Organellar Inheritance in the Green Lineage: Insights from Ostreococcus tauri
Romain Blanc-Mathieu, Sophie Sanchez-Ferandin, Adam Eyre-Walker, Gwenael Piganeau

To cite this version:
Romain Blanc-Mathieu, Sophie Sanchez-Ferandin, Adam Eyre-Walker, Gwenael Piganeau. Organellar Inheritance in the Green Lineage: Insights from Ostreococcus tauri. Genome Biology and Evolution, Society for Molecular Biology and Evolution, 2013, 5 (8), pp.1503-1511. <10.1093/gbe/evt106>. <hal-01579089>

HAL Id: hal-01579089
https://hal.sorbonne-universite.fr/hal-01579089
Submitted on 30 Aug 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
Organellar Inheritance in the Green Lineage: Insights from Ostreococcus tauri

Romain Blanc-Mathieu1,2,*, Sophie Sanchez-Ferandin1,2, Adam Eyre-Walker3, and Gwenael Piganeau1,2

1UPMC Univ Paris 06, UMR7232, Observatoire Océanologique, Banyuls-sur-Mer, France
2CNRS, UMR 7232, Observatoire Océanologique, Banyuls-sur-Mer, France
3School of Life Sciences, Sussex University, Falmer, United Kingdom

*Corresponding author: E-mail: blancmathieu@obs-banyuls.fr.
Accepted: July 9, 2013

Data deposition: The thirteen complete mtDNA and cpDNA genome sequences obtained from this study have been deposited at GenBank under the accession numbers KC967294–KC967306 for the mtDNA and KC990831, KF285522–KF285533 for the cpDNA.

Abstract

Along the green lineage (Chlorophyta and Streptophyta), mitochondria and chloroplast are mainly uniparentally transmitted and their evolution is thus clonal. The mode of organellar inheritance in their ancestor is less certain. The inability to make clear phylogenetic inference is partly due to a lack of information for deep branching organisms in this lineage. Here, we investigate organellar evolution in the early branching green alga Ostreococcus tauri using population genomics data from the complete mitochondrial and chloroplast genomes. The haplotype structure is consistent with clonal evolution in mitochondria, while we find evidence for recombination in the chloroplast genome. The number of recombination events in the genealogy of the chloroplast suggests that recombination, and thus biparental inheritance, is not rare. Consistent with the evidence of recombination, we find that the ratio of the number of nonsynonymous to the synonymous polymorphisms per site is lower in chloroplast than in the mitochondria genome. We also find evidence for the segregation of two selfish genetic elements in the chloroplast. These results shed light on the role of recombination and the evolutionary history of organellar inheritance in the green lineage.

Key words: organellar inheritance, population genomics, recombination, effective population size, picoeukaryotes, large indel polymorphisms.

Introduction

Early in the last century, Baur (1909) and Correns (1909) independently observed that leaf color patterns did not follow Mendelian inheritance rules in Mirabilis and Pelargonium cultivars, paving the way for future studies of cytoplasmic and organellar inheritance. After the identification of DNA as the purveyor of genetic information, early studies in the unicellular model green alga Chlamydomonas provided evidence that chloroplast (Sager and Lane 1972; Sager and Ramanis 1963) and mitochondrial (Boynton et al. 1987) genomes (hereafter cpDNA and mtDNA) are transmitted to all progeny from one parent only. Since, the mode of organelle inheritance has been investigated in many groups (fig. 1); within the Chlorophyta, uniparental inheritance of both mtDNA and cpDNA has been reported in the Ulvophyceae, Chlorophyceae, and the Nephroselmisphycyeae (Miyamura 2010). Uniparental inheritance of cpDNA has also been reported in the Trebouxiophyceae (Miyamura 2010). Within the Streptophytes, extensive literature exists for land plants and converges to the overall conclusion that their organelles are mainly maternally transmitted with the exception of the cpDNA that is mainly paternally transmitted in the Gymnosperms (Birky 1995). In the Charales and Zygmenatales, two groups of aquatic Streptophytes, the cpDNA is uniparentally inherited (Miyamura 2010). However, although mtDNA and cpDNA are uniparentally inherited in Chlamydomonas, under UV radiation biparental inheritance and recombination can be induced (Sager and Ramanis 1967) providing evidence that Chlamydomonas has the machinery for recombination and chloroplast fusion. Furthermore, indirect evidence of recombination has been observed in natural populations from population genetic signatures of homologous recombination between diverged...
haplotypes in a few species. It has been detected in the mtDNA of *Silene acaulis* (Städler and Delph 2002), and *S. vulgaris* (Houlston and Olson 2006) and in the cpDNA of *Pinus concentra* (Marshall et al. 2001) and *Cycas taitungensis* (Huang et al. 2001). The frequency of recombination could not be estimated from these observations and biparental inheritance has been suggested to be rare (Houlston and Olson 2006). In summary, although exceptions to uniparental inheritance have been reported in some of these groups, the literature indicates that organelles are mainly transmitted asexually, usually from only one parent, generally the mother in anisogamous species, or the mt+ mating type in isogamous species, such as *Chlamydomonas* (fig. 1). One might therefore infer that organelle inheritance of the most recent common ancestor of the green lineage was uniparental, and was therefore evolving asexually. However, we lack information from deep branching lineages such as Mesostigmatophyceae (Streptophyta) and Mamiellophyceae (Chlorophyta) to support this hypothesis.

Here, we tested for evidence of recombination and estimated its frequency in the complete chloroplast and mitochondrial genomes of the early diverged unicellular green algae, *Ostreococcus tauri* (Chlorophyta, Mamiellophyceae), using population genetic tests of recombination. These tests can detect homologous recombination between the mutations that have occurred in the genealogy of the sequences.

Although, *O. tauri* is a haploid species, several indirect lines of evidence suggest that its nuclear genome undergoes recombination, which suggest that its life cycle includes a sexual stage. First, it harbors coding sequences with high similarity to several core meiotic genes (Derelle et al. 2006). Second, evidence for recombination has been detected from a population genetic data set of eight nuclear intergenic regions from two chromosomes (Grimsley et al. 2010). And third, the chromosomal GC content is negatively correlated with chromosomes size (Jancek et al. 2008), as expected if there is biased gene conversion, a process that is the result of meiotic crossing overs (Duret and Galtier 2009).

In *O. tauri*, there is one single mitochondrial and chloroplast per cell. Their genomes are circular chromosomes, 44- and 72-kb long, respectively. Both genomes contain an internal segmental duplication, and contain 43 and 61 protein-coding sequences, representing 69% and 64% of the genome, respectively (Robbens et al. 2007). We have combined Illumina Paired End (PE) sequencing data and targeted polymerase chain reaction (PCR) sequencing to obtain the genome-wide polymorphisms from mitochondria and chloroplast from thirteen strains of *O. tauri* sampled from the North-West Mediterranean sea (Grimsley et al. 2010). We used these data to test for indirect evidence of homologous recombination and estimate its frequency.

### Materials and Methods

#### Data

We analyzed data from 13 *O. tauri* strains that had been sampled from the North-West Mediterranean sea and have been described previously (Grimsley et al. 2010). These strains were established from clonal culture and identified as *O. tauri* based on 100% identity in the 18S encoding DNA. Their whole nuclear and cytoplasmic genomes have been sequenced using Illumina GA at the Joint Genome Institute (http://www.jgi.doe.gov/, Community Sequencing Program CSP-129). We aligned the PE-reads data sets (76 bp for the strains RCC1108, RCC1114, RCC1115, RCC1116, RCC1558, RCC1559, and RCC745 and 101 bp for the strains RCC1110, RCC1112, RCC1117, RCC1118, RCC1123, and RCC1561) against the reference chloroplast (GenBank accession: CR954199.2) and mitochondrial (CR954200.2) genome sequences using the Burrow–Wheeler Aligner (BWA) with parameters $n = 6$, $l = 35$, $k = 3$, and $e = 3$ (Li and Durbin 2009). We screened these 13 alignments with in-house C programs to look for single nucleotide (SNPs) and insertion–deletion (indels) polymorphisms. To call an SNP or an indel polymorphism, the coverage had to be a minimum 10×. Because these strains are haploid, the frequency of the variant at this position had to be $\geq 0.9$. Large deletion polymorphisms (relative to the reference) were detected by looking for regions with zero-coverage on the reference genome sequence. The presence of large insertions (relative to reference) was suggested by the presence of nonaligned sequences against the reference genome sequence in DNAdiff report files (see [fig. 1](#)).

![fig. 1](#)

**Fig. 1.**—Organellar inheritance along the green lineage. The phylogeny is simplified from Rodríguez-Ezpeleta et al. (2007) and De Clerck et al. (2012). Modes of organellar inheritance are reviewed in Miyamura (2010) and Birky (1995).
next paragraph) (supplementary table S1, Supplementary Material online). Although large deletion polymorphisms are easily inferred from zero-coverage regions of the reference genome sequence, large insertions cannot be directly inferred from BWA alignments and require further sequencing (PCR). Alignment of all 13 chloroplasts genomes (see next paragraph) using clustalw2 (gap extension lowered to 0.05) (Larkin et al. 2007) allowed us to characterize three large indel polymorphisms from three zero-coverage regions, named X, Y, and Z. Regions Y and Z are located at the edge of the internal duplication of the cpDNA. Because the assembly did not cover the whole insertion for one of the strains and to check our indel predictions, chloroplast regions X, Y, and Z were amplified by PCR and sequenced (primers available in supplementary table S2, Supplementary Material online). We used chloroplast regions Y and Z and one mitochondrial region to validate 22, 57, and 9 SNPs, respectively (supplementary table S2, Supplementary Material online).

De Novo Assembly of Organelles Genomes

Genomes were assembled de novo from the PE-reads data sets of each strain using Abyss (Simpson et al. 2009) with kmer = 41, 10 PE-reads to join two contigs into one scaffold, and additional parameters were kept as default. To extract organellar contigs, we blasted them against the chloroplast and mitochondrial genomes, using thresholds of 95% nucleotide identity over a minimum of 500 bp. We ended with Z to 10 contigs for the mitochondria and with 2 to 7 contigs for the chloroplast (supplementary table S1, Supplementary Material online). We then aligned de novo contigs onto the reference genome sequence, large insertions cannot be directly inferred from zero-coverage regions of the reference genome sequence, large insertions cannot be directly inferred from BWA alignments and require further sequencing (PCR). Alignment of all 13 chloroplasts genomes (see next paragraph) using clustalw2 (gap extension lowered to 0.05) (Larkin et al. 2007) allowed us to characterize three large indel polymorphisms from three zero-coverage regions, named X, Y, and Z. Regions Y and Z are located at the edge of the internal duplication of the cpDNA. Because the assembly did not cover the whole insertion for one of the strains and to check our indel predictions, chloroplast regions X, Y, and Z were amplified by PCR and sequenced (primers available in supplementary table S2, Supplementary Material online). We used chloroplast regions Y and Z and one mitochondrial region to validate 22, 57, and 9 SNPs, respectively (supplementary table S2, Supplementary Material online).

Phylogenetic Reconstruction

We used three different methods to reconstruct the phylogenies of the mitochondrial and chloroplast genomes of our samples. Evolutionary models with appropriate parameters were searched out of 24 models using Mega5 tool (Tamura et al. 2011). It came out with the TN93 (Tamura and Nei 1993) substitution model with a proportion of invariant sites for cpDNA and the HKY (Hasegawa et al. 1985) model for the mtDNA. Transitions to transversions ratio were estimated to be 1.43 for the cpDNA and 4.92 for mtDNA. Three different methods were used for the phylogeny reconstructions: the neighbor-joining (NJ) method with the above substitution models for each data set, the maximum parsimony (MP) method with SPR search method and the maximum likelihood approach (PhyML trees) (Guindon and Gascuel 2003) with the above mentioned substitution models for each data set as implemented in Geneious (Geneious version 6.1.3 created by Biomatters; available from http://www.geneious.com/). For all phylogenetic analyses, branch supports were computed over 500 bootstraps. We used Kishino–Hasegawa (KH) and Shimodaira–Hasegawa (SH) tests implemented in PAUP 4.0 beta 10 Win (Swofford 2002), both with parsimony or
likelihood criteria, to test the null hypothesis of no difference between the two maximum likelihood topologies obtained from cpDNA and mtDNA.

Testing Recombination and Its Prevalence

We tested for recombination in both mitochondrial and chloroplast genomes. We excluded duplicated regions for both genomes and additionally excluded a region with a polymorphism hotspot in the chloroplast. This is because rate variation among sites generates homoplasies that may increase the false positive rate of recombination detection methods (Sun et al. 2011). This left us with 71 SNPs in the mitochondria and 203 SNPs in the chloroplast. Indirect test of recombination were performed using five different tests of recombination: 1) \( \chi^2 \) test of Maynard Smith (1992) with improvements suggested by Piganeau et al. (2004); 2) \( \Phi_W \), or pairwise homoplasy index (Bruen et al. 2006); 3) the correlation between linkage disequilibrium, as measured by \( r^2 \) (Hill and Robertson 1968) or \( D' \) (Lewontin 1964), and distance (Spearman correlation); and 4) \( p_{LDhat} \) estimated using a composite-likelihood coalescent-based method implemented in LDhat (McVean et al. 2002). Max \( \chi^2 \) and \( D' \) are used as described in Piganeau et al. (2004).

Max \( \chi^2 \) and \( \Phi_W \) are structure based tests that estimate the probability of observing a mosaic haplotype structure under the null hypothesis of no recombination. The two LD-based methods test whether there is a significantly negative correlation between linkage disequilibrium and distance (which is expected when recombination is operating in a genome).

The coalescent method LDhat estimates the parameter \( p_{LDhat} = 2N_e r \), where \( N_e \) is the effective population size and \( r \) is the genetic map distance across the region analyzed; it gives the number of recombination events that occurred in the genealogy of the sample. We ran LDhat with the gene conversion model and an average track length of 500 bp. LDhat tests the null hypothesis of no recombination (\( 2N_e r = 0 \)) using the likelihood permutation test described in McVean et al. (2002).

Polymorphism Analysis

The amount of polymorphisms was measured as the nucleotide diversity \( \pi \) (Nei and Li 1979).

\[
\pi = \frac{n}{n-1} \sum_{i=1}^{L} 2f_i(1-f_i)
\]

where \( f_i \) is the frequency of the polymorphism at site \( i \) and \( n \) the number of strains. We measured \( \pi \) at nonsynonymous, synonymous, 4-fold degenerated, and intergenic sites (\( \pi_N, \pi_S, \pi_{4-fold}, \) and \( \pi_i \)), using all segregating sites for each of these four categories (\( P_N, P_S, P_{4-fold}, \) and \( P_i \)) using an in-house C code program (available from the authors upon request).

Results

What Is the Level of Genetic Diversity in the Organellar Genomes of O. tauri?

From the alignment of Illumina PE-reads, we obtained an average observed coverage between 435× and 5882× for the chloroplast DNA and 359× and 4697× for the mitochondrial DNA. We found 135 (133 bi-allelic and 2 tri-allelic) and 318 (314 bi-allelic and 4 tri-allelic) SNPs in the mtDNA and cpDNA, respectively (supplementary tables S4 and S5, Supplementary Material online). No indels were identified in the mitochondrial genome, whereas 19 short (<100 bp) and 3 large (≥100 bp) indel polymorphisms were detected in the chloroplast genome (table 1). Levels of nucleotide diversity \( \pi \) together with the locations of the three large indel polymorphisms along the chloroplast genome are shown in table 1 and figure 2. De novo assemblies and PCR sequencing provided the sequence for the three large indel polymorphisms in the chloroplast genome, corresponding to zero-coverage regions X, Y, and Z (supplementary table S2, Supplementary Material online), respectively. Region X is a 3,735 bp indel polymorphism that corresponds to a class II intron located in the ATP8 gene. As compared with the reference, this class II intron is absent in strains RCC1108, RCC1112, RCC1117, RCC1118, RCC1123, RCC1158, RCC1559, and RCC1561 and partially absent in strain RCC1114. However, it is present at very low frequency (coverage <6%) in 7 of these 9 strains (fig. 3A). PCR detects this intra-strain polymorphism in only two strains.

All statistical analyses were performed with R Development Core Team (2010); http://www.R-project.org.

Table 1

Levels of DNA Sequence Diversity in the Organelles Excluding the Two Large Duplicated Regions

| Statistics | mtDNA | cpDNA |
|------------|-------|-------|
| \( P_N \): Nonsynonymous segregating sites | 12 | 16 |
| \( L_N \): Nonsynonymous sites | 14,784 | 26,513 |
| \( \pi_N \): Nonsynonymous nucleotide diversity | 0.0003 | 0.0002 |
| \( P_S \): Synonymous segregating sites | 55 | 119 |
| \( L_S \): Synonymous sites | 6,336 | 11,363 |
| \( \pi_S \): Synonymous nucleotide diversity | 0.003 | 0.004 |
| \( P_{4-fold} \): 4-fold segregating sites | 33 | 91 |
| \( L_{4-fold} \): 4-fold sites | 3,035 | 6,224 |
| \( \pi_{4-fold} \): 4-fold nucleotide diversity | 0.004 | 0.005 |
| \( P_I \): Intergenic segregating sites | 2 | 70 |
| \( L_I \): Intergenic sites | 1,223 | 8,220 |
| \( \pi_I \): Intergenic nucleotide diversity | 0.0005 | 0.003 |
| Indel (1 nt) | 0 | 11 |
| Indel (2–5 nt) | 0 | 4 |
| Indel (14–123 nt) | 0 | 5 |
| Indel >1 kbp | 0 | 2 |
(RCC1116 and RCC117) (fig. 3B), suggesting a higher sensitivity of Illumina sequencing to detect low-frequency variants. Region Y is located in the intergenic region between PsbA1 and Ycf9, and corresponds to a 123 bp deletion in four strains (RCC1558, RCC1115, RCC1116, and RCC1123) (fig. 3A). Region Z is a 3,873 bp insertion for two strains (RCC1115 and RCC1116), which contains a putative homing endonuclease, whereas it is a 90 bp deletion for one strain (RCC1558). This third indel polymorphism and 57 SNPs are clustered in a region of 1,754 bp that corresponds to an intergenic region bordered by genes PetG and PsbA, and constitute a hotspot of polymorphism (fig. 2).

Is There Evidence for Nonclonal Evolution of Organelles?

Whether mitochondria and chloroplasts evolve clonally was investigated by testing for recombination using SNP data (71 SNPs for the mitochondria and 203 for the chloroplast). Because different methods detect different signatures left by recombination in the data, previous studies have suggested the use of several different methods to reject the null hypothesis of clonal evolution (Posada and Crandall 2001;
None of the tests enabled us to reject the null hypothesis of clonal evolution in the mitochondrial genome, whereas all tests consistently rejected the null hypothesis in the chloroplast (table 2), with the exception of the LD\(D_0\) test, where the relationship of linkage disequilibrium with distance is negative, as expected under recombination, but not significant (\(P\) value \(= 0.16\)).

To quantify the level of recombination in the chloroplast, we used a coalescent-based maximum likelihood method as implemented in LDhat (McVean et al. 2002). We estimated the rate of recombination \(\rho_{\text{LDhat}} = 0.96\) in the genealogy of the cpDNA. As expected, the mitochondrial data set showed no evidence of recombination using this method.

If chloroplast and mitochondria are inherited together clonally, we would expect their genomes to contain congruent phylogenetic signals (Olson and McCauley 2000). We constructed the phylogenies for each organelle (fig. 4) and tested for congruence using KH and SH tests implemented in PAUP 4.0 beta 10 Win (Swofford 2002). Both tests rejected congruence (KH, \(P\) value \(< 10^{-4}\); SH, \(P\) value \(< 10^{-4}\)) between cpDNA and mtDNA phylogenies. In agreement with the absence of recombination in the mitochondrial genome, we observed two distinct mitochondrial haplotypes in our population. The minor haplotype is shared by strains RCC1114, RCC1118, RCC1558, and RCC1561 (supplementary table S4, Supplementary Material online).

### Table 2
Population Genetics Tests of Recombination Performed on SNPs of the mtDNA and cpDNA of 13 Ostreococcus tauri Strains

| Genome | SNPs | Max \(\chi^2\) | \(\Phi_{w}\) | LD\(r^2\) | LD\(D_0\) | LDhat |
|--------|------|---------------|-------------|----------|----------|--------|
| mtDNA  | 71   | ns            | ns          | 0.01     | ns       | 0      |
| cpDNA  | 203  | \(< 0.001\)   | ns          | -0.04    | \(< 0.001\) | 0      |

**NOTE.**—Tests performed on whole organelle genomes without the two internal duplicated regions and without the polymorphism hotspot in the chloroplast. \(\rho\), Spearman correlation coefficient between linkage disequilibrium (measured as \(r^2\) or \(D_0\)) and distance; ns, nonsignificant.

**FIG. 4.**—Maximum likelihood phylogenies of mtDNA and cpDNA of 13 Ostreococcus tauri strains. Incongruent mtDNA and cpDNA phylogenetic positions are indicated with dashed lines. Bootstrap values supporting nodes are shown for the NJ, MP, and ML phylogenies.
Discussion

Two Organelles Two Different Modes of Inheritance?

Although O. tauri has never been observed to undergo sexual reproduction in the laboratory, several lines of evidence suggest that it undergoes sex in its natural habitat; it has genes involved in meiosis within its genome (Derelle et al. 2006), population genetic evidence suggests that its nuclear genome undergoes intrachromosomal and interchromosomal recombination (Grimsley et al. 2010), as expected after meiosis, and there is a correlation between chromosome size and GC-content (Jancek et al. 2008), as we would expect if recombination was occurring and biased gene conversion was in operation (Duret and Galtier 2009). Our analysis of population genetic data suggests that recombination has also occurred in the genealogy of the chloroplast genome. The presence of recombination between the alleles segregating in the population is the consequence of homologous recombination between sequences carrying different mutations from the two parental cells. This implies that chloroplasts are, at least occasionally, biparentally inherited in O. tauri.

To estimate the relative prevalence of recombination in cpDNA as compared with nuclear DNA, we compared the estimated recombination rate in cpDNA and nuclear DNA for the same 13 strains (Grimsley et al. 2010) using the LDhat software (McVean et al. 2002). The number of recombination events in the genealogy of the cpDNA is 0.96 while an estimation from nuclear markers of the same strains is 2.9 (Grimsley et al. 2010). This suggests that recombination in cpDNA is approximately one-third as frequent as recombination in the nuclear genome and that chloroplast biparental inheritance is not exceptional. There is evidence that chloroplasts contain the apparatus for recombination since rearrangements have been observed between duplicated regions in both mitochondrial and chloroplast genomes (Maréchal and Brison 2010).

We can be less certain about the inheritance of mitochondria; they might be uniparentally or biparentally inherited. In the case of biparental inheritance, our data suggest that there is no detectable homologous recombination between the two parental genomes.

We observed two clear mitochondrial haplotypes in our population. Two recent estimates of the spontaneous mutation rate in the nuclear genome of Chlamydomonas reinhardtii (Ness et al. 2012: $\mu = 3.23 \times 10^{-10}$ per site per generation; Sung et al. 2012: $\mu = 6.76 \times 10^{-11}$) enable us to estimate the divergence between these two haplotypes. Let us assume one cell division per day in O. tauri, as observed under night:day conditions in the lab and that the mtDNA mutation rate, $\mu$, is the same as the nuclear mutation rate as seen for Mesostigma and Chlamydomonas (Hua et al. 2012). Thus, the divergence between the two haplotypes can be dated between 61,000 and 294,000 years ago.

It has been proposed that the earliest organelles (anterior to the green lineage ancestor) were probably biparentally inherited but did not recombine (Birky 1995). As discussed in the Introduction, organelles are generally transmitted uniparentally along the green lineage (with varying degrees of transmission from both parents, especially in land plants). The evolutionary history argues therefore in favor of a uniparental inheritance of organelles in the green lineage ancestor. Given the phylogenetic position of Ostreococcus at the base of the green lineage (Levis and McCourt 2004; Robbens et al. 2007; Marin and Melkonian 2010), our results suggest that cpDNA may have been largely biparentally inherited in the green lineage ancestor. Uniparental cpDNA inheritance may have evolved later and independently in the Chlorophyta and the Streptophyta lineages. Alternatively, the biparental inheritance of the chloroplast that we have observed in O. tauri may be a derived state from a lineage with uniparental chloroplast transmission. Information on the mode of inheritance in an early diverged Streptophyte such as Mesostigma sp. would greatly help to resolve the ancestral state in the green lineage. In contrast to cpDNA, inheritance of mtDNA may have been uniparental in the common ancestor of all Archaeplastida.

Advantages of Clonal Evolution of Organelles

There are several reasons why organelles might be inherited from one parent. Uniparentally inherited organelles form asexual lineages. Asexual evolution of organelles may be the simplest to maintain, and sexual reproduction may not have been maintained because the advantages of sexual reproduction that apply for nuclear genes (Müller’s ratchet, parental care, repair of chromosomal damage) are not so important to the organelar genomes (Birky 1995). Alternatively, uniparental inheritance may have evolved to avoid risks of cytoplasmic conflicts (Hurst and Hamilton 1992; Hutson and Law 1993). This is because there are many copies of the organelar genomes in one cell and, without mechanisms to synchronize their replication, a selfish faster replicating genome may spread in the population. As an illustration, the petite mitochondria in budding yeast, in which there is biparental inheritance of mitochondria, increase metabolic efficiency of the yeast. However, because they replicate faster than nonmutant mitochondria on media that contain glucose, they may invade the population despite their cost for the cell (MacAlpine et al. 2001). Uniparental transmission contains the spread of such mutants by locking them into one lineage, increasing the probability of counter-selection at the individual level. However, selection in favor of uniparental inheritance only exists whilst a selfish elements is segregating.

The prerequisite for uniparental inheritance is the evolution of, at least, two cell types: one transmitting its organelles while the other does not and a mechanism of recognition between these two cell types. The two different cell types can be mating-type in their most primitive form, so that organelar
inheritance and sexual dimorphism have intertwined evolutionary histories (Hurst and Hamilton 1992).

**Evolutionary Consequences of Recombination in Organelles: Halting Müller’s Ratchet on Amino Acid Mutations while Transmitting Selfish Elements**

We detected two large (>3,500 bp) indel polymorphisms within our chloroplast data (they correspond to chloroplast regions X and Z). The first indel polymorphism is a class II intron, a class of selfish elements that may contain a reverse transcriptase (Bonen and Vogel 2001). Within the 3,735 bp of the first indel, we detected one ORF of 613 codons that codes for a reverse transcriptase and an H-N-H endonuclease domain. It is therefore likely that the intronic sequence can be maintained as an RNA after deletion of the corresponding DNA fragment and may thus be re-inserted back into the genome. The second indel polymorphism is within an intergenic region of the repeated region of the chloroplast. It is 3,873-bp long and contains an ORF predicted to belong to the homing endonuclease superfamily. This suggests that this second insertion is also a selfish element.

Exposing the genome to the spread of selfish elements is one of the costs of biparental inheritance; however, we also provide evidence of one beneficial effect of recombination: slowing the accumulation of slightly deleterious mutations, a hallmark of biparental inheritance for this organelle. The frequency of recombination in chloroplast genome is of the same order of magnitude as previous nuclear-based estimates, suggesting that biparental inheritance is common. In contrast to the cpDNA, there is no evidence of recombination in the mitochondrial genome. These results are consistent with ancestral biparental inheritance of chloroplast and uniparental inheritance of mitochondria. The recombinating chloroplast genome contains fewer nonsynonymous relative to synonymous polymorphisms and harbors selfish elements: illustrating both the advantages and disadvantages of sex.

**Supplementary Material**

Supplementary tables S1–S5 are available at Genome Biology and Evolution online (http://www.gbe.oxfordjournals.org/).

**Acknowledgments**

The authors acknowledge the Genotoul bioinformatic platform (http://bioinfo.genotoul.fr/) for access to sequence analysis tools and computer cluster. They thank the Genomic of Phytoplankton group, especially Nigel Grimsley and Hervé Moreau for support. The Illumina sequence data analyzed in this study were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/ within the Community Sequencing Program CSP-129. This work was supported by the European Community’s 7th Framework program FP7 under grant agreement no. 254619 to G.P and A.E.W. and the AAP-FRB2009-PICOPOP (http://www.fondationbiodiversite.fr/) to G.P.

**Literature Cited**

Baur E. 1909. Das Wesen und die Erblichkeitsverhältnisse der “Varietates albomarginatae hort.” von Pelargonium zonale. Z. Für Indukt. Abstammungs-Vererbungslehre. 1:330–351.

Birky CW. 1995. Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. Proc Natl Acad Sci U S A. 92:11331–11338.

Boen L, Vogel J. 2001. The ins and outs of group II introns. Trends Genet. 17:322–331.

Boytonton JE, Harris EH, Burkhart BD, Lamerson PM, Gillham NW. 1987. Transmission of mitochondrial and chloroplast genomes in crosses of Chlamydomonas. Proc Natl Acad Sci U S A. 84:2391–2395.

Bruen TC, Philippe H, Bryant D. 2006. A simple and robust statistical test for detecting the presence of recombination. Genetics 172:2665–2681.

Correns C. 1909. Vererbungsversuche mit blass (gelb) grünen und bunt-blättrigen Sippen bei Mirabilis Jalapa, Urtica pilulifera un Lunaria annua. Z. Für Indukt. Abstammungs-Vererbungslehre. 1:291–329.
De Clerck O, Bogaert KA, Lelaert F. 2012. Diversity and evolution of algae: primary endosymbiosis. In: Piganeau G, editor. Genomics insights into the biology of algae, Vol. 64. London: Academic Press Ltd-Elsevier Science Ltd. p. 55–86.

Dere e E, et al. 2006. Genome analysis of the smallest free-living eukaryote Ostreococcus tauri unveils many unique features. Proc Natl Acad Sci U S A. 103:11647–11652.

Duret L, Galtier N. 2009. Biased gene conversion and the evolution of mammalian genomic landscapes. Annu Rev Genomics Hum Genet. 10:285–311.

Grimsley N, Pequin B, Bachy C, Moreau H, Piganeau G. 2010. Cryptic sex in the smallest eukaryotic marine green alga. Mol Biol Evol. 27:47–54.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol. 52: 696–704.

Hasegawa M, Kishino H, Yano T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol. 22:160–174.

Hill WG, Robertson A. 1966. The effect of linkage on limits to artificial selection. Genet Res. 8:269–294.

Hill WG, Robertson A. 1968. Linkage disequilibrium in finite populations. Theor Appl Genet. 38:226–231.

Houliston GJ, Olson MS. 2006. Nonneutral evolution of organelle genes in Silene vulgaris. Genetics 174:1983–1994.

Hua J, Smith DR, Borza T, Lee RW. 2012. Similar relative mutation rates in the three genetic compartments of Mesostigma and Chlamydomonas. Protist 163:105–115.

Huang S, Chiang Y-C, Schaal BA, Chou C-H, Chiang T-Y. 2001. Organelle DNA phylogography of Cycas taitungensis, a relict species in Taiwan. Mol Ecol. 10:2669–2681.

Hurst LD, Hamilton WD. 1992. Cytoplasmic fusion and the nature of sexes. Proc Biol Sci. 247:189–194.

Hutson V, Law R. 1993. Four steps to two sexes. Proc Biol Sci. 253:43–51.

Jancek S, Gourbie`re S, Moreau H, Piganeau G. 2008. Clues about the biology of algae, Vol. 64. London: Academic Press Ltd-Elsevier Science Ltd. p. 55–86.

John Archibald

Miyamura S. 2010. Cytoplasmic inheritance in green algae: patterns, mechanisms and relation to sex type. J Plant Res. 123:171–184.

Muller HJ. 1964. The relation of recombination to mutational advance. Mutat Res Mol Mech Mutagen. 1:2–9.

Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci U S A. 76. 5269–5273.

Ness RW, Morgan AD, Colegrave N, Kightley PD. 2012. Estimate of the spontaneous mutation rate in Chlamydomonas reinhardtii. Genetics 192:1447–1454.

Olson MS, McCauley DE. 2000. Linkage disequilibrium and phylogenetic congruence between chloroplast and mitochondrial haplotypes in Silene vulgaris. Proc Biol Sci. 267:1801–1808.

Piganeau G, Gardner M, Eyre-Walker A. 2004. A broad survey of recombination in animal mitochondria. Mol Biol Evol. 21:2319–2325.

Posada D. 2002. Evaluation of methods for detecting recombination from DNA sequences: empirical data. Mol Biol Evol. 19:708–717.

Posada D, Crandall KA. 2001. Evaluation of methods for detecting recombination from DNA sequences: computer simulations. Proc Natl Acad Sci U S A. 98:13757–13762.

R Development Core Team. 2010. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.

Robbens S, et al. 2007. The complete chloroplast and mitochondrial DNA sequence of Ostreococcus tauri: organelle genomes of the smallest eukaryote are examples of compaction. Mol Biol Evol. 24: 956–968.

Rodríguez-Espeleta N, Philippe H, Brinkmann H, Becker B, Melkonian M. 2007. Phylogenetic analyses of nuclear, mitochondrial, and plastid mtgenome data sets support the placement of mesostigma in the streptophyta. Mol Biol Evol. 24:723–731.

Rager S, Lane D. 1972. Molecular basis of maternal inheritance. Proc Natl Acad Sci U S A. 69:2410–2413.

Rager S, Ramanis Z. 1963. The particulate nature of nonchromosomal genes in Chlamydomonas. Proc Natl Acad Sci U S A. 50:260–268.

Rager S, Ramanis Z. 1967. Biparental inheritance of nonchromosomal genes induced by ultraviolet irradiation. Proc Natl Acad Sci U S A. 58:931.

Simpson JT, et al. 2009. ABYSS: a parallel assembler for short read sequence data. Genome Res. 19:1117–1123.

Städler T, Delph LF. 2002. Ancient mitochondrial haplotypes and evidence for intragenic recombination in a Gynodioecious plant. Proc Natl Acad Sci U S A. 99:11730–11735.

Sung W, Ackerman MS, Miller SF, Doak TG, Lynch M. 2012. Drift-barrier hypothesis and mutation-rate evolution. Proc Natl Acad Sci U S A. 109: 18488–18492.

Swofford DL. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland (MA): Sinauer Associates.

Tamura K, Nii M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol. 10:512–526.

Tamura K, et al. 2011. MEGAS: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 28:2549–2559.

Tsa l I, Bensasson D, Burt A, Koufopanou V. 2008. Population genomics of the wild yeast Saccharomyces paradoxus: quantifying the life cycle. Proc Natl Acad Sci U S A. 105:4957–4962.

Associate editor: John Archibald

Organellar Inheritance in the Green Lineage

Tamura K, Nei M. 1997. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol. 10:512–526.

Tamura K, et al. 2011. MEGAS: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 28:2549–2559.

Tsa l I, Bensasson D, Burt A, Koufopanou V. 2008. Population genomics of the wild yeast Saccharomyces paradoxus: quantifying the life cycle. Proc Natl Acad Sci U S A. 105:4957–4962.