The chloroplast and mitochondrial genome sequences of the charophyte *Chaetosphaeridium globosum*: Insights into the timing of the events that restructured organelle DNAs within the green algal lineage that led to land plants

Monique Turmel*, Christian Otis, and Claude Lemieux

Canadian Institute for Advanced Research, Program in Evolutionary Biology, and Département de Biochimie et de Microbiologie, Université Laval, Québec, QC, Canada G1K 7P4

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The land plants and their immediate green algal ancestors, the charophytes, form the Streptophyta. There is evidence that both the chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) underwent substantial changes in their architecture (intron insertions, gene losses, scrambling in gene order, and genome expansion in the case of mtDNA) during the evolution of streptophytes; however, because no charophyte organelle DNAs have been sequenced completely thus far, the suite of events that shaped streptophyte organelle genomes remains largely unknown. Here, we have determined the complete cpDNA (131,183 bp) and mtDNA (56,574 bp) sequences of the charophyte *Chaetosphaeridium globosum* (Coleochaetales). At the levels of gene content (124 genes), intron composition (18 introns), and gene order, *Chaetosphaeridium* cpDNA is remarkably similar to land-plant cpDNAs, implying that most of the features characteristic of land-plant lineages were gained during the evolution of charophytes. Although the gene content of *Chaetosphaeridium* mtDNA (67 genes) closely resembles that of the bryophyte *Marchantia polymorpha* (69 genes), this charophyte mtDNA differs substantially from its land-plant relatives at the levels of size, intron composition (11 introns), and gene order. Our finding that it shares only one intron with its land-plant counterparts supports the idea that the vast majority of mitochondrial introns in land plants appeared after the emergence of these organisms. Our results also suggest that the events accounting for the spacious intergenic spacers found in land-plant mtDNAs took place late during the evolution of charophytes or coincided with the transition from charophytes to land plants.

It is well recognized that land plants arose from green algae belonging to the Charophyta (1). Land plants and charophytes form the Streptophyta, a lineage sister to the Chlorophyta, which comprises the rest of green algae (2–4). Of the five orders recognized in the Charophyta (5), the Charales have been shown recently to be the closest relatives of land plants (6). A third green-plant lineage, at the base of the split of the Chlorophyta and Streptophyta, is represented possibly by the green alga *Mesostigma viride* (7–9). This lineage remains controversial, because some phylogenetic analyses placed *Mesostigma* within the Streptophyta (2, 6, 10). Whatever the exact position of *Mesostigma*, there is no doubt that this alga belongs to a deeply diverging lineage, because it represents the most basal branch in trees inferred from sequences of land plants and charophytes from all five orders (6).

An understanding of the evolution of chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) within the Streptophyta is of great interest, considering that comparative analyses of complete organelle DNA sequences from *Mesostigma* and land plants highlight considerable differences at the organizational level, with cpDNA showing more conservation than mtDNA (7, 8). Because there exist only fragmentary data on the organization of charophyte organelle DNAs (mainly for the cpDNA of *Spirogyra maxima*, a member of the Zygnematales; refs. 11–13), it is difficult to predict the timing of the major events that shaped the architectures of land-plant cpDNAs and mtDNAs.

Mesostigma cpDNA is highly similar in size and gene organization to the cpDNAs of the 10 photosynthetic land plants examined thus far (the bryophyte *Marchantia polymorpha* and nine vascular plants; see www.ncbi.nlm.nih.gov/PMGifs/Genomes/organelles.html), but lacks any introns and contains ≈20 extra genes (7). Chloroplast gene loss is an ongoing process in the Streptophyta, with independent losses occurring in multiple lineages (14, 15). *Mesostigma* and most land-plant cpDNAs share a quadripartite structure that is characterized by the presence of two copies of a rRNA-containing inverted repeat (IR) separated by large and small single-copy regions. All the genes they have in common, with a few exceptions, reside in corresponding genomic regions, and the great majority are part of conserved clusters.

In contrast, *Mesostigma* mtDNA greatly differs at the levels of size, gene organization, and intron content from the completely sequenced mtDNAs of the bryophyte (*M. polymorpha*) and the two angiosperms (*Arabidopsis thaliana* and *Beta vulgaris*) that have been investigated thus far (8). During the evolutionary transition from *Mesostigma* to *Marchantia*, mtDNA underwent a 4-fold increase in size, was rearranged extensively, and gained many introns while maintaining a similar gene content. After the emergence of bryophytes, mtDNA grew larger via the duplication of noncoding regions and the capture of cpDNA and nuclear DNA sequences, sustained loss of numerous genes, and acquired a highly dynamic genome structure as exemplified by the existence of angiosperm mtDNA as a mixture of recombinational isomers (16, 17). *Mesostigma* and land-plant mtDNAs share no introns, and only 1 of the 32 introns in *Marchantia* mtDNA (18) is conserved in *A. thaliana* (19) and *B. vulgaris* mtDNAs (20), suggesting that most of the liverwort mitochondrial introns have arisen independently from those present in angiosperms. The distribution patterns of mitochondrial introns among basal land plants are consistent with this hypothesis and also indicate that all five trans-spliced introns conserved among angiosperm mtDNAs arose from cis-spliced intron homologs (21–25). RNA-editing events involving mainly the conversions of cytidine into uridine have been observed in the mitochondria of basal land plants and angiosperms (26–29) as well as in their chloro-

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Abbreviations: cpDNA, chloroplast DNA; mtDNA, mitochondrial DNA; IR, inverted repeat.

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*To whom reprint requests should be addressed. E-mail: monique.turmel@rsvs.ulaval.ca.

Table 1. General features of cpDNA

Feature	*Mesostigma*	*Chaetosphaeridium*	*Marchantia*	*Arabidopsis*
Size, bp	118,360	131,183	121,024	154,478
A + T content, %	69.9	70.4	71.2	63.7
Gene content*	136	125	120	110
Introns				
Group I	0	1	1	1
Group II				
cis-spliced	0	16	18	19
trans-spliced	0	1	1	1

*Unique ORFs, intron ORFs, and pseudogenes were not taken into account.

plasts (26, 30–32) but appear to be absent in both organelles of *Marchantia* and the few algae examined thus far. These events are more frequent in mitochondria, where they affect essentially every protein-encoding mRNA in angiosperms.

Here, we report the complete cpDNA and mtDNA sequences of the charophyte *Chaetosphaeridium globosum*, a member of the order (Coleochaetales) that has been identified as the sister group of the Charales and land plants (6). Our comparative analyses of the *Chaetosphaeridium* organelle genomes with their *Mesostigma* and land-plant counterparts have allowed us to trace the origins of some of the events that restructured the cpDNA and mtDNA within the Streptophyta.

Materials and Methods

The axenic strain M1311 of *C. globosum* (Nordstedt) Klebahn was obtained from M. Melkonian (University of Köln, Köln, Germany). Cultures were grown at 18°C under alternating 12-h light/12-h dark periods in medium AF-6 (33). The cpDNA and mtDNA were sequenced in parallel by using a plasmid library prepared from an AT-rich fraction. The methods used were those reported previously (7) except that nucleotide sequences were determined with the PRISM Big Dye terminator cycle-sequencing ready-reaction kit (Applied Biosystems), the PRISM dGTP Big Dye terminator ready-reaction kit (Applied Biosystems), and the DYEnamic ET terminator cycle-sequencing kit (Amersham Pharmacia) on the ABI model 377 DNA sequencer (Applied Biosystems). Sequence analysis was carried out as described (8). The program DERANGE2 (M. Blanchette and D. Sankoff, Université de Montréal, Montréal) and that developed by N. El-Mabrouk and Y. Ajana (Université de Montréal) were used to infer the number of gene permutations by inversions. Maximum-likelihood analysis of combined protein sequences was carried out with PROTML (34) and AAML (35) by using the JTT model and gamma-distributed rates of substitutions across sites. The chloroplast and mitochondrial data sets (11,482 and 4,259 positions, respectively) consisted of the proteins analyzed in refs. 7 and 8, respectively. The homologous sequences of *Cyanophora paradoxa* were used as outgroup in the chloroplast analysis, whereas those of *Porphyra purpurea*, *Chondrus crispus*, and *Cyanidioschyzon merolae* were used to root the mitochondrial trees.

Results and Discussion

Features of *Chaetosphaeridium* cpDNA and mtDNA. In terms of size, gene content, and intron composition, *Chaetosphaeridium* cpDNA (131,183 bp) closely resembles *Marchantia* cpDNA (Table 1 and Fig. 1*A*). It encodes 124 genes, all of which have been identified in previously investigated green-plant cpDNAs, and features a quadripartite structure in which the IR displays a pair of extra genes (*chlL* and *chlN*) as compared with the corresponding genomic region in *Marchantia* cpDNA. The *Chaetosphaeridium chlL/chlN* cluster is located at one end of the IR, whereas this cluster is located at the immediate border of the small single-copy region in *Marchantia*. This observation indicates that expansion/contraction of the IR, a common event in land plants (36), led to the increased size and gene content of the *Chaetosphaeridium* IR. All the other genes in *Chaetosphaeridium* cpDNA that are shared with land-plant and *Mesostigma* cpDNAs are partitioned within the same genomic regions. In contrast, there is no rRNA-encoding IR in the 129.9-kb *Spirogyra* cpDNA (11). Seventeen group II introns and a unique group I intron are found in *Chaetosphaeridium* cpDNA; all are similar positionally and structurally to land-plant chloroplast introns. Similar to its land-plant homologs, *Chaetosphaeridium* cpDNA contains a single cis-spliced intron [in *trnK*(uuu)] with an ORF (*matK*) as well as a single trans-spliced intron (*rps12*.i1 intron). The latter contains an ORF in domain IV, which is missing in land-plant cpDNAs. Before our study, homologs of some land-plant chloroplast introns had been identified in other charophytes (12, 13, 37).

Chaetosphaeridium mtDNA (56,574 bp) is more alike *Mesostigma* mtDNA than *Marchantia* mtDNA at the levels of size and intron composition (Table 2). Its 67 genes, all previously identified in green-plant mtDNAs, are tightly packed in a 48.3-kb segment, the gene density of which is comparable to that found in *Mesostigma* mtDNA (Fig. 1*B*). The remaining 8.3-kb segment mainly accounts for the increased size of *Chaetosphaeridium* mtDNA relative to its *Mesostigma* homolog. Two of the four ORFs found in this segment, those lying at the borders and on opposite strands (orf101 and orf202), show sequence homology with phage integrase/recombinase genes and with orf304 in the mtDNA of the chlorophyte *Prototheca wickerhamii*. Given this observation and the fact that none of the potential coding sequences of the 8.3-kb segment are commonly found in mtDNA, it is possible that this segment took residence in mtDNA via horizontal transfer of phage or bacterial DNA. Nine group I introns and two group II introns were identified in six mitochondrial genes of *Chaetosphaeridium*. Only one (*cox1*.i2) of these introns is homologous positionally and structurally to an intron in land-plant mtDNAs (in *Marchantia* mtDNA), and only one (*cox1*.i4) has a known homolog in *Mesostigma* mtDNA (Table 3).

Patterns of Gene Losses in Streptophyte cpDNAs and mtDNAs. We compared the gene contents of *Mesostigma* (7, 8), *Chaetosphaeridium*, *Marchantia* (18, 38), and *Arabidopsis* (19, 39) organelle DNAs and inferred the events of gene loss from the cpDNA and mtDNA by using the single-tree topology that was retrieved after separate maximum-likelihood analyses of the combined protein sequences predicted from 53 chloroplast genes and 19 mitochondrial genes (Fig. 2). Sequences from chlorophyte taxa were not included in the data sets, because the current controversy regarding the position of *Mesostigma* relative to chlorophytes could not be resolved by the addition of the *Chaetosphaeridium* taxon. When chlorophyte sequences were included in the data sets, trees based on chloroplast proteins identified *Mesostigma* as the earliest green-plant divergence, but mitochondrial trees gave low support for this position (data not shown). All phylogenetic analyses unambiguously placed *Chaetosphaeridium* at the base of land-plant lineages.

Of the 140 genes predicted to have been present in the cpDNA

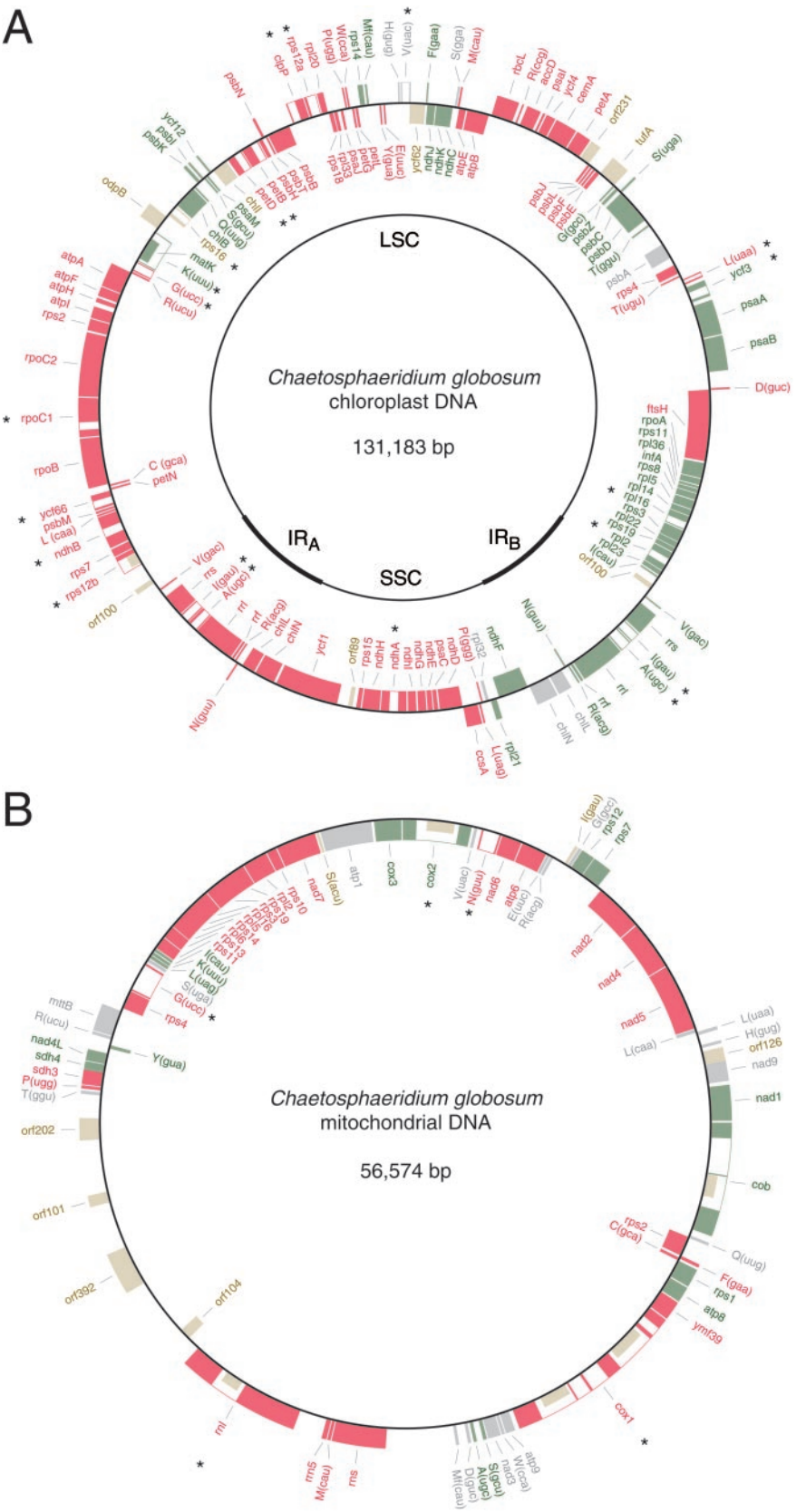

Fig. 1. Gene maps of *Chaetosphaeridium* cpDNA (*A*) and mtDNA (*B*). Genes outside each map are transcribed clockwise. Genes absent from *Marchantia* cpDNA and mtDNA are represented in beige. Gene clusters shared with *Marchantia* are shown as series of green and red boxes. Genes present in *Marchantia* but located outside conserved clusters are shown in gray. tRNA genes are indicated by the one-letter amino acid code followed by the anticodon in parentheses. Intron-containing genes are denoted by asterisks, with the introns represented as open boxes. The intron sequences bordering the *rps12* exons (*rps12*a and *rps12*b) are spliced in trans at the RNA level.

Table 2. General features of mtDNA

Feature	*Mesostigma*	*Chaetosphaeridium*	*Marchantia*	*Arabidopsis*
Size, bp	42,424	56,574	186,609	366,924
A + T content, %	67.8	65.6	57.6	55.2
Coding sequences,* %	86.6	76.3	65.0	45.5
Gene content†	65	67	69	50
Introns				
Group I	4	9	7	0
Group II				
cis-spliced	1	2	25	18
trans-spliced	2	0	0	5

For *Mesostigma* mtDNA, the data were taken from ref. 8, whereas those for the *Marchantia* and *Arabidopsis* mtDNAs were taken from ref. 47.

*Conserved genes, unique ORFs, introns, and intron ORFs were considered as coding sequences.

†Unique ORFs, intron ORFs, and pseudogenes were not taken into account.

of the last common ancestor of *Mesostigma* and *Chaetosphaeridium* (Table 4, which is published as supporting information on the PNAS web site, www.pnas.org), 19 were lost during the transition from this ancestor to the common ancestor of all land plants, with 14 of these loss events recorded during the interval separating the *Mesostigma* and *Chaetosphaeridium* lineages (Fig. 2*A*). We included *tufA* among the genes that were lost, although a sequence homologous to this gene was detected in *Chaetosphaeridium* cpDNA. Similar to the *tufA* sequence in the cpDNA of another member of the Coleochaetales (*Coleochaete orbicularis*; ref. 40), that of *Chaetosphaeridium* is highly divergent from those of other green plants and most probably codes for a nonfunctional protein. Because *tufA* is present in the nuclear genome of *Arabidopsis* (41) and also because *tufA*-like sequences have been detected by hybridization in the nuclear DNA of certain charophytes (including *Coleochaete*; ref. 40), the function of this gene in the Coleochaetales most probably has been replaced by a copy of the same gene that was transferred to the nucleus early during charophyte evolution (40).

Mitochondrial genes were lost at a significantly lower frequency than chloroplast genes during the transition from the last common ancestor of *Mesostigma* and *Chaetosphaeridium* to the common ancestor of all land plants (Fig. 2*B*). Only three of the 74 mitochondrial genes predicted in the former ancestor (Table 5, which is published as supporting information on the PNAS web site) were lost during this evolutionary period. Conversely, a higher proportion of mitochondrial genes suffered loss in the lineages leading to *Mesostigma* and *Chaetosphaeridium*, with five genes lost independently in these two lineages.

Table 3. *Chaetosphaeridium* mitochondrial introns

Intron	Group*	ORF size, codons	ORF location†	Source of homologous introns‡
cob.i1	ID	—	—	*Nephroselmis olivacea* mt (i1)
				Chlamydomonas smithii mt (i1)
cob.i2	ID	207	L2	*Saccharomyces cerevisiae* mt (i2)
coxI.i1	IB2	—	—	*Allomyces macrogynus* mt (i2)
				Emericella nidulans mt (i1)
				Podospora anserina mt (i3)
coxI.i2	IB4	303	L8	*Agrocybe aegerita* mt (i1)
				Allomyces macrogynus mt (i5)
				Marchantia polymorpha mt (i4)
				Prototheca wickerhamii mt (i1)
				Saccharomyces douglasii mt (i1)
				Schizosaccharomyces pombe mt (i1)
coxI.i3	IB1	—	—	*Podospora anserina* mt (i11)
coxI.i4	IB2	—	—	*Allomyces macrogynus* mt (i8)
				Chlorogonium elongatum mt (i1)
				Mesostigma viride mt (i1)
				Podospora anserina mt (i12)
coxI.i5	IB2	267	L6	*Podospora anserina* mt (i14)
cox2.i1	ID	269	L2	*Dictyostelium discoideum* mt (i4)
rnl.i1§	IB4	170	L6	*Chlorosarcina brevispinosa* cp
trnG(*ucc*).i1	IIA	—	—	—
trnN(*guu*).i1¶	—	—	—	

*Classification of group I and II introns was done according to refs. 48 and 49, respectively.

†L followed by a number refers to the loop extending the base-paired region identified by the number. Each of the ORFs identified potentially codes for a protein with the LAGLIDADG motif.

‡Introns inserted at identical gene locations (mt, mitochondria; cp, chloroplast).

§Insertion site corresponding to position 1917 in *Escherichia coli* 23S rRNA.

¶The absence of domain VI, which carries the highly conserved bulged adenosine that acts as the nucleophile in the first step of splicing by transesterification, prevented the determination of the sequence signatures that distinguish IIA and IIB introns. It is possible that splicing of this intron proceeds through a pathway in which water is the nucleophile (50).

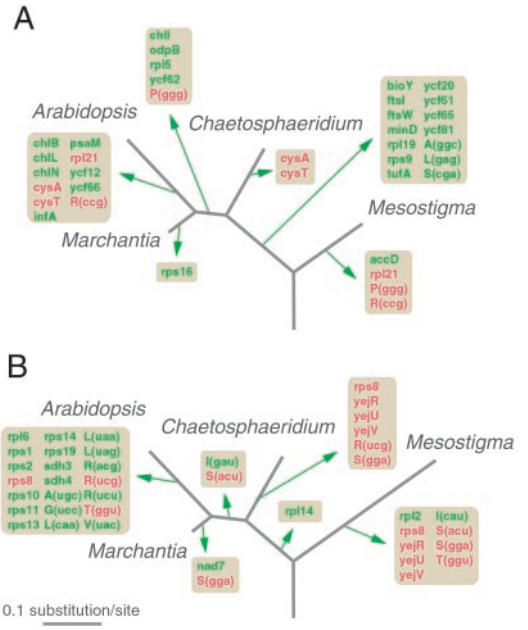

Fig. 2. Phylogenetic distribution of gene loss from cpDNA (*A*) and mtDNA (*B*) in the Streptophyta. The genes that were lost independently in different lineages are indicated in red. The predicted genes in the organelle DNAs of the common ancestor of *Mesostigma*, *Chaetosphaeridium*, and land plants are listed in Tables 4 and 5. In the lineage leading to *Arabidopsis*, the tRNA genes that were lost from mtDNA after the insertion of homologous cpDNA sequences (17) are not indicated. The chloroplast and mitochondrial trees were drawn on the same scale. For both phylogenetic analyses, the *Arabidopsis* protein sequences were predicted from the corresponding gene sequences without taking into account the edited sites in mRNA.

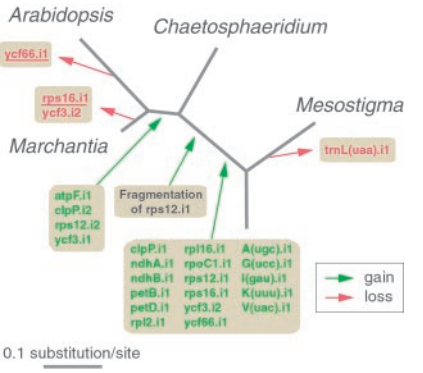

Fig. 3. Phylogenetic distribution of chloroplast intron gain and loss in the Streptophyta. The chloroplast tree presented in Fig. 2 is shown. The introns that are indicated in red and are underlined were lost from cpDNA together with the genes they interrupt.

Changes in Chloroplast and Mitochondrial Gene Orders in Streptophyta. The similarity in gene order between *Chaetosphaeridium* and *Marchantia* cpDNAs is remarkable. These cpDNAs share 12 blocks of colinear sequences (Fig. 1*A*), and up to 40 genes are present in individual blocks, the average number being 10. Only six genes, four of which are tRNA genes, are not comprised within common blocks. The differences in gene order in the small single-copy region can be explained by a single inversion, whereas those in the large single-copy region are attributable to 11 inversions that share 7 endpoints. As in rearranged land-plant cpDNAs (42, 43), tRNA genes often occur at the rearrangement breakpoints (at 12 of the 17 different endpoints identified), suggesting that chloroplast gene order in both charophyte and land-plant cpDNAs is scrambled by recombination across short indirect repeats found within or near tRNA genes. In contrast, *Chaetosphaeridium* cpDNA differs from its *Mesostigma* homolog by many rearrangements. These cpDNAs share 24 blocks of colinear sequences, each containing four genes in average, and 44 inversions (7 in the small single-copy region and 37 in the large single-copy region) account for the observed structural differences. Interestingly, a smaller number (37) of inversions are required for converting the gene order of *Marchantia* cpDNA into that of *Mesostigma* cpDNA; this observation is consistent with our finding that *Chaetosphaeridium* cpDNA has not retained some of the ancestral gene clusters shared by *Mesostigma* and land-plant cpDNAs (Table 6, which is published as supporting information on the PNAS web site). We find an identical gene order in the *Chaetosphaeridium*, *Marchantia*, and *Mesostigma* IRs when we disregard the differences in gene content arising from the expansion/contraction of the IR. As judged from the few genes that have been mapped on *S. maxima* cpDNA (11), this genome is rearranged more extensively than its *Chaetosphaeridium* homolog relative to *Mesostigma* and land-plant cpDNAs, supporting the hypothesis that the IR stabilizes the structure of cpDNA by decreasing intramolecular recombination (44).

Gene order has been preserved also in *Chaetosphaeridium* and *Marchantia* mtDNAs but to a lesser extent than in the cpDNAs of these green plants. Fifteen blocks of colinear mitochondrial sequences containing three genes on average were identified (Fig. 1*B*). This level of conservation was unexpected given that all previously analyzed green-plant mtDNAs bear little resemblance with one another at the gene-order level. Only two pairs of genes (*rpl6-rps13* and *rps12-rps7*) are shared between *Chaetosphaeridium* and *Mesostigma* mtDNAs. A total of 27 inversions (using 34 different endpoints) would be required to convert the gene order of *Chaetosphaeridium* mtDNA into that of *Marchantia* mtDNA, whereas 59 and 61 inversions account for the gene rearrangements displayed by the *Mesostigma*/*Marchantia* and *Chaetosphaeridium*/*Mesostigma* mtDNA pairs, respectively. As aforementioned for cpDNA, a large fraction of the inversion endpoints (27/34) inferred in the *Chaetosphaeridium*/*Marchantia* mtDNA comparison is associated with tRNA genes.

Patterns of Intron Gains and Losses in Streptophyte cpDNAs and mtDNAs. The single group I intron in *Chaetosphaeridium* and land-plant cpDNAs, which resides in *trnL*(uaa), is believed to be the most ancient intron found yet. Phylogenetic analyses (37, 45) indicate that it was present in the cyanobacterial ancestor of all chloroplasts and that it originated early during the evolution of cyanobacteria; its absence from some algal cpDNAs (e.g., *Mesostigma* cpDNA) is attributed to independent losses from *trnL*(uaa). With regards to the origin of the 21 different group II introns identified in land-plant cpDNAs, the pattern of intron distribution among green plants (Table 7, which is published as supporting information on the PNAS web site) predicts that 17 of these introns were acquired during the interval separating the *Mesostigma* and *Chaetosphaeridium* lineages (Fig. 3). Considering that trans-spliced introns have been shown to evolve from cis-spliced orthologs (23, 24), the gain of a cis-spliced version of the trans-spliced *rps*12.i1 intron must have preceded fragmentation of the intron and relocalization of the resulting intron–exon pieces. Available cpDNA sequences from zygnematalean charophytes (9, 12, 13) including *Spirogyra* indicate that the latter events as well as the insertions of the *trnI*(gau) and *trnA*(ugc) introns occurred before the emergence of the Zygnematales. The four group II introns missing from *Chaetosphaeridium* cpDNA might have been acquired by streptophytes after the emergence of the *Chaetosphaeridium* lineage, or alternatively, they might

have been gained earlier and lost subsequently. It is possible that proliferation of a few founding group II introns gave rise to the large intron population found in *Chaetosphaeridium* and land-plant cpDNAs. Analysis of cpDNAs from other charophyte lineages would be helpful in testing this hypothesis.

The pattern of intron distribution in land-plant mtDNAs together with our finding that *Chaetosphaeridium* mtDNA shares only one intron (*cox1*.i2) with its land-plant counterparts are consistent with, but do not prove, the idea that the great majority of land-plant mitochondrial introns originated after the emergence of land plants. Analysis of mtDNAs from other charophyte lineages, especially the Charales, would be required to identify the complete subset of *Marchantia* and/or angiosperm mitochondrial introns that took their origin during charophyte evolution. The *cox1*.i4 intron in *Chaetosphaeridium* mtDNA seems to have been inherited by vertical descent from a common ancestor of *Mesostigma* and charophytes. The remaining 10 *Chaetosphaeridium* introns likely were gained through horizontal transfers, because homologs of all these introns, except the two group II introns interrupting tRNA genes, have been identified in the mtDNAs of distantly related eukaryotes, mostly fungi and chlorophyte green algae (Table 3).

Prediction of Protein Sequence Alignments About RNA Editing in *Chaetosphaeridium* Organelles. The predicted proteins encoded by *Chaetosphaeridium* organelle genes were compared with their homologs in plants, algae, and bacteria. No unusual amino acids were identified at sites that are known to be edited in tobacco, rice, and maize chloroplast ORFs (46) as well as in *Arabidopsis* mitochondrial ORFs (29), and outside these sites only exceptional ones revealed potential mismatch corrections that could be corrected by C-to-U or U-to-C editing. Therefore, our analyses provide no compelling evidence for the occurrence of RNA editing in *Chaetosphaeridium* organelle transcripts. Sequence analysis of the RNA population in *Chaetosphaeridium* chloroplasts and mitochondria would be necessary to reject with confidence the possibility that scarce sites are edited.

Concluding Remarks. By disclosing details on the cpDNA and mtDNA architectures of a charophyte, our study provides a better understanding of how organelle genomes have evolved in the Streptophyta. We have shown that the chloroplast genome acquired the features characteristic of land-plant lineages at an earlier stage than the mitochondrial genome. At various levels, *Chaetosphaeridium* cpDNA is strikingly similar to land-plant cpDNAs; however, *Chaetosphaeridium* mtDNA is more compact than its land-plant relatives and features a very different intron composition. The events that gave rise to the spacious intergenic spacers found in land-plant mtDNAs took place either late during the evolution of charophytes or coincided with the transition from charophytes to land plants.

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