. Relative rates of synonymous substitutions in the mitochondrial, chloroplast and nuclear genomes of seed plants. Mol. Phylogenet. Evol. 49, 827–831.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.
Goldman, N., Yang, Z., 1994. A codon-based model of nucleotide substitution for protein-coding DNA sequences. Mol. Biol. Evol. 11, 725–736.

Hershberg, R., Petrov, D.A., 2008. Selection on codon bias. Annu. Rev. Genet. 42, 287–299.
Hua, J., Smith, D.R., Borza, T., Lee, R.W., 2012. Similar relative mutation rates in the three genetic compartments of *Mesostigma* and *Chlamydomonas*. Protist 163, 105–115.
Keeling, P.J., 2010. The endosymbiotic origin, diversification and fate of plastids. Philos. Trans. Roy. Soc. B. 365, 729–748.
Kimura, M., 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge, UK.
Klein, A.S., Mathieson, A.C., Neefus, C.D., Cain, D.F., Taylor, H.A., Teasdale, B.W., West, A.L., Hehre, E.J., Brodie, J., Yarish, C., Wallace, A.L., 2003. Identification of north-western Atlantic *Porphyra* (Bangiaceae, Bangiales) based on sequence variation in nuclear SSU and plastid rbcL genes. Phycologia 42, 109–122.
Kondrashov, F.A., Kondrashov, A.S., 2010. Measurements of spontaneous rates of mutations in the recent past and the near future. Philos. Trans. Roy. Soc. B 365, 1169–1176.
Leliaert, F., Smith, D.R., Moreau, H., Herron, M., Verbruggen, H., Delwiche, C.F., De Clerk, O., 2012. Phylogeny and molecular evolution of the green algae. Crit. Rev. Plant Sci. 31, 1–46.
Li, W.H., 1997. Molecular Evolution. Sinauer, Sunderland, MA.
Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Lindstrom, S.C., Fredericq, S., 2003. RbcL gene sequences reveal relationships among north-east Pacific species of Porphyra (Bangiales, Rhodophyta) and a new species, *P. aestivalis*. Phycol. Res. 51, 211–224.
Lynch, M., 2007. The Origins of Genome Architecture. Sinauer, Sunderland, MA.
Lynch, M., Koskella, B., Schaack, S., 2006. Mutation pressure and the evolution of organelle genomic architecture. Science 311, 1727–1730.
Milstein, D., Oliveira, M.C., Martins, F.M., Matioli, S.R., 2008. Group I introns and associated homing endonuclease genes reveals a clinal structure for *Porphyra spiralis* var. *amplifolia* (Bangiales, Rhodophyta) along the eastern coast of South America. BMC Evol. Biol. 8, 308.
Mitman, G.G., van der Meer, J.P., 1994. Meiosis, blade development, and sex determination in *Porphyra purpurea* (Rhodophyta). J. Phycol. 30, 147–159.
Mower, J.P., Touzet, P., Gummow, J.S., Delph, J.S., Palmer, J.D., 2007. Extensive variation in synonymous substitution rates in mitochondrial genes of seed plants. BMC Evol. Biol. 7, 135.
Mumford, T.F., Miura, A., 1988. *Porphyra* as food: cultivation and economics. In: Lembi, C.A., Waaland, J.R. (Eds.), Algae and Human Affairs. Cambridge University Press, Cambridge, UK, pp. 87–117.
Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, NY.
Niwa, K., Kobiyama, A., Sakamoto, T., 2010. Interspecific hybridization in the haploid blade-forming marine crop *Porphyra* (Bangiales, Rhodophyta): occurrence of allodiploidy in surviving F1 gametophytic blades. J. Phycol. 46, 693–702.
Palmer, J.D., Herbon, L.A., 1988. Plant mitochondrial DNA evolves rapidly in structure, but slowly in sequence. J. Mol. Evol. 28, 87–97.
Popescu, C.E., Lee, R.W., 2007. Mitochondrial genome sequence evolution in Chlamydomonas. Genetics 175, 819–826.
Reith, M., Munholland, J., 1995. Complete nucleotide sequence of the *Porphyra purpurea* chloroplast genome. Plant Mol. Biol. Rep. 13, 333–335.
Robba, L., Russell, S.J., Barker, G.L., Brodie, J., 2006. Assessing the use of the mitochondrial *cox*1 marker for use in DNA barcoding of red algae (Rhodophyta). Am. J. Bot. 93, 1101–1108.
Saunders, G.W., Hommersand, M.H., 2004. Assessing red algal supraordinal diversity and taxonomy in the context of contemporary systematic data. Am. J. Bot. 91, 1494–1507.
Sloan, D.B., Alverson, A.J., Chuckalovcak, J.P., Wu, M., McCauley, D.E., Palmer, J.D., Taylor, D.R., 2012. Rapid evolution of enormous, multichromosomal genomes in flowering plant mitochondria with exceptionally high mutation rates. PLoS Biol. 10, e1001241.
Sloan, D.B., Barr, C.M., Olson, M.S., Keller, S.R., Taylor, D.R., 2008. Evolutionary rate variation at multiple levels of biological organization in plant mitochondrial DNA. Mol. Biol. Evol. 25, 243–246.
Smith, D.R., Keeling, P.J., 2012. Twenty-fold difference in evolutionary rates between the mitochondrial and plastid genomes of species with secondary red plastids. J. Eukaryot. Microbiol. 59, 181–184.
Smith, D.R., Lee, R.W., 2010. Low nucleotide diversity for the expanded organelle and nuclear genomes of *Volvox carteri* supports the mutational-hazard hypothesis. Mol. Biol. Evol. 27, 2244–2256.
Sutherland, J.E., Lindstrom, S.C., Nelson, W.A., Brodie, J., Lynch, M.D.J., Hwang, M.S., Choi, H., Miyata, M., Kikuchi, N., Oliveira, M.C., Farr, T., Neefus, C., Mols-Mortensen, A., Milstein, D., Müller, K.M., 2011. A new look at an ancient order: generic revision of the Bangiales (Rhodophyta) 1. J. Phycol. 47, 1131–1151.
Teasdale, B.W., Klein, A.S., 2010. Genetic variation and biogeographical boundaries within the red alga *Porphyra umbilicalis* (Bangiales, Rhodophyta). Bot. Mar. 53, 417–431.
Teich, R., Zauner, S., Baurain, D., Brinkmann, H., Petersen, J., 2007. Origin and distribution of Calvin cycle fructose and sedoheptulose bisphosphatases in plantae and complex algae: a single secondary origin of complex red plastids and subsequent propagation via tertiary endosymbioses. Protist 158, 263–276.
Verbruggen, H., Maggs, C.A., Saunders, G.W., Gall, L.L., Yoon, H., De Clerck, O., 2010. Data mining approach identifies research priorities and data requirements for resolving the red algal tree of life. BMC Evol. Biol. 10, 16.
Wolfe, K.H., Li, W.H., Sharp, P.M., 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proc. Natl. Acad. Sci. USA 84, 9054–9058.
Yang, Z., 2007. PAML 4: phylogenetic analysis by maximum likelihood. Mol. Biol. Evol. 24, 1586–1591.