The Journal of
Eukaryotic Microbiology

Published by the International Society of Protistologists

J. Eukaryot. Microbiol., ■■(■■), pp. 1–3

DOI: 10.1111/j.1550-7408.2011.00569.x

Nucleotide Diversity of the Colorless Green Alga *Polytomella parva* (Chlorophyceae, Chlorophyta): High for the Mitochondrial Telomeres, Surprisingly Low Everywhere Else*

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ABSTRACT. Silent-site nucleotide diversity data (π_{silent}) can provide insights into the forces driving genome evolution. Here we present π_{silent} statistics for the mitochondrial and nuclear DNAs of *Polytomella parva*, a nonphotosynthetic green alga with a highly reduced, linear fragmented mitochondrial genome. We show that this species harbors very little genetic diversity, with the exception of the mitochondrial telomeres, which have an excess of polymorphic sites. These data are compared with previously published π_{silent} values from the mitochondrial and nuclear genomes of the model species *Chlamydomonas reinhardtii* and *Volvox carteri*, which are close relatives of *P. parva*, and are used to understand the modes and tempos of genome evolution within green algae.

Key Words. *Chlamydomonas*, Chlorophyte, genetic diversity, mitochondrial DNA, *Volvox*.

THE nucleotide diversity at silent sites (π_{silent}), which include noncoding positions and the synonymous sites of protein-coding DNA, can provide insights into the combined contributions of genetic drift and mutation acting on a genome—parameters that are arguably among the key determinants driving the evolution of genomic architecture (Lynch 2007). Smith and Lee (2008, 2009, 2010) measured genetic diversity of the model green algae *Chlamydomonas reinhardtii* and *Volvox carteri* and found that π_{silent} is moderate to high for the organelle and nuclear genomes of *C. reinhardtii* but unprecedentedly low for those of *V. carteri*. These findings helped explain why the organelle and nuclear DNAs of *V. carteri* are more expanded and embellished than those of *C. reinhardtii* (Smith and Lee 2010).

We present nucleotide diversity data from the mitochondrial and nuclear compartments of the unicellular, nonphotosynthetic green alga *Polytomella parva*. This species is an excellent candidate for diversity studies because it is closely related to *C. reinhardtii* and *V. carteri* (Nakada, Misawa, and Nozaki 2008), allowing for meaningful comparisons of the π_{silent} data, and because it boasts an atypical mitochondrial genome. Indeed, the *P. parva* mitochondrial DNA (mtDNA) is fragmented into two linear chromosomes of 13 and 3 kb (Fan and Lee 2002), both of which have 1.3-kb hairpin-loop telomeres (Smith, Hua, and Lee 2010), and it contains only 10 genes.

MATERIALS AND METHODS

Polytomella strains were obtained from The Culture Collection of Algae at the University of Göttingen (SAG, Göttingen, Germany) in April of 2010. Cultures were made axenic and grown as described by Mallet and Lee (2006). Total cellular DNA was extracted, amplified, and sequenced using the methods described for *V. carteri* in Smith and Lee (2010). Nucleotide diversity was calculated with DnaSP v5.10.01 (Librado and Rozas 2009). The mtDNA and nucDNA sequence data used to measure nucleotide diversity within the *P. parva* lineage are deposited in GenBank under accession numbers HQ585000-HQ585014 and HQ584989-HQ584998, respectively.

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*Sequence data from this article have been deposited in GenBank under accession numbers HQ584989-HQ585014.

RESULTS AND DISCUSSION

For measuring nucleotide diversity we used the following *Polytomella* SAG strains, with origins of isolation listed in brackets: SAG 63-2 (Glasgow, Scotland), 63-2b (Bohemia, Czech Republic), 63-3 (Shelford, UK), 64-4 (Grantchester, UK), and 198.80 (unknown). From each isolate we sequenced ~8.5 kb of mtDNA and ~3 kb of nucDNA. The sequenced mtDNA regions encompassed 7 kb of coding DNA and ~1.5 kb of noncoding DNA, giving 2.8 kb of silent sites for our nucleotide diversity analyses. The nucDNA sequence data included ~2 kb of exons and ~1 kb of introns, from *cox3* and the 18S rRNA gene, providing 1.1 kb of silent sites.

The only nucleotide polymorphisms within the sequenced mtDNA and nucDNA regions were in the mitochondrial telomeres (the 1.3 kb gene-less regions at the ends of the two mitochondrial chromosomes), which contained 49 segregating sites ($\pi_{telomere} = 17.5 \times 10^{-3}$) (Table 1). This was ~2.3 times more nucleotide diversity than was observed for the *C. reinhardtii* mitochondrial telomeres (Smith and Lee 2008) (Table 1). As the *V. carteri* mtDNA assembles as a circular molecule, no telomeres were identified (Smith and Lee 2010). When all types of silent sites were considered, including telomeric, synonymous, and intergenic positions, the overall value of π_{silent} for the *P. parva* lineage mitochondrial compartment was 7×10^{-3}, which is similar to that of *C. reinhardtii* ($\pi_{silent} = 8 \times 10^{-3}$) and more than an order of magnitude greater than that of *V. carteri* ($\pi_{silent} = 0.4 \times 10^{-3}$). However, all of the silent-site variation came from the mitochondrial telomeres for the *P. parva* lineage, whereas for *C. reinhardtii* and *V. carteri* the levels of mtDNA and nucDNA diversity were relatively constant across different types of silent sites (Smith and Lee 2008, 2010). Among the five *P. parva* lineage strains, the Czech isolate (SAG 63-2b) had the most divergent mtDNA sequence. When it was removed from our diversity analyses, $\pi_{telomere}$ and π_{silent} dropped to 2.5×10^{-3} and 0.8×10^{-3}, respectively.

When a population is at mutation-drift equilibrium, π_{silent} should approximate $2N_g\mu$ (i.e. twice the effective number of genes per locus in the population times the mutation rate per nucleotide site per generation) (Lynch 2007). Therefore, the general absence of genetic diversity within the mtDNA and nucDNA of the *P. parva* lineage isolates may reflect a low mutation rate and/or a small N_g in both of these compartments. Little is known about the mitochondrial or nuclear mutation rates within the various *Polytomella* lineages, but one might expect *Polytomella* species to have a low N_g. Based on earlier descriptions, many *Polytomella* taxa, including those from the *P. parva* lineage, inhabit putrid ditches that are prone to drying out. Under such conditions, the survival and propagation of *Polytomella* is dependent on its ability to form a resisting cyst that can withstand desiccation (Pringsheim

Table 1. Silent-site nucleotide diversity estimates for isolates of the *Polytomella parva* lineage compared with those of *Chlamydomonas reinhardtii* and *Volvox carteri*.

	Pp		*Cr*		*Vc*	
	mtDNA	nucDNA	mtDNA	nucDNA	mtDNA	nucDNA
Synonymous, intergenic, and intronic sites						
N	1,692	1,100	4,602	5,051	2,984	6,519
S	0	0	85	406	7	104
Indels	0	0	9	48	0	12
$\pi \times 10^{-3}$	0	0	8.72	32.29	0.38	5.28
SD $\times 10^{-3}$	NA	NA	1.45	(3.01)	(0.17)	(3.24)
Telomeric sites[a]						
N	1,175	ND	948	ND	NA	ND
S	49	ND	19	ND	NA	ND
Indels	8	ND	2	ND	NA	ND
$\pi \times 10^{-3}$	17.55	ND	7.59	ND	NA	ND
SD $\times 10^{-3}$	9.00	ND	1.95	ND	NA	ND

[a]The *P. parva* lineage isolates and *C. reinhardtii* have 4 and 2 mitochondrial telomeres, respectively; the data shown are for 1 telomere.

Pp, *Polytomella parva* lineage; *Cr*, *Chlamydomonas reinhardtii*; *Vc*, *Volvox carteri*; *N*, number of sites (comprises all sites in the nucleotide alignment, including those with indels); *S*, number of segregating sites (i.e. polymorphic sites); Indels, insertion–deletion events (indels involving more than one nucleotide are counted as a single event); π, nucleotide diversity; SD, standard deviation of π; NA, not applicable; ND, not determined. Note, the nucleotide diversity data for *C. reinhardtii* and *V. carteri* come from Smith and Lee (2008, 2009, 2010). All estimates of genetic diversity and its standard deviation were calculated with DnaSP v5.10.01 (Librado and Rozas 2009).

1955). Their small ephemeral habitat and the necessity of encystment could predispose *Polytomella* species to relatively small effective population sizes and genetic bottlenecks, two factors that can lower N_g and thereby reduce π_{silent} (Lynch 2007, Chapter 4). Given the relatively compact nature of the *P. parva* mitochondrial genome, the mutational-hazard hypothesis (Lynch 2007) would have forecasted it to have a high degree of silent-site nucleotide diversity, which could suggest that the streamlined mtDNA in *P. parva* is the result of selection for rapid growth (but see p. 35 in Lynch 2007): *P. parva* can achieve a mean generation time of about 4.7 h at 25 °C (Sheeler, Cantor, and Moore 1970).

But why was the genetic diversity so much higher for the mitochondrial telomeres of *P. parva* as compared with the other mtDNA regions? High levels of telomeric polymorphism have been observed in both the nuclear (Broun, Ganal, and Tanksley 1992; Cohn, McEachern, and Blackburn 1998; McCormick-Graham, Haynes, and Romero 1997) and mitochondrial genomes (Morin and Cech 1988) from other species, and it has been suggested that this heterogeneity plays an important role in telomere evolution (Tomaska and Nosek 2009). The ends of linear chromosomes, particularly telomeres, tend to have higher recombination rates than more centrally located chromosomal regions (Eichler and Sankoff 2003; See et al. 2006), and because telomeres are repetitive elements, they can recombine with one another promiscuously. Moreover, one of the primary strategies for the maintenance and lengthening of telomeres in telomerase-independent systems, such as mitochondria, is through recombination (Tomaska and Nosek 2009). These points are significant because recombination rates are often positively correlated with genetic diversity, substitution rates, and mutation rates (Eyre-Walker 1993; Hellmann et al. 2003; Nachman 2001), and they also tend to scale positively with N_g (Lynch 2007). Furthermore, the ends of linear chromosomes are prone to exonucleolytic degradation—a feature that would presumably increase their mutation rate relative to more interiorly positioned loci (Nosek, Tomaska, and Kucejová 2004).

If elevated frequencies of recombination are largely responsible for the high values of π_{silent} in the mitochondrial telomeres as compared with the internal mtDNA regions in *P. parva*, then one might have expected a parallel trend for the *C. reinhardtii* mtDNA. We found, however, that π_{silent} values in the telomeric and nontelomeric mtDNA of this species were similar to each other (Table 1), suggesting that one or more other important contributing factors might be involved in the elevated π_{silent} of the *P. parva* telomeric mtDNA. It may be significant, in this connection, that the mtDNA of *P. parva* is fragmented into two subgenomic elements whereas that of *C. reinhardtii* is a monomeric linear molecule. Thus, the number of telomeres per mtDNA haplotype in *P. parva* should be twice that of *C. reinhardtii* and 4 times the number for nontelomeric mtDNA loci in each of these algae. The proportion of telomeric mtDNA in *P. parva* could be even greater if additional small putative mtDNA elements identified in *P. parva* are contributing telomeres to the mtDNA haplotype—a situation that is probable given that these elements hybridize with telomeric mtDNA in Southern-blot analyses (Mallet and Lee 2006). Ultimately, the potential for the mitochondrial telomeres having both a disproportionately high effective number of copies (a high N_g) in the *P. parva* population and increased levels of mutation-prone recombination (due to a high copy number per cell) could result in elevated levels of π_{silent}.

ACKNOWLEDGMENTS

We thank Jimeng Hua for helpful discussion. This work was supported by a grant to RWL from the Natural Sciences and Engineering Research Council (NSERC) of Canada. D.R.S. was an Izaak Walton Killam Memorial Scholar and held a Canada Graduate Scholarship from NSERC.

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Received: 03/22/11. 05/19/11; accepted: 06/03/11