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Twenty-Fold Difference in Evolutionary Rates between the Mitochondrial and Plastid Genomes of Species with Secondary Red Plastids[1]

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ABSTRACT. Within plastid-bearing species, the relative rates of evolution between mitochondrial and plastid genomes are poorly studied, but for the few lineages in which they have been explored, including land plants and green algae, the mitochondrial DNA mutation rate is nearly always estimated to be lower than or equal to that of the plastid DNA. Here, we show that in protists from three distinct lineages with secondary, red algal-derived plastids, the opposite is true: their mitochondrial genomes are evolving 5–30 times faster than their plastid genomes, even when the plastid is nonphotosynthetic. These findings have implications for understanding the origins and evolution of organelle genome architecture and the genes they encode.

Key Words. *Babesia bovis*, *Emiliania huxleyi*, genetic diversity, haptophyte, *Heterosigma akashiwo*.

MITOCHONDRIAL and plastid DNAs (mtDNAs and ptDNAs) boast an impressive and often puzzling array of genomic architectures, which has been hypothesized to be a consequence of differing organelle mutation rates (Lynch 2007; Lynch, Koskella, and Schaack 2006). There are, however, limited data on the rates of mtDNA and ptDNA evolution. Nonetheless, if silent nucleotide sites, here defined as noncoding and synonymous sites, are assumed to be neutral, then the silent-site divergence between members from distinct populations, as long as it has not reached saturation, can be used to infer mutation rate (Kimura 1983), but such data have historically been restricted to land plants and green algae.

The relative levels of silent-site divergence between mitochondrial and plastid genomes in land plants and green algae have suggested that the mutation rate of the mtDNA is, with some exceptions (Mower et al. 2007; Parkinson et al. 2005), much lower than or equal to that of the ptDNA (Drouin, Daoud, and Xia 2008; Hua, Smith, and Lee 2011; Wolfe, Li, and Sharp 1987). Indeed, land plant mtDNAs are one of the slowest evolving genomes observed to date. But outside of the green lineage, little is known about the relative or absolute evolutionary rates of mtDNA vs. ptDNA. In particular, no such data exist for species with secondary plastids derived from red algae, which include dinoflagellates, apicomplexans, cryptophytes, haptophytes, and stramenopiles—often collectively referred to as the chromalveolate lineages, although their monophyly is a point of contention (Cavalier-Smith 1999; Delwiche 1999; Keeling 2009). Data from these lineages would provide an important point of comparison with the primary plastids of plants and green algae, particularly given that apicomplexans and dinoflagellates have among the most bizarre organelle genomes characterized to date, but until recently organelle DNA sequences for species with secondary red plastids came from distantly related taxa and were therefore too divergent from one another to measure silent-site substitution rates. New protist genome projects now make it possible to collect, and in some cases assemble, mtDNAs and ptDNAs from closely related chromalveolates and measure their relative rates of silent-site divergence.

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[1]The *Emiliania huxleyi* CCMP1516 mitochondrial and plastid genome sequences have been deposited in GenBank under accession numbers JN022704 and JN022705, respectively.

MATERIALS AND METHODS

We obtained complete mtDNA and ptDNA sequences from two geographically distinct isolates of the unicellular marine haptophyte *Emiliania huxleyi*: CCMP373 (isolated from the North Atlantic in 1960) and CCMP1516 (isolated from the South Pacific in 1991). The organelle DNAs of CCMP373 were downloaded from GenBank (accessions: AY342361 and AY741371) (Sánchez-Puerta, Bachvaroff, and Delwiche 2004, 2005) and those of CCMP1516 were assembled using Sanger sequencing reads produced by the *E. huxleyi* genome project, funded by the U.S. Department of Energy Joint Genome Institute (DOE JGI). Reads were mined from the GenBank Trace Archive, using the CCMP373 organelle DNAs as BlastN (v2.2.25+) queries (default settings), and assembled as described by Smith et al. (2010, 2011), giving complete CCMP1516 mitochondrial and plastid genome sequences with greater than 50-fold coverage (GenBank accessions: JN022704 and JN022705) (see Supplementary Fig. S1 for genetic maps). We assembled complete mtDNA and ptDNA sequences from the *Babesia bovis* C9.1 clonal line (Allred et al. 1994) using Illumina DNA sequence data derived from the *B. bovis* Genome Project, funded by the Wellcome Trust Sanger Institute (GenBank Sequence Read Archive accession number ERX007786) (see Suppl. Fig. 2 for genetic maps and assembly details). Illumina reads were mapped onto the organelle DNAs of the T2Bo isolate of *B. bovis* (GenBank accessions: NC_009902 and NC_011395) with the "Assemble to Reference" program from the Geneious v5.4.4 software suite (Biomatters Ltd., Auckland, New Zealand), using default parameters, a sensitivity setting of "medium" and a fine tuning setting of "some." The C9.1 reads mapped the entire length of the T2Bo organelle genomes with > 200-fold coverage. The *Heterosigma akashiwo* CCMP452 and NIES293 organelle DNAs were downloaded from GenBank (accessions: EU168190–1 and GQ222227–8) (Cattolico et al. 2008; Karol et al. 2010). Nucleotide divergences were calculated with DnaSP v5.10.01 and MEGA5 (Librado and Rozas 2009; Tamura et al. 2011), using the Jukes and Cantor correction, which was chosen instead of a more complex model because all pairs of sequences showed less than 1.5% overall divergence (far from nucleotide saturation). The numbers of synonymous and nonsynonymous sites were estimated using the methods of Nei and Gojobori (1986) with adjustments for changes in the mitochondrial genetic code. Consecutive indels were counted as a single event.

RESULTS AND DISCUSSION

The mitochondrial and plastid genomes of *E. huxleyi* CCMP373 (North Atlantic), which are ~29 and ~105 kilobases

(kb), respectively, are identical in structure, gene content, and gene organization to those of *E. huxleyi* CCMP1516 (South Pacific). When we aligned the CCMP1516 organelle genomes to those of CCMP373, the average number of nucleotide substitutions per site for the mtDNA was 16 times that of the ptDNA (i.e. 7.5×10^{-3} vs. 0.46×10^{-3}, Table 1). For both genomes, > 85% of the polymorphisms were silent, with the average silent-site divergence of the mtDNA being 18 times that of the ptDNA (i.e. 20.6×10^{-3} vs. 1.1×10^{-3}, Table 1), and when only intergenic sites were considered, the mtDNA was 30 times more divergent than the ptDNA (Table 1). The genetic divergence at nonsilent sites (i.e. amino acid replacement, rRNA-, and tRNA-coding positions) was $< 1.5 \times 10^{-3}$ in both compartments, but was 3–10 times greater in the mtDNA than the ptDNA. Among the different protein-coding loci within the mtDNA and ptDNA, the nucleotide substitution levels ranged from $0–10 \times 10^{-3}$ (average = 3.2×10^{-3}) and from $0–7.5 \times 10^{-3}$ (average = 0.4×10^{-3}), respectively.

Recently, mitochondrial and plastid genomes were sequenced and compared from two disparate strains of the unicellular marine stramenopile *H. akashiwo*: CCMP452 isolated from the North Atlantic in 1952 and NIES293 isolated from the North Pacific in 1984 (Cattolico et al. 2008; Karol et al. 2010). Our relative rate analyses of these organelle sequences, which are ~ 39 kb (mtDNA) and ~ 160 kb (ptDNA), revealed similar trends to those observed for *E. huxleyi* (Table 1). For CCMP452 and NIES293, the overall nucleotide divergence within the mtDNA was ~ 6 times that of the ptDNA (i.e. 13×10^{-3} vs. 2.3×10^{-3}); at silent sites, the mtDNA was between 7 and 16 times more divergent than the ptDNA (i.e. 51.3×10^{-3} vs. 7.2×10^{-3} for the intergenic regions and 16.9×10^{-3} vs. 1.1×10^{-3} for synonymous sites, Table 1). As with *E. huxleyi*, diversity at nonsilent sites was low for both the mtDNA and ptDNA of *H. akashiwo*, but was still ~ 3 times greater in the mitochondrial genome. Again, the levels of divergence among the different regions within the mtDNA and ptDNA were relatively constant, but see Cattolico et al. (2008) and Karol et al. (2010) for further discussion.

We were also able to glean interstrain organelle sequence data from the apicomplexan parasite *B. bovis* (Alveolata), which also has a secondary, red algal-derived plastid (Fichera and Roos 1997; Janouškovec et al. 2010), but in this case is an especially interesting comparison because the plastid is nonphotosynthetic and its genome is regarded as rapidly evolving. Complete mtDNA and ptDNA sequences from the *B. bovis* C9.1 clonal line (Allred et al. 1994), which originates from the "Mexico" isolate (Hines et al. 1989), were compared with those of the unrelated "Texas" T2Bo isolate (Goff, Johnson, and Cluff 1998). Surprisingly, not a single polymorphism was found in the ptDNA, which is ~ 35-kb long and has ~ 15 kb of silent sites, whereas for the mtDNA, which is ~ 6-kb long with ~ 3 kb of silent sites, the overall level of genetic divergence was 1.2×10^{-3}, with average synonymous- and amino-acid-replacement-site divergences of 5.5×10^{-3} and 0.8×10^{-3}, respectively (Table 1).

Thus, for *E. huxleyi*, *H. akashiwo*, and *B. bovis*, which represent three distinct and distantly related lineages with secondary red algal plastids, the silent-site divergence of the mtDNA is at least 5–30 times greater than that of the ptDNA (Table 1), suggesting that for each of these taxa, the mutation rate of the mitochondrial genome greatly exceeds that of the plastid genome. To the best of our knowledge, these are three of only a few examples of a plastid genome that is evolving more slowly than its mitochondrial counterpart, contrasting data from the green lineage where the evolutionary rate of mtDNA is almost universally estimated to be lower than or

Table 1. Interstrain genetic divergences of organelle genomes from *Emiliania huxleyi*, *Heterosigma akashiwo*, and *Babesia bovis*, all of which have red algal-derived secondary plastids.

	Emiliania huxleyi CCMP1516 vs. CCMP373				*Heterosigma akashiwo* CCMP452 vs. NIES293				*Babesia bovis* T2Bo vs. C9.1			
	Number of sites (nt)[a]	Number of polymorphic sites	Indels	Nucleotide divergence ($\times 10^{-3}$)[b]	Number of sites (nt)[a]	Number of polymorphic sites	Indels	Nucleotide divergence ($\times 10^{-3}$)[b]	Number of sites (nt)[a]	Number of polymorphic sites	Indels	Nucleotide divergence ($\times 10^{-3}$)[b]
PtDNA												
Full genome	100,521	46	7	0.46	137,595	311	36	2.27	35,104	0	2	0
Synonymous sites	18,485	26	–	1.41	20,943	23	–	1.10	3,804	0	0	0
Nonsynonymous sites	60,376	7	0	0.12	72,258	23	3	0.32	14,372	0	0	0
Structural RNAs[c]	6,586	1	0	0.15	7,017	0	0	0	5,809	0	0	0
Intergenic	15,074	12	7	0.80	37,330	265	33	7.17	11,130	0	2	0
Silent sites[d]	33,559	38	7	1.13	58,273	288	33	4.97	14,934	0	2	0
MtDNA												
Full genome	29,037	215	31	7.51	38,763	503	19	13.03	5,970	7	0	1.17
Synonymous sites	3,757	50	–	13.3	5,815	98	–	16.85	731	4	0	5.49
Nonsynonymous sites	12,737	5	1	0.39	19,547	21	0	1.07	2,434	2	0	0.82
Structural RNAs[c]	6,130	8	0	1.31	6,019	12	0	1.99	636	1	0	1.57
Intergenic	6,413	152	30	25.2	7,413	372	19	51.30	2,169	0	0	0
Silent sites[d]	10,170	202	30	20.6	13,228	470	19	35.97	2,900	4	0	1.38

[a]Comprises all sites in the nucleotide alignment, including those with indels. Consecutive indels were counted as a single indel event. For ptDNA data, loci in the inverted repeat were considered only once. Nonstandard and hypothetical open reading frames were classified as noncoding DNA.
[b]Nucleotide divergence (average number of nucleotide substitutions per site).
[c]Includes rRNA- and tRNA-coding regions.
[d]Noncoding and synonymous sites.

approximately equal to that of the ptDNA (Drouin et al. 2008; Hua et al. 2011; Smith and Lee 2010; Wolfe et al. 1987), with the exception of certain angiosperms with unusual mitochondrial substitution rates (Mower et al. 2007; Parkinson et al. 2005; Sloan et al. 2008).

Nevertheless, when interpreting these data, one should keep in mind that the silent-site divergence between members from separate populations provides an entrée into the mutation rate, but the within-population silent-site diversity—when that population is at mutation-drift equilibrium—reflects the combined effects of mutation *and* genetic drift acting on a genome (Lynch 2007; Nei 1987). Given the large geographical and temporal distances separating the different isolates that we used to measure silent-site divergence, one might expect them to represent members of distinct populations. But even if they are from the same population, it has been shown that within populations, the effective number of genes per locus (N_g), which defines the power of random genetic drift, is similar for mtDNA and ptDNA loci, providing that they both follow the same mode of inheritance (Lynch et al. 2006; Wright et al. 2008). The mtDNA and ptDNA of apicomplexan parasites, like *B. bovis,* are thought to be uniparentally inherited (Creasey et al. 1993, 1994), and although the modes of organelle inheritance for *E. huxleyi* and *H. akashiwo* are unknown, and it is not even known if the latter has sex, in most eukaryotes, including protists, uniparental inheritance of the mitochondrial and plastid genomes is the norm (Birky 1995; Harris 2009; Kuroiwa and Uchida 1996). However, it should be emphasized that our interpretation about differences in evolutionary rates assumes that the pairs of mitochondrial and plastid genomes have the same divergence times, which in turn assumes that they have the same genealogy. If there were opportunities for re-association of organelle haplotypes, it could greatly affect the interpretations of the data presented above.

Finally, the results of this study may help explain certain aspects of the organelle genome architectures of *E. huxleyi*, *H. akashiwo*, and *B. bovis*. These three taxa, and chromalveolates as whole, have mitochondrial genomes that are smaller and more reduced than their plastid genomes, whereas in land plants the opposite is true. This disparity in genomic complexity between the mtDNAs and ptDNAs of land plants is hypothesized to be a consequence of differing organelle DNA mutation rates, where the lower relative mutation rate of the mitochondrial compartment makes for a more permissive environment for the accumulation of excess DNA compared with the plastid compartment (Lynch et al. 2006). Similarly, one could argue that the reverse is occurring in the species studied here: a high mutation rate in mtDNA relative to the ptDNA is resulting in a more reduced mtDNA architecture compared with the plastid genome. It will be interesting to see whether the relative rates of mtDNA vs. ptDNA from other lineages with secondary red plastids show similar trends to those described here.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Genetic maps and assembly methods of the *Emiliania huxleyi* CCMP1516 mitochondrial (inner; 28.7 kb) and plastid (outer; 105.3 kb) genomes—GenBank accession numbers JN022704 and JN022705, respectively. Genomes were assembled using Sanger sequencing reads produced by the DOE JGI *E. huxleyi* genome project. Reads were data-mined from the GenBank Trace Archive and assembled as previously described (Smith et al. 2011, *PLoS ONE* 6:e23624).

Fig. S2. Genetic maps and assembly methods of the *Babesia bovis* C9.1 mitochondrial (inner; linear molecule; ~ 6 kb) and plastid (outer; ~ 35 kb) genomes. Genomes were assembled using Illumina DNA sequence data derived from the Wellcome Trust Sanger Institute *B. bovis* Genome Project (GenBank Sequence Read Archive accession number ERX007786); reads were aligned to the *B. bovis* T2Bo mitochondrial and plastid genomes (GenBank accession numbers NC_009902 and NC_011395, respectively) using Geneious v5.4.4 (Biomatters Ltd., Auckland, New Zealand).

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