Chapter 7

Conservative and Dynamic Evolution of Mitochondrial Genomes in Early Land Plants

Yang Liu, Bin Wang, Libo Li, and Yin-Long Qiu*
Department of Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109-1048, USA

Jiayu Xue
Department of Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109-1048, USA
and School of Life Sciences, Nanjing University, Nanjing 210093, China

Summary

Early land plant mitochondrial genomes (chondromes) might have captured important changes of mitochondrial genome evolution when photosynthetic eukaryotes colonized land in a unprecedented scale, and thus deserve special attention in investigation of plant mitochondrial genomes. The chondromes of land plants that are well adapted to the terrestrial environment, namely seed plants, show many derived characteristics, including large genome size variation, frequent occurrence of intra-genomic rearrangements, abundant introns and high levels of RNA editing. In contrast, the chondromes of charophytes, the closest algal relatives of land plants, are still largely ancestral in these aspects, resembling chondromes of early eukaryotes. Several recently sequenced chondromes from basal land plants including liverworts, mosses, hornworts and lycophytes have provided fresh insights into mitochondrial genome evolution of early land plants. Comparative analyses of these genomes have identified lycophytes, which represent the most ancient extant vascular plants, as the major

* Author for correspondence, e-mail: ylqiu@umich.edu

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point of change in plant mitochondrial genome evolution, with long conserved mitochondrial gene synteny largely disrupted. The chondromes of bryophytes are conservative in gene order, but rather dynamic in intron content. The gene contents and RNA editing levels also show wide variation from lineage to lineage. Overall, the mitochondrial genomes experienced dynamic evolutionary changes during the origin and early evolution of land plants when the major lineages of bryophytes and vascular plants appeared, but have remained relatively conservative afterwards except in vascular plants.

I. Introduction

Among the major clades of eukaryotes, land plants (embryophytes) are outstanding in having mitochondrial genomes that show dramatic size variation, experience highly frequent intra-genomic rearrangement, harbor abundant introns and promiscuous DNA originating from nuclear and chloroplast genomes, and undergo heavy RNA editing during gene expression (Schuster and Brennicke 1994; Gray et al. 1999; Palmer et al. 2000; Knoop 2004). When and how these evolutionary novelties arose has remained unknown until very recently. The first plant mitochondrial genome was sequenced two decades ago from a liverwort, *Marchantia polymorpha* (Oda et al. 1992). In the 20 years that followed, over a dozen angiosperm chondromes (see Chap. 8) were sequenced (Unseld et al. 1997; Kubo et al. 2000; Notsu et al. 2002; Handa 2003; Clifton et al. 2004; Sugiyama et al. 2005; Allen et al. 2007; Goremykin et al. 2009; Alverson et al. 2010; Sloan et al. 2010; Alverson et al. 2011a; Rice et al. 2011). These data provided complete genome sequence information, confirmed the above evolutionary phenomena that had been described in earlier small-scale studies of individual gene and heightened the interest in pursuing the questions when and how these phenomena came into being. Reports of several charophytic algal mitochondrial genomes at the same time indicated that the *Marchantia* chondrome had not diverged very far from the charophyte chondromes and clearly represented an ancestral condition of plant mitochondrial genomes (Turmel et al. 1999; Turmel et al. 2002a, b, 2003, 2007). More recently, complete or partial sequencing of several bryophyte, lycophyte and gymnosperm chondromes (Terasawa et al. 2007; Chaw et al. 2008; Grewe et al. 2009; Li et al. 2009; Wang et al. 2009; Xue et al. 2010; Hecht et al. 2011) revealed that the major change in mitochondrial genome evolution of land plants took place during the origin of vascular plants, though some changes such as genome size increase, intron acquisition, and RNA editing already occurred during the origin and early evolution of land plants. In this chapter, we briefly review the progress in research on the mitochondrial genome evolution in early land plants.

Abbreviations: bp – Base pairs; kbp – Kilobase pairs

II. Genome Size and Gene Content

In bryophytes, the mitochondrial genomes of two liverworts, one moss and two hornworts have been completely sequenced, and their size range varies roughly by two times (Table 7.1). The smallest chondrome sequenced so far (105,340 base pairs (bp)) is found in the moss *Physcomitrella patens* (Terasawa et al. 2007), and the largest one (209,482 bp) occurs in the hornwort *Phaeoceros laevis* (Xue et al. 2010). This size range is much smaller than the long-known wide size variation of angiosperm chondromes, which can be up to an order of magnitude within a single family (Ward et al. 1981; Alverson et al. 2010). In comparison with the alga *Chara vulgaris*, which is a member of the sister group of land plants (Karol et al. 2001; Qiu et al. 2006) and has a chondrome of 68 kbp (Turmel et al. 2003),

Table 7.1. Genome sizes and proportions of the various sequence types in the mitochondrial genomes of *Chara vulgaris* and five bryophytes[a]

Species	Genome size (bp)	Genes (%)	Exons (%)	Introns (%)	Intergenic spacers (%)
Chara vulgaris	67,737	91	52	39	9
Marchantia polymorpha	186,609	51	23	28	49
Pleurozia purpurea	168,526	52	29	23	48
Physcomitrella patens	105,340	65	37	28	35
Megaceros aenigmaticus	184,908	50	16	34	50
Phaeoceros laevis	209,482	47	11	36	53

[a]The rows are shaded alternately by plant clades (Charales, liverworts, mosses and hornworts)

the bryophytes have significantly larger chondromes, whose size likely increased by 2–3 times during the origin of land plants. This estimate is supported by the fact that both liverworts and hornworts have large mitochondrial genomes, the former representing the first diverging lineage of land plants and the latter being sister to vascular plants (Qiu et al. 2006). The 100 kbp chondrome in *P. patens* likely resulted from secondary size reduction that happened early in moss evolution, as another moss, *Anomodon rugelii,* a distant relative of *P. patens*, has a chondrome of similar size (Liu et al. 2011).

The genome size increase is not the result of an increase in the number of genes (Table 7.2). Intron contents vary among these chondromes, but their number per genome fluctuates only slightly (Table 7.1). What changes greatly is the amount of intergenic spacer sequences, which seem to be the main determinant of genome size changes in the mitochondria of these organisms. This phenomenon has also been observed recently in the dramatic size increase of chondromes within a single angiosperm family, the Cucurbitaceae (Alverson et al. 2010).

The gene contents are remarkably similar in chondromes of *C. vulgaris*, the two liverworts and the moss (Table 7.2), which indicates that this aspect of mitochondrial genome evolution did not experience major changes during the origin of land plants. The two hornwort chondromes, in contrast, have lost or are in the process of losing many genes. The main groups of genes affected are those encoding ATP synthase (*atp8*), enzymes for cytochrome *c* biogenesis (*ccmFC* and *ccmFN*), ribosomal proteins (*rpl* and *rps*), succinate:ubiquinone oxidoreductase (*sdh3*), and transfer RNAs (tRNA genes). An intriguing observation is that many pseudogenes are present in the two hornwort chondromes. The two species sequenced for chondromes span nearly the entire phylogenetic diversity of hornworts (Duff et al. 2007), which originated at least in the Silurian (444 million years ago) (Stewart 1983). Thus, shared presence of these nonfunctional gene copies may indicate either independent pseudogenization events in the two species or retention of ancient pseudogenes for some not yet understood reasons (Xue et al. 2010). The absence of many tRNA genes may be explained by their straightforward replacement by tRNAs imported from the cytosol. The loss of these several categories of genes also fits the pattern that has been observed before. In a large survey of angiosperm mitochondrial genes, *rpl, rps* and *sdh* genes were shown to be most prone to loss from the chondromes of some angiosperm lineages (Adams et al. 2001; Adams et al. 2002). The dramatically reduced mitochondrial genomes of some green and red algae, for example, *Chlamydomonas eugametos* and *Porphyra purpurea,* have also lost some or even most genes in these categories (Denovan-Wright et al. 1998; Burger et al. 1999). Likewise, the highly reduced mitochondrial genomes of animals and most fungi have lost almost all *sdh, ccm, rpl* and *rps* genes (Gray et al. 1999). In land plants,

Table 7.2. Gene contents in mitochondrial genomes of *Chara vulgaris* and some early land plants[a]

Gene/ species[b]		*Ch. vu.*	*Ma. po.*	*Pl. pu.*	*Ph. pa.*	*Me. ae.*	*Ph. la.*	*Is. en.*	*Se. mo.*
atp1	a1	+	+	+	+	+	+	+	+
atp4	a4	+	+	+	+	+	+	+	
atp6	a6	+	+	+	+	+	+	+	+
atp8	a8	+	+	+	+	ψ	ψ	+	+
atp9	a9	+	+	+	+	+	+	+	+
ccmB	mb	+	+	+	+				
ccmC	mc	+	+	+	+				
ccmF[c]	mf	+							
ccmFC[c]	my		+	+	+	ψ	ψ		
ccmFN[c]	mz		+	+	+				
cob	cb	+	+	+	+	+	+	+	+
cox1	c1	+	+	+	+	+	+	+	+
cox2	c2	+	+	+	+	+	+	+	+
cox3	c3	+	+	+	+	+	+	+	+
nad1	n1	+	+	+	+	+	+	+	+
nad2	n2	+	+	+	+	+	+	+	+
nad3	n3	+	+	+	+	+	+	+	+
nad4	n4	+	+	+	+	+	+	+	+
nad4L	na	+	+	+	+	+	+	+	+
nad5	n5	+	+	+	+	+	+	+	+
nad6	n6	+	+	+	+	+	+	+	+
nad7	n7	+	ψ	ψ	+			+	+
nad9	n9	+	+	+	+	+	+	+	+
rpl2	l2	+	+	+	+				
rpl5	l5	+	+	+	+	ψ		+	
rpl6	l6	+	+	+	+	ψ	ψ		
rpl10	l10		+	+	+	+	+		
rpl14	l14	+							
rpl16	l16	+	+	+	+				
rps1	s1	+	+	+	+	ψ	ψ	ψ	
rps2	s2	+	+	+	+		ψ	+	
rps3	s3	+	+	+	+			+	
rps4	s4	+	+	+	+	ψ	ψ	+	
rps7	s7	+	+	+	+	ψ	ψ		
rps8	s8		+	+	ψ	ψ			
rps10	s10	+	+	+	ψ				
rps11	s11	+	+	+	+	ψ	ψ		
rps12	s12	+	+	+	+	ψ	ψ		
rps13	s13		+	+	+	+	+		
rps14	s14	+	+	+	+	+			
rps19	s19	+	+	+	+				
rrn5	r5	+	+	+	+	+	+	+	
rrn18	r18	+	+	+	+	+	+	+	+
rrn26	r26	+	+	+	+	+	+	+	+
rtl	x1		+	ψ	ψ				
sdh3	d3	+	+	+	+	ψ	ψ	+	
sdh4	d4	+	+	+	+	+	+		
tatC	w2	+	+	+	+	+	+	+	+
trnAugc	ta	+	+	+	+	+	+		
trnCgca	tc	+	+	+	+	+	+	+	

(continued)

Table 7.2. (continued)

Gene/ species[b]		*Ch. vu.*	*Ma. po.*	*Pl. pu.*	*Ph. pa.*	*Me. ae.*	*Ph. la.*	*Is. en.*	*Se. mo.*
trnDguc	td	+	+	+	+	+	+		
trnEuuc	te	+	+	+	+	+	+	+	
trnFgaa	tf	+	+	+	+	+	+	+	
trnGgcc	tg	+	+	+	+	+	+	+	
trnGucc	t2	+	+	+	+				
trnHgug	th	+	+	+	+	+	+		
trnIcau	ti	+	+	+	+	+	+	+	
trnIgau	t3	+							
trnKuuu	tk	+	+	+	+	+	+	+	
trnLcaa	t5	+	+	+	+	+	+		
trnLuaa	t7	+	+	+	+	+	+		
trnLuag	t8	+	+	+	+		+	ψ	
trnMcau	tm	+	+	+	+	+	+	+	
trnMfcau	t9	+	+	+	+	+	+	+	
trnNguu	tn	+	+	+					
trnPugg	tp	+	+	+	+	+	+	+	
trnQuug	t10	+	+	+	+	+	+	+	
trnRacg	tr	+	+	+	+				
trnRucg	t12		+						
trnRucu	t13	+	+	+	+				
trnSgcu	t14	+	+	+					
trnSuga	t15	+	+	+	+			+	
trnTggu	tt	+	+		+	+	+		
trnVuac	tv	+	+	+	+		+		
trnWcca	tw	+	+	+	+	+	+	+	
trnYgua	ty	+	+	+	+	+	+	+	

[a]"+" and "ψ" indicate presence of functional gene and pseudogene, respectively. No sign indicates gene absence. The data columns are shaded alternately by plant clades (Charales, liverworts, mosses, hornworts and lycophytes)

[b]Abbreviated gene names listed in the second column are used in Fig. 7.1. Full species names are as follows (in the order of their appearance): *Chara vulgaris*, *Marchantia polymorpha, Pleurozia purpurea, Physcomitrella patens, Megaceros aenigmaticus, Phaeoceros laevis*, *Isoetes engelmannii* and *Selaginella moellendorffii*

[c]The genes *ccmFC* and *ccmFN* in land plant chondromes appear as a single gene *ccmF* in *Chara vulgaris*

the loss of *ccm* genes has been less well known until now. The loss of tRNA genes may be related to the phenomenon of some mitochondrial tRNA genes being replaced by their chloroplast counterparts at some stages of vascular plant evolution (Li et al. 2009).

An interesting case of a tRNA gene loss and regain by modifying a duplicated copy of a different tRNA gene was uncovered in the study of the chondrome of the liverwort *Pleurozia purpurea* (Wang et al. 2009), where a *trnRucg* gene was found missing in comparison to *M. polymorpha*. The gene *trnRucg* was probably lost shortly after the endosymbiotic origin of mitochondria, as it is absent in chondromes of *Reclinomonas americana*, an early diverging eukaryote with the most ancestral form of mitochondrial DNA (Lang et al. 1997), and from many other protist mtDNAs (Wang et al. 2009). In the liverwort *M. polymorpha* (Oda et al. 1992), the charophytic algae *Chlorokybus atmophyticus* (Turmel et al. 2007) and *Mesostigma viride*, (Turmel et al. 2002b), and the prasinophycean alga *Nephroselmis olivacea* (Turmel et al. 1999), however, this gene is present. In a comparative analysis of

all *trnR* genes from a broad diversity of protists and photosynthetic eukaryotes, it was determined that *trnRucg* in *M. polymorpha* and *C. atmophyticus* was derived from modification of a duplicated copy of *trnRucu*, whereas the gene in *M. viride* and *N. olivacea* was a modified copy of *trnRacg* (Wang et al. 2009). Three examined liverworts provided particularly convincing evidence to demonstrate the origin of the *trnRucg* gene from a gene duplication. In *Treubia lacunosa*, a member of the Haplomitriopsida lineage that is sister to all other liverworts, and *P. purpurea*, a member of the simple thalloid ("metzgeriid") liverworts, two copies of *trnRucu* were found, one located between *nad2* and *trnYgua*, and the other between *tatC* and *trnYgua in* a repeat sequence environment. *M. polymorpha* also has these two gene clusters, but the *trnR* gene between *tatC* and *trnYuga* is *trnRucg*, not *trnRucu*. Sequence comparison clearly shows that *trnRucg* is actually a modified *trnRucu*, with only three nucleotides changed, one being the U → G change in the anticodon. Hence it appears that an early gene duplication of *trnRucu* in the liverworts laid the foundation for neofunctionalization through conversion of one of the copies into a *trnRucg* in *Marchantia*. Previously, it has been shown that some tRNA genes in seed plant chondromes (see next chapter) originated from modification of chloroplast originated tRNA gene copies now located in the mitochondria (Maréchal-Drouard et al. 1990; Li et al. 2009). In seed plant chondromes that often have large chunks of chloroplast-originated sequences (Unseld et al. 1997; Chaw et al. 2008; Alverson et al. 2010), it seems a natural way to derive new tRNA genes from their chloroplast counterparts. In bryophyte chondromes, no such intracellular inter-organelle DNA movement has been detected so far. Hence, different ways can be used to re-create tRNA genes that were lost from plant mitochondria: from a related tRNA gene that still resides in the chondrome. These two evolutionary pathways of re-creating long-lost tRNA genes add to the long list of peculiar molecular evolutionary phenomena characterizing plant mitochondrial genomes that has been compiled since the 1980s.

The two recently sequenced chondromes of the lycophytes *Isoetes engelmanii* (Grewe et al. 2009) and *Selaginella moellendorffii* (Hecht et al. 2011) allow a glimpse into gene contents of mitochondrial genomes in the most basal lineage of vascular plants (Raubeson and Jansen 1992; Qiu et al. 2006). It is rather shocking to see that these genomes have also lost many of the genes that are gone in the hornwort chondromes (Table 7.2). The *Selaginella* case is particularly impressive, as this is the only land plant chondrome known so far that apparently has lost all ribosomal protein genes and all tRNA genes. Previously, mitochondrial genomes of animals and some green algae have been known to lack the entire set of ribosomal protein genes (Denovan-Wright et al. 1998; Dellaporta et al. 2006). Given that hornworts and lycophytes span the important evolutionary transition from gametophyte to sporophyte as the independent, free-living, dominating generation in the life cycle (Qiu et al. 2006), it is tempting to suggest that these gene losses may be related to this life cycle change. However, a completely sequenced chondrome from *Huperzia squarrosa,* which is a member of the third order (Lycopodiales) and the most basal lineage of lycophytes, shows that it actually has many ribosomal protein genes and tRNA genes. Its gene content is in fact not very different from that of the liverworts and the mosses (Liu et al. 2011). The widespread gene losses in chondromes of hornworts and lycophytes are very likely due to independent events, since the lost genes are obviously retained during the main course of plant evolution (in that they reside in seed plant mtDNAs; see next chapters). Given recurrent losses of these categories of genes in many eukaryotic lineages (discussed above), such a scenario does not seem unlikely.

Finally, there are many open reading frames (ORFs) in the bryophyte chondromes that are longer than 100 codons. Some are

conserved between the two liverworts or between the two hornworts, both in their position and at the sequence level. Given that the identity of *rpl10,* previously only annotated as an ORF in several chondromes, was just revealed recently (Mower and Bonen 2009; Kubo and Arimura 2010), it is likely that some of these ORFs may represent uncharacterized genes. The gene *rtl*, encoding a reverse transcriptase, represents another interesting case. It has been known as ORF732 and ORF-721 in the chondromes *M. polymorpha* and *P. purpurea* respectively, and in both taxa, this gene exists as a free-standing gene located between *cob* and *nad9* (Oda et al. 1992; Wang et al. 2009). However, this gene is located within a group II intron in *nad9* of the chondromes of *P. patens* (Terasawa et al. 2007) and *A. rugelii* (Liu et al. 2011). The gene may have also been pseudogenized in some taxa, and more investigation is needed to determine the exact status.

III. Genome Rearrangement and Gene Order

High levels of synteny, i.e., the same gene orders among chondromes of different species, due to low rates of recombination and presence of polycistronic operons, are a major characteristic of organellar genomes (Palmer 1985; Gillham 1994). It was thus a surprise when angiosperm mitochondrial genomes were found to evolve extremely rapidly in structure (Palmer and Herbon 1988). Recently, genome level sequence data have shown that chondromes of two *Zea mays* cytotypes experience as many as 16 rearrangements (Allen et al. 2007). On the other hand, comparison of the *Marchantia* chondrome (Oda et al. 1992) and the chondromes of two closely related charophytic green algae, *C. vulgaris* and *Chaetosphaeridium globosum* (Turmel et al. 2002a, 2003), indicates that these genomes have experienced conservative structural evolution in early land plants (Fig. 7.1). Hence, the question arises as to when the mitochondrial genome acquired the ability to undergo rapid genome rearrangement during plant evolution.

Four recently sequenced bryophyte chondromes, which cover all three major lineages of bryophytes – liverworts, mosses, and hornworts (Terasawa et al. 2007; Li et al. 2009; Wang et al. 2009; Xue et al. 2010) – show that the structural evolution of mitochondrial genome is highly conservative not only within each individual lineage but also across bryophytes. It takes 16 rearrangements to bring the *Marchantia* and *Physcomitrella* chondromes into complete synteny (Fig. 7.1), the same number of rearrangements that chondromes of two *Z. mays* cytotypes have experienced (Allen et al. 2007). The readers are reminded here that liverworts and mosses diverged at least 375 million years ago according to the age of the oldest fossil of a clearly identifiable liverwort (Hernick et al. 2008). Likewise, only seven changes are required to bring all genes of the *Physcomitrella* and *Megaceros* chondromes into the same order (Fig. 7.1). The high level of structural conservation in bryophyte mitochondrial genomes is further confirmed by gene order comparison among chondromes of distantly related species within liverworts, mosses, and hornworts. The chondromes of two species that represent complex thalloid liverworts (*M. polymorpha* (Oda et al. 1992)) and simple thalloid liverworts (*Pleurozia purpurea* (Wang et al. 2009)) have identical gene orders. Likewise, the chondromes of two mosses, *P. patens* (Terasawa et al. 2007) and *A. rugelii* (Liu et al. 2011), which represent the diversity of almost the entire clade of peristomate mosses (Goffinet et al. 2001; Qiu et al. 2006), have identical gene orders. Finally, the chondromes of two hornworts, *Megaceros aenigmaticus* (Li et al. 2009) and *P. laevis* (Xue et al. 2010), which span the diversity of the entire clade, differ by only four rearrangements. This level of genome structural conservation shows that the mitochondrial genome in bryophytes still behaves like the typical

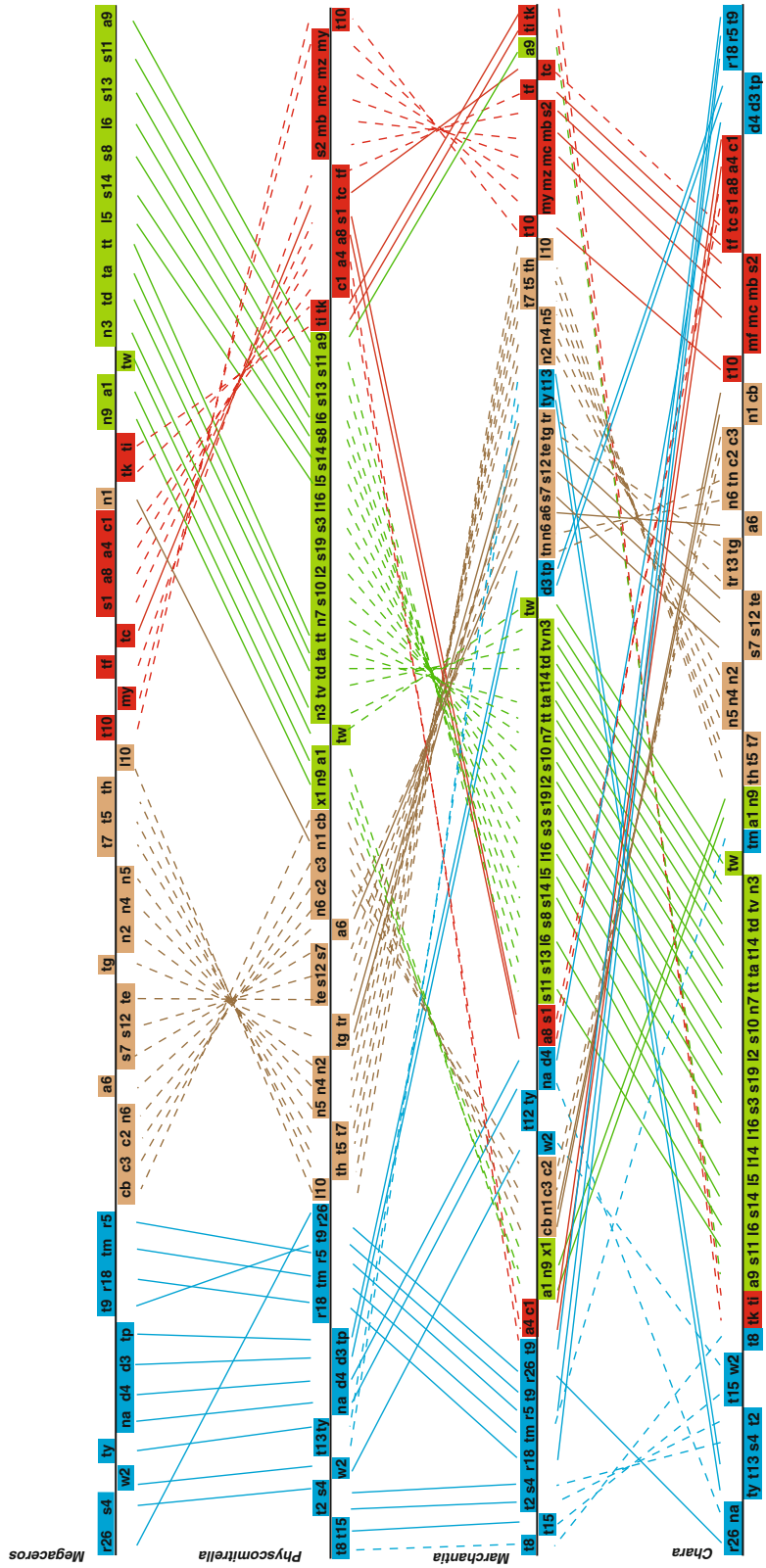

Fig. 7.1. Gene order comparison among chondromes of *Chara vulgaris, Marchantia polymorpha, Physcomitrella patens* and *Megaceros aenigmaticus*. *Solid lines* connect orthologous genes between species with the same gene orientation, whereas *dashed lines* connect those with inverted gene orientation. The gene *rtl* (reverse transcriptase) is located between *cob* and *nad9* in the *M. polymorpha* chondrome, but within a group II intron in *nad9* of the *P. patens* chondrome.

Table 7.3. Intron contents in mitochondrial genomes of *Chara vulgaris* and some early land plants[a]

Intron/species[b]	*Ch. vu.*	*Ma. po.*	*Pl. pu.*	*Ph. pa.*	*Me. ae.*	*Ph. la.*	*Is. en.*	*Se. mo.*
atp1i805g2					+	+		
atp1i989g2		+	+					
atp1i1019g2					+	+		
atp1i1050g2*		+	+		+	+		
atp1i1129g2				+				
atp6i80g2*				+	+	+		
atp6i439g2*					+	+	+	+
atp9i21g2*				+			+	*trans*
atp9i87g2**		+	+	+			+	+
atp9i95g2**				+	+	+		+
atp9i145g2	+							
atp9i214g2	+							
ccmFCi829g2*				+	+	+		
cobi274g2	+							
cobi372g2		+	+					
cobi420g1*				+		+		
cobi537g2	+							
cobi688g2	+							
cobi693g2							+	+
cobi783g2		+	+					
cobi787g2*					+	+	+	*trans*
cobi824g2		+	+					
cobi838g2					+	+		
cox1i44g2*		+	+		+	+		
cox1i150g2					+	+		
cox1i178g2		+	+					
cox1i211g2	+							
cox1i227g2							+	+
cox1i266g2							+	+
cox1i323g2							+	
cox1i375g1		+	+					
cox1i395g1*		+	+				+	
cox1i511g2**		+	+	+				+
cox1i624g1*		+	+	+				
cox1i729g1*	+	+	+					
cox1i732g2				+				
cox1i740g1	+							
cox1i835g2	+							
cox1i876g1*	+							+
cox1i909g1	+							
cox1i995g2							+	+
cox1i1064g2				+				
cox1i1116g1		+	+					
cox1i1149g2								+
cox1i1298g2					+	+		
cox1i1305g1*		+	+				*trans*	*trans*
cox2i94g2							+	+
cox2i97g2		+	+					
cox2i104g2*	+			+				
cox2i250g2		+	+					
cox2i281g2					+	+		

(continued)

Table 7.3. (continued)

Intron/species[b]	*Ch. vu.*	*Ma. po.*	*Pl. pu.*	*Ph. pa.*	*Me. ae.*	*Ph. la.*	*Is. en.*	*Se. mo.*
cox2i373g2***				+	+	+		*trans*
cox2i564g2						+		
cox2i691g2*				+		+		
cox3i109g2						+		
cox3i171g2		+	+					
cox3i506g2				+				
cox3i625g2		+	+					
nad1i287g2*				+	+	+		
nad1i348g2					+	+		
nad1i394g2							+	+
nad1i477g2								+
nad1i669g2								+
nad1i728g2**				+	+	+		+
nad2i156g2*				+			+	+
nad2i542g2							+	+
nad2i709g2**		+	+		+	+	+	+
nad2i830g2							+	+
nad2i1282g2					+	+		
nad3i52g2*					+	+	+	+
nad3i140g2**	+	+	+		+	+	+	+
nad3i211g2	+							
nad4i461g2**				+	+	+	+	+
nad4i548g2		+	+					
nad4i976g2**	+				+	+		+
nad4i1399g2							+	+
nad4Li100g2		+	+					
nad4Li283g2*		+	+	+				
nad5i230g2*				+	+	+		
nad5i242g2							+	+
nad5i753g1*		+	+	+				
nad5i1455g2*				+	+	+	+	+
nad5i1477g2*					+	+	+	+
nad6i444g2					+	+		
nad7i140g2*				+				+
nad7i209g2*				+			+	+
nad7i336g2		+	+					
nad7i676g2							+	+
nad7i917g2							+	+
nad7i1113g2*		+	+				+	
nad9i246g2					+	+		
nad9i283g2				+				
nad9i502g2					+	+		
rpl2i28g2		+	+					
rps3i74g2*	+						+	
rps14i114g2		+	+					
rrn18i839g1							+	+
rrn18i1065g2		+						
rrn26i819g1	+							
rrn26i827g2		+	+					
rrn26i1871g1	+							

(continued)

Table 7.3. (continued)

Intron/species[b]	*Ch. vu.*	*Ma. po.*	*Pl. pu.*	*Ph. pa.*	*Me. ae.*	*Ph. la.*	*Is. en.*	*Se. mo.*
rrn26i1879g1	+							
rrn26i1891g1	+							
rrn26i2191g1	+							
rrn26i2429g1	+							
rrn26i2462g1	+							
rrn26i2500g1	+							
rrn26i2513g1	+							
sdh3i100g2*				+	+	+		
trnNguui38g2	+							
trnSgcui43g2		+	+					

[a]"+" and "*trans*" indicate presence of *cis*- and *trans*-spliced introns, respectively. No sign indicates intron absence. The data cells are shaded alternately by plant clades (Charales, liverworts, mosses, hornworts and lycophytes). Group I introns are also shaded. Introns present in more than one clades are bold-faced, and the number of stars indicates the number of plants clades in which the intron is present

[b]Intron nomenclature follows Dombrovska and Qiu 2004, and Knoop 2004. Full species names are as follows (in the order as they appear): *Chara vulgaris, Marchantia polymorpha, Pleurozia purpurea, Physcomitrella patens, Megaceros aenigmaticus, Phaeoceros laevis, Isoetes engelmannii* and *Selaginella moellendorffii*

organellar genome as previously known in most other eukaryotes (Gray et al. 1999), and has not yet acquired the ability to undergo rapid rearrangement.

The lycophyte chondrome sequences from *I. engelmanii* (Grewe et al. 2009) and *S. moellendorffii* (Hecht et al. 2011), however, tell a different story. The two genomes not only show highly rearranged gene order from each other, but also differ significantly from the conservative bryophyte chondriomes. These data suggest that the lycophytes may represent the beginning of rapid structural evolution in plant mitochondrial genomes. However, the extent of mtDNA rearrangement in lycophytes is not necessarily as dramatic as seen in these two chondromes, because the chondrome of *H. squarrosa* (Liu et al. 2011) is less reshuffled in comparison to the bryophyte chondromes. One particular additional indicator is that the *H. squarrosa* chondrome has no single *trans*-spliced intron whereas both *I. engelmanii* and *S. moellendorffii* have *trans*-spliced introns in their chondromes.

With regard to mechanisms of intragenomic rearrangement, repeat sequences may have been involved as hypothesized earlier (Andre et al. 1992). Comparison of gene orders between the two hornwort chondromes detected several pairs of repeat sequences (Xue et al. 2010). Similarly, numerous large repeat sequences were found in the highly rearranged chondrome of the lycophyte *S. moellendorffii* (Hecht et al. 2011).

IV. Introns

Both groups I and II introns are present in chondromes of bryophytes and lycophytes (Table 7.3). Their distribution patterns reflect their nature as mobile genetic elements. On the one hand, many introns are conserved within liverworts, hornworts or lycophytes, or even larger clades that include vascular plants and more than one major clade of bryophytes. On the other hand, some introns appear to have experienced lateral movement. Broad surveys including a large number of taxa and phylogenetic analyses of both exon and intron sequences are needed to determine whether an intron has been vertically inherited or laterally transferred (Malek and Knoop 1998; Qiu et al. 1998; Dombrovska and Qiu 2004).

Despite their seemingly idiosyncratic distribution patterns, a few generalizations can be made about these introns. First, it is clear

that they experienced active transposition during major evolutionary events such as the origins of land plants, individual bryophyte clades and vascular plants, but were stably inherited afterwards, because most of these introns show plant clade-specific distribution patterns (Table 7.3). Second, the host gene seems to be a factor in determining intron distribution. Group II introns have a broad distribution, but they are mostly present in respiratory protein genes. Genes for genetic information processing, such as rRNA genes, ribosomal protein genes and tRNA genes, are greatly under-represented among the host genes of introns. Intriguingly, group II introns in chloroplast genomes of charophytes and land plants do not show such strong host gene preference (Ohyama et al. 1986; Turmel et al. 2002a). Group I introns are mostly found in only two genes, *cox1* and *rrn26*, and thus the host gene diversity is too low to allow detection of any meaningful pattern. Third, *trans*-splicing is highly correlated to the level of recombination within the genome. No *trans*-spliced intron has been found in any bryophyte chondrome, consistent with the high level of structural conservation in these genomes (Oda et al. 1992; Terasawa et al. 2007; Li et al. 2009; Wang et al. 2009; Xue et al. 2010). In contrast, *trans*-spliced group II introns have been detected in the highly recombinogenic *Selaginella* chondrome, and a first ever *trans*-spliced group I intron has been found in the chondromes of both *Selaginella* and *Isoetes* (Grewe et al. 2009; Hecht et al. 2011). This observation is consistent with a previous report of multiple independent evolution of *trans*-splicing from a *cis*-spliced homologous intron in the highly recombinogenic vascular plant chondromes (Qiu and Palmer 2004). These non-random distribution patterns, as idiosyncratic as they are, probably reflect the interplay of historical processes during plant evolution, mechanistic preference of transposition mediated by both endonucleases and recognition motifs, recombination activity within a genome, and some as yet poorly understood functional adaptive mechanisms.

Previously, intron distribution patterns have been used to resolve difficult phylogenetic issues in land plants (Qiu et al. 1998; Groth-Malonek et al. 2005; Qiu et al. 2006). Knowledge of the full sets of introns (as gained from sequencing of entire chondromes) constitutes the prime data for this purpose, although taxon sampling at this stage is often not yet dense enough. Nevertheless, it seems clear that no intron is uniquely present in chondromes of all three bryophyte lineages, lending strong support to the paraphyly hypothesis of bryophytes (Mishler and Churchill 1984; Kenrick and Crane 1997; Qiu et al. 2006).

V. RNA Editing

RNA editing was originally not detected in bryophyte chondromes (Hiesel et al. 1994), due to lack or low levels of editing in the small number of taxa investigated: *Marchantia, Sphagnum* and *Physcomitrella*. The two bryophyte chondromes that were sequenced first happened to be non- or low-editing taxa, *M. polymorpha* and *P. patens* (Oda et al. 1992; Terasawa et al. 2007). Later surveys including many more taxa, based on indirect evidence from codon conservation analysis, suggested occurrence of RNA editing, sometimes at high levels, in bryophyte chondromes (Steinhauser et al. 1999; Dombrovska and Qiu 2004). Direct evidence for genome-wide occurrence of RNA editing was obtained only recently from cDNA sequencing in the moss *P. patens* (Rüdinger et al. 2009) and the hornwort *M. aenigmaticus* (Xue et al. 2010). Annotation of completely sequenced chondromes from the liverwort *P. purpurea* and the hornwort *P. laevis* also invokes a significant number of editing events to create proper start and stop codons and to remove internal stop codons (Li et al. 2009; Wang et al. 2009). Thus, RNA editing is clearly present throughout land plants (with the exception of an apparent secondary loss in the marchantiid liverworts (Groth-Malonek et al. 2007)) and the editing machinery likely originated in the common

ancestor of land plants. No editing has been detected so far in charophyte chondromes (Turmel et al. 2002a, b, 2003, 2007).

In early vascular plant chondromes, RNA editing has been known for a long time (Hiesel et al. 1994), but the extent of editing at a genome-wide level was not known until very recently, when transcriptome analyses of the lycophytes *I. engelmanii* and *S. moellendorffii* were performed (Grewe et al. 2009, 2010; Hecht et al. 2011). Over 2000 sites of editing in apparently not very large genomes set the record for perhaps the highest levels of RNA editing in any genome known so far. While RNA editing has been shown to follow adaptive distribution patterns (Jobson and Qiu 2008), its highly lineage-specific occurrence remains poorly understood. It seems that life history characteristics of organisms need to be considered when seeking explanations of such a bizarre molecular evolutionary phenomenon that is often lineage-specific.

VI. Concluding Remarks

Mitochondrial genomes of early land plants have occupied a unique position in our quest to understand evolution of this important organellar genome. Sequencing of the *Marchantia* chondrome 20 years ago provided a wealth of information for the characterization of mitochondrial genes in other basal land plants and the exploration of information for phylogenetic reconstructions. Recent sequencing of several chondromes that cover all major lineages of bryophytes and lycophytes allowed detailed examination of various aspects of this genome as well as identification of the major point of genomic structural change in plant mitochondrial genome evolution. These studies helped to develop a more complete understanding of mitochondrial genome evolution in plants and eukaryotes. In the future, it will be desirable to obtain chondrome sequences of some ferns and more bryophytes, so that a comprehensive understanding of this genome and its various intriguing aspects, such as gene loss, intron acquisition and RNA editing, can be systematically developed and correlated to the major transitions in land plant evolution.

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