

# Angiosperm mitochondrial genomes and mutations

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## Abstract

Flowering plants harbor the largest mitochondrial genomes reported so far. At present, the nucleotide sequences of 15 mitochondrial genomes from seven angiosperm species are available, making detailed comparative analysis feasible. The gene content is variable among the species, but the most striking feature is the fluidity of intergenic regions, where species-specific sequences predominate. Additionally, angiosperm mitochondrial genomes, even within a species, show a remarkable amount of rearrangement. We also review mitochondrial mutants in angiosperms from a genomic viewpoint, and discuss how they have arisen. The involvement of nuclear genes in mitochondrial genome stability and organization is currently being revealed through the analysis of mutants.

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## 1. Introduction

The largest mitochondrial genomes reported so far are those of flowering plants, whose sizes are estimated to range from 200 to more than 2400 kbp (reviewed in Schefler, 1999). From investigations of both plant and non-plant mitochondrial genomes (Knoop, 2004; Gray et al., 2004; Terasawa et al., 2007), it has been inferred that the sizes of angiosperm mitochondrial genomes have expanded since plants colonized the land although the genomes have lost some genes during this evolutionary period. This means that intergenic regions have expanded in angiosperm mitochondria. Therefore, the evolutionary trend of the angiosperm mitochondrial genome is counter to that of the mammalian mitochondrial genome, which has become smaller and more compact since the endosymbiotic origin of mitochondria (Scheffler, 1999). Another characteristic of angiosperm mitochondrial genomes is the high degree of variation in terms of the genomic organization, because frequent rearrangements are still ongoing in

almost every angiosperm lineage. During this process, unique mitochondrial mutants have arisen as by-products.

Due to initial technical difficulties in sequencing the complex and large DNA molecules, the entire nucleotide sequence of an angiosperm mitochondrial genome was not known until 1997, when Brennicke's group reported the 366,924 nucleotides of the model plant *Arabidopsis thaliana* (Unsold et al., 1997). Subsequently, the complete nucleotide sequences of 15 mitochondrial genomes from seven angiosperm species have been made available (Table 1) (Kubo et al., 2000; Notsu et al., 2002; Handa, 2003; Satoh et al., 2004; Clifton et al., 2004; Sugiyama et al., 2005; Ogihara et al., 2005; Tian et al., 2006; Allen et al., 2007). In this review, we will first describe briefly the organization of angiosperm mitochondrial genomes. Then, we will give an overview of angiosperm mitochondrial mutants classified on the basis of their associated mutations. More detailed information has been presented in earlier reviews on the first topic (Kubo and Mikami, 2007; Kmiec et al., 2006; Knoop, 2004; Bullerwell and Gray, 2004; Adams and Palmer, 2003; Marienfeld et al., 1999). For details about cytoplasmic male sterility and nuclear–mitochondrial interaction, see Carlsson et al.

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Table 1  
Comparison of gene content among angiosperm mitochondrial genome

Plant:	Sugar beet		Tobacco	Arabidopsis	Rapeseed	Rice				Maize					Wheat
Cultivar/strain:	TK81-O	TK81-MS	Bright Yellow04	Col-0	Wester	Japonica, Nipponbare	Japonica, Nipponbare-S	Japonica, PA64S	Indica, 93-11	B37N	A188	B37S	B37T	B37C	Chinese spring
Name of cytoplasm or genome type		Owen cytoplasm			<i>nap</i> cytoplasm					NB	NA	S (USDA) cytoplasm	T (Texas) cytoplasm	C (Charrua) cytoplasm	
Size of master circle (nucleotides)	368,801	501,020	430,597	366,924	221,853	490,520	490,669	490,673	491,515	569,630	701,046	557,162	535,825	739,719	452,528
Accession number	BA000009	BA000024	BA000042	Y08501	AP006444	BA000029	DQ167400	DQ167807	DQ167399	AY506529	DQ490952	DQ490951	DQ490953	DQ645536	AP008982
Complex I	<i>nad1</i> , 2, 3, 4, 4L, 5, 6, 7, 9	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Complex II	<i>sdh3</i>	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	<i>sdh4</i>	ψ	ψ	+	ψ	ψ	-	-	-	-	-	-	-	-	-
Complex III	<i>cob</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Complex IV	<i>cox1</i> , 2, 3	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Complex V	<i>atp1</i> , 4, 6, 8, 9	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cytochrome <i>c</i> biogenesis	<i>ccmB</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	<i>ccmC</i>	+*1	+*1	+	+	+	+	+	+	+	+	+	+	+	+
	<i>ccmFC</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	<i>ccmFN</i>	+	+	+	+*2	+*2	+	+	+	+	+	+	+	+	+
Ribosomal protein	<i>rpl2</i>	-	-	+	+	+	+	+	+	-	-	-	-	-	ψ
	<i>rpl5</i>	+	+	+	+	+	+	+	+	-	-	-	-	-	+
	<i>rpl16</i>	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	<i>rps1</i>	-	-	+	-	-	+	+	+	+	+	+	+	+	+
	<i>rps2</i>	-	-	-	-	-	+	+	+	+	+	+	+	+	+
	<i>rps3</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	<i>rps4</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	<i>rps7</i>	+	+	-	+	+	+	+	+	+	+	+	+	+	+
	<i>rps10</i>	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	<i>rps11</i>	-	-	-	-	-	ψ	ψ	ψ	-	-	-	-	-	-
	<i>rps12</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	<i>rps13</i>	+	+	+	-	-	+	+	+	+	+	+	+	+	+
	<i>rps14</i>	-	-	ψ	ψ	+	ψ	ψ	ψ	-	-	-	-	-	ψ
	<i>rps19</i>	-	-	+	ψ	-	+	+	+	-	-	-	-	-	ψ
Protein translocator	<i>mttB</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intronic ORF in the fourth intron of <i>nad1</i>	<i>mat-R</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+
rRNA	<i>rrn5</i> , 18, 26	+	+	+	+	+	+	+	+	+	+	+	+	+	+
tRNA	Native	11	11	12	11	11	10	10	10	10	10	10	10	10	10
	Chloroplast-like	6	6	9	4	6	7	7	7	6	6	6	6	6	6
	Origin unknown	1	1	0	0	0	0	0	0	0	0	0	0	0	0
CMS-associated ORF			<i>preSatp6</i>				<i>orf222</i>					<i>orf355/</i> <i>orf77</i>	<i>T-urf13</i>		

+, presence of the gene; -, absence of the gene; ψ, pseudo gene; \*1, unusual N-terminal extension; \*2, split into two genes.



## 2.2. Genetic information

The sizes of the master chromosomes of the sequenced mitochondrial genomes range from 221,853 (rapeseed) to 739,719 bp (maize CMS-C; Fig. 1) (Table 1). However, because these figures include repeated sequences or large duplications ranging from 0.2 to 120 kbp, the net genetic complexity (i.e., the size following removal of one copy of each large repeat) is much smaller. In addition to these long repeats, short repeated sequences can be found frequently. The difference between the large repeats and short repeats is not only the length but also the activity of recombination. Whereas many of the large repeats appear to actively and frequently recombine, the short repeats are usually inactive, but they appear to play a central role in the evolution of angiosperm mitochondrial genomes (Andre et al., 1992; Conklin and Hanson, 1994; see below).

The number of mitochondrial genes in angiosperms is 50–60 (not considering copy number), as summarized in Table 1. The differential number of genes is due to the differential gene content for the subunits of Complex II, and especially, ribosomal proteins and tRNAs. When the content of ribosomal protein genes is compared among angiosperms, one can realize how often genes have been lost from the mitochondrial genome during angiosperm evolution. Most of the genes that are lost from the mitochondrion appear to have been transferred to the nuclear genome (Adams and Palmer, 2003), but this is not always the case. For example, one of the duplicated *rps13* genes of plastid origin in the *A. thaliana* nuclear genome has been recruited to encode a mitochondrially targeted polypeptide (Mollier et al., 2002).

Plant mitochondrial genes are translated according to the universal genetic code. However, the composition of transfer RNA genes in angiosperm mitochondria is quite unique. Of the 15–21 tRNA genes encoded, 10–12 are orthologous to those residing in moss mitochondria, and thus are considered to be descendants of those harbored by the initial endosymbiont from the origin of mitochondria: this class of tRNA genes is called ‘native’ (Dietrich et al., 1992; Marienfeld et al., 1999; Kubo and Mikami, 2007). The remaining tRNA genes either have high homology to chloroplast DNA or their origin is unknown; i.e., their sequences do not match tRNAs from any known source. Differential allocation of tRNA genes to these classes is frequently found: for example, whereas tRNA<sup>Cys</sup> (GCA) is encoded by a native class gene in many dicotyledonous plants, an origin-unknown gene is the only functional tRNA<sup>Cys</sup> (GCA) gene in sugar beet, and grass family members use a chloroplast-like gene (Kubo et al., 2000). This suggests that recruitment of tRNA genes has occurred independently in several angiosperm lineages. Another example is tRNA<sup>His</sup> (GUG): although most of the angiosperms have a corresponding chloroplast-like gene, no tRNA<sup>His</sup> (GUG) gene is found in wheat mitochondria (Ogihara et al., 2005). Lack of this tRNA gene

is compensated for by the import of cytosolic tRNA<sup>His</sup> (GUG) molecules (Dietrich et al., 1992; Kumar et al., 1996).

In general, the import of tRNA molecules from the cytosol is common in angiosperm mitochondria. Sequence analysis of mitochondrial genomes revealed that tRNAs for alanine, arginine, leucine, threonine, and valine are commonly lost (Marienfeld et al., 1999; Kubo and Mikami, 2007), so the corresponding tRNAs would be expected to be imported. Additional tRNA molecules have to be imported in some plant lineages: for example, tRNAs for phenylalanine, methionine (elongator), and tryptophan are needed in *A. thaliana* mitochondria (Marienfeld et al., 1999; Duchene and Marechal-Drouard, 2001). tRNA molecules are presumed to pass through the mitochondrial membrane through voltage dependent anion channels (Salinas et al., 2006).

Some of the mitochondrial genes in angiosperms are interrupted by introns. In each of the sequenced genomes, the total number of the introns is 20–24, constituting 4–13% of the genome. All the introns in the sequenced mitochondrial genomes are classified as group II type; however, a horizontally transferred group I intron has also been documented (Cho et al., 1998). One of the characteristics of angiosperm mitochondrial introns is the presence of trans splicing, particularly for genes encoding Complex I subunits. This was first reported for *nad1* (Chapdelaine and Bonen, 1991; Wissinger et al., 1991); then *nad2* and *nad5* were revealed to contain trans-splicing introns (Binder et al., 1992; Knoop et al., 1991; Pereira de Souza et al., 1991). That there was a transition from a cis to a trans arrangement of introns during their evolution was supported by the finding that all the trans introns in angiosperms are in cis arrangements in non-angiosperm plants (Malek and Knoop, 1998). Interestingly, sometimes the transition seems to have occurred independently in multiple lineages of angiosperms. For example, although sugar beet, petunia, and tobacco have a trans-splicing intron between the fourth and fifth exons of *nad1*, careful phylogenetic analysis concluded that they have independent origins (Qiu and Palmer, 2004). For details on plant introns and splicing, see Bonen (2007) in this issue.

RNA editing is a necessary step for angiosperm mitochondrial gene expression, which converts cytidine residues to uridine and, rarely, uridine to cytidine. Most of the RNA editing sites are found in the protein-coding genes, and a few are in tRNAs, untranslated regions, and introns (Shikanai, 2006). The numbers of editing sites have been determined for some of the sequenced angiosperm species, ranging from a total of 357 in sugar beet (Mower and Palmer, 2006) to 491 in rice (Notsu et al., 2002). Comparative analysis of editing sites revealed that there are a number of species-specific editing sites: for example, *A. thaliana* and rapeseed have species-specific editing at 83 and 69 sites, respectively (Handa, 2003). For details about RNA editing, see Takenaka et al. (2008) in this issue.

### 2.3. 'Promiscuous' DNA in intergenic regions

Because the gene-coding regions constitute only 7–17% of the mitochondrial genome in the angiosperms sequenced to date, the remaining regions (intergenic regions) were examined to discover how the genome sizes had expanded in angiosperms. However, no conclusive mechanism has been proposed yet. Database searching using intergenic regions as a query revealed that some intergenic regions contain chloroplast DNA sequences (1.6–6.2% of the genome) and nuclear DNA sequences (0.1–13.4% of the genome) (Kubo and Mikami, 2007; Allen et al., 2007). Nuclear or chloroplast DNA that has incorporated into the mitochondrial genome has been called 'promiscuous' DNA (see Timmis et al., 2004). Promiscuous DNA has never been reported in animal mitochondrial genomes (Scheffler, 1999). As was mentioned earlier, chloroplast DNA sequences in mtDNA can be sources of some functional tRNA genes, such as *trnD*-GAC, *trnH*-GUG, *trnM*-CAU, *trnN*-GUU, *trnS*-GGA and *trnW*-CCA (Marienfeld et al., 1999; Kubo and Mikami, 2007). It should be noted that chloroplast DNA sequences have also been reported to serve as the promoter for a mitochondrial gene (Nakazono et al., 1996); however, this is not commonly found. Other possible roles are currently unknown.

Nuclear DNA sequences have been found by database searches as well as by hybridization analyses, but there are no reports to identify any functions for these nuclear DNA sequences in mitochondria. Overall, the functional significance of promiscuous DNA does not seem great except for the above-mentioned examples. Promiscuous DNA is rarely conserved among angiosperm mitochondrial genomes.

### 2.4. Intergenic DNA "of unknown origin" in plant mitochondrial genomes

Outside of genes, which themselves are highly conserved, and after accounting for chloroplast, nuclear and plasmid DNA insertions, the majority of the DNA in the sequenced plant mitochondrial genomes is of unrecognizable origin. Considering the compact and conserved nature of animal mitochondrial genomes, it was truly surprising to find that, in the first sequenced angiosperm mitochondrial genomes, over half of each genome had no homology to any sequences in the public databases (Unsold et al., 1997; Kubo et al., 2000; Notsu et al., 2002; Handa, 2003; Clifton et al., 2004).

An unusual case of large amounts of *recognizable* sequences in intergenic mtDNA has been discovered in the basal angiosperm, *Amborella trichopoda* (Bergthorsson et al., 2004). It contains over 250 mitochondrial gene sequences with high sequence similarity to other, usually eudicot, mtDNA. Palmer and colleagues have proposed that this situation results from relatively recent mitochondrion-to-mitochondrion gene transfer (Richardson and Palmer, 2007). Multiple and ongoing horizontal gene trans-

fer events may be responsible for the expansion of the *A. trichopoda* mitochondrial genome to over 4000 kb in size (J. Palmer, personal communication). A number of other sporadic mitochondrial horizontal gene transfer (HGT) events have been recently reported in a variety of plant species (reviewed in Richardson and Palmer, 2007).

The ways that plant mitochondria from distant relatives could come in contact with one another to transmit information is currently a matter of speculation and could include illegitimate pollination, insect vectors, and parasitic and epiphytic growth on host plants (Richardson and Palmer, 2007). Thus, although *A. trichopoda* may represent the extreme case, rare HGT among mitochondria over evolutionary time spans could be one of the possible sources of the additional mtDNA present in angiosperm mitochondrial genomes. After this extra, unneeded DNA is incorporated, it would be subject to rearrangement and loss such that it becomes unrecognizable with the passage of time.

### 2.5. Genome rearrangements and acquisition/loss of nucleotide sequences

It is well known that the arrangement of mitochondrial genes can vary, even within a single angiosperm species (e.g., Satoh et al., 2004; see also Fig. 1). This is because angiosperm mitochondrial genomes have undergone frequent genome rearrangement over the course of time. In this process, DNA recombination leading to inversions or deletions has played a major role. For example, some but not all the rearrangements between two sugar beet genomes may involve homologous recombination via 9–376 bp repeated sequences (Satoh et al., 2006; see also below).

The distribution of rearrangement points was investigated through comparative analysis of five maize genomes (Allen et al., 2007). Interestingly, rearrangement points are unevenly distributed in the genome, but the reason for this is unclear. Most of the rearrangements do not affect gene expression; i.e., they just produce a polymorphism between the genomes. In rare cases they are critical for mitochondrial gene expression (see below).

During successive genome rearrangements, angiosperm mitochondrial genomes have lost and acquired some sequences. When the first two sequenced angiosperm genomes, *A. thaliana* and sugar beet, were compared, shared sequences between the two were found to comprise only 21% of each genome, consisting of gene-coding regions and their flanks (Kubo et al., 2000). It is also surprising that intra-specific comparison of angiosperm mitochondrial genomes revealed 0.48–13.6% of the genome to be specific to either of the genomes (Satoh et al., 2004, 2006; Allen et al., 2007). Much of the 'mitotype-specific sequence' has been determined to originate from the chloroplast, the nucleus or from plasmids resident in the mitochondria; however, DNA whose origin is unknown can also be specific to a single mitotype (Satoh et al., 2006; Allen et al., 2007).

### 3. Mitochondrial mutants in angiosperms

#### 3.1. Caused by point mutation

Whereas a number of point mutations in mitochondrial genomes are reported to be associated with human diseases (Moraes, 1998), very few point mutations that impair mitochondrial function have been described in angiosperm mitochondria. A unique mitochondrial mutant has been found in wild beet populations in which the activity of cytochrome *c* oxidase is decreased by 50% compared to normal beet; its COXII polypeptide is missing the final eight amino acids due to truncation by a nonsense mutation in the *cox2* ORF (Ducos et al., 2001). Interestingly, this mutated *cox2* is present in a homoplasmic state, i.e., no normal copy of the *cox2* gene is detected, and only a shorter, less stable COX2 protein is synthesized (Ducos et al., 2001). The phenotype is male sterile but otherwise normal. How does such a mitochondrial mutant survive in wild populations despite such a large decrease in mitochondrial electron transport activity? One answer is that plants have an alternative respiratory pathway (see Rasmusson et al. (2007) in this issue) that appears to be increased in this mutant, which may be sufficient to compensate for the 50% reduction in the cytochrome pathway.

Comparative analyses of nucleotide sequences between normal and mutant mitochondrial genomes that are responsible for maternally inherited male sterility phenotypes (called cytoplasmic male sterility or CMS) has been done in sugar beet (Satoh et al., 2004) and maize (Allen et al., 2007). These analyses revealed a total of 1–24 nucleotide substitutions in gene-coding regions; however, the functional significance for mitochondrial gene expression has not yet been experimentally elucidated. On the other hand, it should be noted that most CMS-related mutations, including the above-mentioned examples in sugar beet and maize, are presumed to be associated with the expression of aberrant ORFs (Yamamoto et al., 2005; Allen et al., 2007; see below).

#### 3.2. Caused by deletion

Some angiosperm mitochondrial mutants are caused by deletions resulting from recombination via rather short (6 to less than 100 nucleotides) repeated sequences, leading to the loss of parts of specific mitochondrial genes. This class of mutants exhibits abnormal phenotypes such as retarded development and, in several cases, leaf variegation due to chlorophyll disorders (a pleiotropic effect of the mitochondrial deficiency). In maize, a series of spontaneous defective-growth mutants, known as non-chromosomal stripe (NCS), have been described that are heteroplasmic for deletions in mitochondrial genes such as *cox2*, *nad4*, and *rps3* (Lauer et al., 1990; Marienfeld and Newton, 1994; Newton et al., 1996). In a tobacco-related species, *Nicotiana sylvestris*, apparently homoplasmic *nad7*-deficient mutants that exhibit male sterility and

slow growth were obtained from protoplast culture (Pineau et al., 2005). These mtDNA deletion mutants have been utilized as a tool for investigating nuclear–mitochondrial interactions (Kuzmin et al., 2004; Vidal et al., 2007). A similar cucumber mutant called mosaic (MSC) was also reported, but it is still unclear which of the mitochondrial genes is deleted (Lilly et al., 2001).

In most of the defective-growth mitochondrial mutants, normal mitochondrial genes are also present in a heteroplasmic state. Cells homoplasmic for the maize NCS6 *cox2* deletion can be obtained by tissue culture, but it is quite difficult to regenerate a plantlet (Gu et al., 1994), suggesting that mitochondrial activity is insufficient for development when the *cox2* deletion mutation is homoplasmic. Homoplasmic NCS2 maize plants lacking functional *nad4* have been found on rare occasions in the field, but such plants exhibit both male and female sterility and severely depressed growth (Yamato and Newton, 1999).

#### 3.3. Caused by an aberrant ORF

In angiosperms, the most commonly seen class of mitochondrial mutant is cytoplasmic male sterility (CMS), in which male gametophytic development is impaired but the plant is otherwise normal (Chase, 2007; Hanson and Bentolila, 2004). Mitochondrial genomes of CMS plants have an identical set of genes to that of normal mitochondria (Satoh et al., 2004; Allen et al., 2007). Efforts to identify the responsible genes in mitochondrial genomes resulted in the discovery of aberrant open reading frames, consisting of fragments of mitochondrial genes and/or unknown sequences, usually with a chimeric structure.

In a few cases, aberrant ORFs are translated into unique polypeptides associated with mutant phenotypes. For example, *pcf*, which encodes a 25 kDa polypeptide associated with petunia CMS, consists of four parts: a fragment of *atp9*, two *cox2* fragments, and a sequence of unknown origin (reviewed by Hanson and Bentolila, 2004). Aberrant ORFs can also exist within gene-coding regions such as the 5' leader of the *atp6* gene. In sugar beet, the 5' leader of *atp6* in a CMS variety is extended to 387 codons and is translated into a CMS-associated 35 kDa polypeptide, whereas the translation product of core *atp6* is unaffected (Yamamoto et al., 2005).

To date, a number of aberrant ORFs, each producing unique polypeptides, have been reported to be associated with CMS (Chase, 2007; Hanson and Bentolila, 2004). Surprisingly, the aberrant ORFs have little in common, even when their phenotype is very similar. This suggests that the CMS-associated aberrant ORFs have arisen independently during the course of angiosperm evolution. In all the sequenced angiosperm mitochondrial genomes, ORFs of unknown function are found and some of them exhibit chimeric organization, although they are unlikely to be translated (Marienfeld et al., 1997; Yamamoto et al., 2005). Whether the ORFs of unknown function are starting to evolve into novel aberrant ORFs conferring some

specific phenotype or whether they are accumulating mutations and becoming degraded is not known.

Although most of the aberrant ORFs are associated only with male-sterile flowers, additional phenotypes can be observed. In maize CMS-T, male sterility is accompanied by sensitivity to specific fungal toxins and an insecticide, which results from pleiotropic effects of its aberrant ORF, *T-urf13-T* (Schnable and Wise, 1998). Another aberrant ORF in citrus, *ACRS*, also seems responsible for fungal-toxin sensitivity, but *ACRS* is not associated with CMS (Ohtani et al., 2002). Unlike the deletion-class mutants, retarded growth or chlorophyll disorders are not seen with the expression of the CMS-associated chimeric genes, even though the unique polypeptides are often accumulated in mitochondria throughout the whole plant. One exception is *pvs-orf239* in common bean, which exhibits anther-specific accumulation of the translation products (Sarría et al., 1998).

Although the phenotypes of vegetative and female reproductive tissues of CMS plants show no obvious defects, physiological and gene expression differences in comparison with male-fertile (normal) plants have been reported for non-anther tissues. These include altered amounts of nucleus-coded mitochondrial proteins in ears of maize (Hochholdinger et al., 2004) and altered electron transport in cell cultures of petunia (Connett and Hanson, 1990). This suggests that accumulation of CMS-associated proteins may affect all the tissues but that there is a mechanism of compensation in non-anther tissues.

Most of the mitochondrially localized proteins that differ quantitatively in their expression between maize CMS and normal mitotypes are nucleus-coded, suggesting differential signaling from mitochondria to the nucleus (Hochholdinger et al., 2004). Differential signaling from mutant mitochondria to alter nuclear gene expression has also been reported for the deletion-class NCS mutants of maize (Karpova et al., 2002; Kuzmin et al., 2004). The topic of “retrograde regulation” by plant mitochondria has been the subject of an extensive review in a previous issue of this journal (Rhoads and Subbaiah, 2007).

It was pointed out that CMS-associated aberrant ORFs often neighbor, and are co-transcribed together with genes for ATPase subunits (Hanson and Bentolila, 2004). It is interesting that decreased activity of  $F_0F_1$ -ATPase was reported in sunflower CMS (Saber et al., 2003), which expresses a chimeric ORF that may compete with the ATP8 subunit. From an evolutionary viewpoint, comparative analyses of sequenced mitochondrial genomes revealed that flanking regions of the genes for ATPase subunits are less well conserved compared to those of other genes (Hazle and Bonen, 2007), suggesting that mutations can accumulate more readily in these regions.

#### 4. Mechanisms leading to mutations

It is apparent that the occurrence of deletions and the emergence of aberrant ORFs depends upon mitochondrial

genome rearrangements in plants. The process of genome rearrangements has been investigated through comparative analyses among closely related mitochondrial genomes, including normal mitochondria, wild relatives, mitochondrial mutants, and mitochondrial revertants (Andre et al., 1992; Conklin and Hanson, 1994). As a result, a model for the emergence of novel mitochondrial DNA molecules with deletions or duplications was proposed as follows. Due to active recombination via large repeated sequences, multiple molecules including master chromosomes and subgenomes co-exist in dynamic equilibrium in mitochondria. If sporadic recombination via short repeated sequences of direct orientation occurs in a master chromosome, unusual subgenomes arise, and if one of the unusual subgenomes recombines with a normal subgenome, then the resultant larger chromosome will have a novel arrangement with a deletion and a duplication (Small et al., 1989). This model can also be applied to small-scale duplications (Woloszynska et al., 2001).

Under certain conditions, the recombinant molecules, initially present at very low (substoichiometric) levels, can be transmitted to the next generation or segregated to the daughter cells, where they can become predominant in mitochondria (Small et al., 1989). One such condition is cell culture as reported by Kanazawa et al. (1994). Another inducer is the nuclear genotype. For example, the maize P2 strain is known to generate multiple independent mitochondrial mutants, reflecting both novel recombination events and amplification of substoichiometric arrangements of mtDNA, and resulting in defective phenotypes (Kuzmin et al., 2005). The mutation-inducing nuclear genotype appears to involve one or more recessive alleles, which have been transferred into other maize lines through the male parent. In common bean, a dominant allele called *Fr* specifically reduces the copy number of a mtDNA molecule containing the CMS-associated *pvs-orf239* sequence to a substoichiometric level, restoring male-fertility to the plant (Kmieć et al., 2006).

Clearly, there are nuclear genes that affect mitochondrial recombination and replication, and that stabilize mitochondrial genome organization in terms of transmission and/or segregation. Such nuclear genes have been isolated in *A. thaliana*. They include *Msh1*, the *A. thaliana* homolog of the *Escherichia coli* *MutS* mismatch repair component, *RecA3*, which is one of the three *A. thaliana* homologs of *Escherichia coli* *RecA*, and *Osb*, a member of a family of plant-specific DNA-binding proteins (Abdelnoor et al., 2003; Shedge et al., 2007; Zaegel et al., 2006). Homozygous mutations in any of the three genes results in the emergence of recombinant mtDNA that is not detectable in the wild type plant. The *atp9* locus was chosen for a detailed investigation of the recombination events in the three mutants. Interestingly, the three mutants utilized the same short repeated sequences in the *atp9* coding region for homologous recombination events, resulted in the emergence of the same new *atp9* locus, although the entire genomic arrangement of the three mutants were different. Based

on these observations, it has been proposed that the function of the normal versions of these nuclear genes is to suppress homologous recombination via short (less than 560 nt) repeated sequences in mtDNA. Although further study is necessary to verify this model, tools are now available for the efficient induction of mitochondrial mutants. RNAi knockdowns of the tobacco and tomato homologues of *Msh1* were shown to induce phenotypic defects, including male sterility, accompanied by novel mitochondrial genome organizations (Sandhu et al., 2007).

The fixation of mitochondrial mutants requires several steps including the transmission and amplification of mutated mtDNA molecules, about which little is known in plants. Initially, the amount of mutated molecules is very small, so means to transmit and amplify the mutated molecule have been proposed. One such mechanism is a bottleneck effect in which there is a decrease in mtDNA copy number during early oogenesis, and, after random segregation of the mtDNA molecules, amplification back to the original level of the pool of transmitted molecules, which may include mutant mtDNAs (Kmiec et al., 2006). To test this model in mice, the copy number of mtDNA was examined during oogenesis. Cao et al. (2007) observed that the copy number of mtDNA exceeded the expectation from a simple bottleneck theory. To explain the rapid transition from heteroplasmic to homoplasmic mtDNA populations after transmission, Cao et al. (2007) proposed that there is a segregation unit which contains several mtDNA copies. Such a study has not been conducted in angiosperms, but it does appear that the organization of mtDNA in rapidly dividing cells (e.g., root meristem) is different from other somatic cells (Kmiec et al., 2006). Obviously, this raises the fundamental question of how mtDNA molecules are organized when mitochondrial genomes are transmitted, as well as how mtDNA molecules are replicated. We reiterate that very little is known yet about these processes in angiosperms.

## 5. Conclusions

The sequencing of multiple angiosperm mitochondrial genomes enables in-depth analysis of mitochondrial genetics and mutation. The size expansion of the mitochondrial genome in angiosperms is clearly due to both duplications and increases in intergenic regions. The intergenic regions are rapidly changing compared to the gene-coding regions. This process includes deletion, inversion, and acquisition of sequences. The fluidity of intergenic regions can be observed even from intra-specific comparisons. However, it should be pointed out that the basic set of genes and genetic complexity is faithfully maintained within a species. For example, the net genetic complexity is 336–351 kbp in sugar beet and 507–537 kbp in maize (Kubo et al., 2000; Satoh et al., 2004; Allen et al., 2007); i.e., the loss and acquisition of sequences is equivalent. This suggests that expansion of the genome is not unbridled but operates under some constraint, at least within a species. The nature

of this constraint is unknown but nuclear genes must be involved, because the stability of genome organization is under control of nuclear genes, as exemplified by the several nuclear mutants mentioned above. If recombination via short repeated sequences is controlled by nuclear genes, the evolution of mitochondrial genomes and emergence of mitochondrial mutants in plants now comes under the purview of the nuclear genome.

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## References

- Abdelnoor, R.V., Yule, R., Elo, A., Christensen, A.C., Meyer-Gauen, G., Mackenzie, S.A., 2003. Substoichiometric shifting in the plant mitochondrial genome is influenced by a gene homologous to *MutS*. *Proc. Natl. Acad. Sci. USA* 100, 5968–5973.
- Adams, K.L., Palmer, J.D., 2003. Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. *Mol. Phylogenet. Evol.* 29, 380–395.
- Allen, J.O., Fauron, C.M., Minx, P., Roark, L., Oddiraju, S., Lin, G.N., Meyer, L., Sun, H., Kim, K., Wang, C., Du, F., Xu, D., Gibson, M., Cifrese, J., Clifton, S.W., Newton, K.J., 2007. Comparisons among two fertile and three male-sterile mitochondrial genomes of maize. *Genetics* 117, 1173–1192.
- Andre, C., Levy, A., Walbot, V., 1992. Small repeated sequences and the structure of plant mitochondrial genomes. *Trends Genet.* 8, 128–132.
- Backert, S., Börner, T., 2000. Phage T4-like intermediates of DNA replication and recombination in the mitochondria of the higher plant *Chenopodium album* (L.). *Curr. Genet.* 37, 304–314.
- Bergthorsson, U., Richardson, A.O., Young, G.J., Goertzen, L.R., Palmer, J.D., 2004. Massive horizontal transfer of mitochondrial genes from diverse land plant donors to the basal angiosperm *Amborella*. *Proc. Natl. Acad. Sci. USA* 101, 17747–17752.
- Binder, S., Marchfelder, A., Brennicke, A., Wissinger, B., 1992. RNA editing in trans-splicing intron sequences of *nad2* mRNAs in *Oenothera*. *J. Biol. Chem.* 267, 7615–7623.
- Bonen, L., 2007. Cis- and trans-splicing of group II introns in plant mitochondria. *Mitochondrion* 8, 26–34.
- Bullerwell, C.E., Gray, M.W., 2004. Evolution of the mitochondrial genome: protist connections to animals, fungi and plants. *Curr. Opin. Microbiol.* 7, 528–534.
- Cao, L., Shitara, H., Horii, T., Nagao, Y., Imai, H., Abe, K., Hara, T., Hayashi, J., Yonekawa, H., 2007. The mitochondrial bottleneck occurs without reduction of mtDNA content in female mouse germ cells. *Nat. Genet.* 39, 386–390.
- Carlsson, J., Leino, M., Sohlberg, J., Sundström, J., Glimelius, K., 2007. Mitochondrial regulation of flower development. *Mitochondrion* 8, 74–86.
- Chapdelaine, Y., Bonen, L., 1991. The wheat mitochondrial gene for subunit I of the NADH dehydrogenase complex: a trans-splicing model for this gene-in-pieces. *Cell* 65, 465–472.
- Chase, C.D., 2007. Cytoplasmic male sterility: a window to the world of plant mitochondrial-nuclear interactions. *Trends Genet.* 23, 81–90.

- Cho, Y., Qiu, Y.-L., Kuhlman, P., Palmer, J.D., 1998. Explosive invasion of plant mitochondria by a group I intron. *Proc. Natl. Acad. Sci. USA* 95, 14244–14249.
- Clifton, S.W., Minx, P., Fauron, C.M., Gibson, M., Allen, J.O., Sun, H., Thompson, M., Barbazuk, W.B., Kanuganti, S., Tayloe, C., Meyer, L., Wilson, R.K., Newton, K.J., 2004. Sequence and comparative analysis of the maize NB mitochondrial genome. *Plant Physiol.* 136, 3486–3503.
- Conklin, P.L., Hanson, M.R., 1994. Recombination of plant mitochondrial genomes. In: Paszkowski, J. (Ed.), *Homologous Recombination and Gene Silencing in Plants*. Kluwer Academic Publishers, Netherlands, pp. 61–81.
- Connett, M.B., Hanson, M.R., 1990. Differential mitochondrial electron transport through the cyanide-sensitive and cyanide-insensitive pathways in isonuclear lines of cytoplasmic male sterile, male fertile, and restored petunia. *Plant Physiol.* 93, 1634–1640.
- Dietrich, A., Weil, J.H., Marechal-Drouard, L., 1992. Nuclear-encoded transfer RNAs in plant mitochondria. *Annu. Rev. Cell Biol.* 8, 115–131.
- Duchene, A.-M., Marechal-Drouard, L., 2001. The chloroplast-derived *trnW* and *trnM-e* genes are not expressed in *Arabidopsis* mitochondria. *Biochem. Biophys. Res. Commun.* 285, 1213–1216.
- Ducos, E., Touzet, P., Boutry, M., 2001. The male sterile *G* cytoplasm of wild beet displays modified mitochondrial respiratory complexes. *Plant J.* 26, 171–180.
- Gray, M.W., Lang, B.F., Burger, G., 2004. Mitochondria of protists. *Annu. Rev. Genet.* 38, 477–524.
- Gu, J., Dempsey, S., Newton, K.J., 1994. Rescue of a maize mitochondrial cytochrome oxidase mutant by tissue culture. *Plant J.* 6, 787–794.
- Handa, H., 2003. The complete nucleotide sequence and RNA editing content of the mitochondrial genome of rapeseed (*Brassica napus* L.): comparative analysis of the mitochondrial genomes of rapeseed and *Arabidopsis thaliana*. *Nucleic Acids Res.* 31, 5907–5916.
- Hanson, M.R., Bentolila, S., 2004. Interaction of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell* 16, S154–S169.
- Hazle, T., Bonen, L., 2007. Comparative analysis of sequences preceding protein-coding mitochondrial genes in flowering plants. *Mol. Biol. Evol.* 24, 1101–1112.
- Hochholdinger, F., Guo, L., Schnable, P.S., 2004. Cytoplasmic regulation of the accumulation of nuclear-encoded proteins in the mitochondrial proteome of maize. *Plant J.* 37, 199–208.
- Kanazawa, A., Tsutsumi, N., Hirai, A., 1994. Reversible changes in the composition of the population of mtDNAs during dedifferentiation and regeneration in tobacco. *Genetics* 138, 865–870.
- Karpova, O.V., Kuzmin, E.V., Elthon, T.E., Newton, K.J., 2002. Differential expression of alternative oxidase genes in maize mitochondrial mutants. *Plant Cell* 14, 3271–3284.
- Kmiec, B., Woloszynska, M., Janska, H., 2006. Heteroplasmy as a common state of mitochondrial genetic information in plants and animals. *Curr. Genet.* 50, 149–159.
- Knoop, V., Schuster, W., Wissinger, B., Brennicke, A., 1991. Trans splicing integrates an exon of 22 nucleotides into the *nad5* mRNA in higher plant mitochondria. *EMBO J.* 10, 3483–3493.
- Knoop, V., 2004. The mitochondrial DNA of land plants: peculiarities in phylogenetic perspective. *Curr. Genet.* 46, 123–139.
- Kubo, T., Nishizawa, S., Mikami, T., 1999. Alterations in organization and transcription of the mitochondrial genome of cytoplasmic male sterile sugar beet (*Beta vulgaris* L.). *Mol. Gen. Genet.* 262, 283–290.
- Kubo, T., Nishizawa, S., Sugawara, A., Itchoda, N., Estiati, A., Mikami, T., 2000. The complete nucleotide sequence of the mitochondrial genome of sugar beet (*Beta vulgaris* L.) reveals a novel gene for tRNA<sup>Cys</sup>(GCA). *Nucleic Acids Res.* 28, 2571–2576.
- Kubo, T., Mikami, T., 2007. Organization and variation of angiosperm mitochondrial genome. *Physiol. Plant.* 129, 6–13.
- Kumar, R., Marechal-Drouard, L., Akama, K., Small, I., 1996. Striking differences in mitochondrial tRNA import between different plant species. *Mol. Gen. Genet.* 252, 404–411.
- Kuzmin, E.V., Karpova, O.V., Elthon, T.E., Newton, K.J., 2004. Mitochondrial respiratory deficiencies signal up-regulation of genes for heat shock proteins. *J. Biol. Chem.* 279, 20672–20677.
- Kuzmin, E.V., Duwick, D.N., Newton, K.J., 2005. A mitochondrial mutator system in maize. *Plant Physiol.* 137, 779–789.
- Lauer, M., Knudsen, C., Newton, K.J., Gabay-Laughnan, S., Laughnan, J., 1990. A partially deleted mitochondrial cytochrome oxidase 2 gene in the NCS6 abnormal growth mutant of maize. *New Biol.* 2, 179–186.
- Lilly, J.W., Bartoszewski, G., Malepszy, S., Havey, M.J., 2001. A major deletion in the cucumber mitochondrial genome sorts with the MSC phenotype. *Curr. Genet.* 40, 144–151.
- Malek, O., Knoop, V., 1998. Trans-splicing group II introns in plant mitochondria: the complete set of *cis*-arranged homologs in ferns, fern allies, and a hornwort. *RNA* 4, 1599–1609.
- Marienfild, J.R., Newton, K.J., 1994. The maize NCS2 abnormal growth mutant has a chimeric *nad4-nad7* mitochondrial gene and is associated with reduced complex I function. *Genetics* 138, 855–863.
- Marienfild, J.R., Unsel, M., Brandt, P., Brennicke, A., 1997. Mosaic open reading frames in the *Arabidopsis thaliana* mitochondrial genome. *Biol. Chem.* 378, 859–862.
- Marienfild, J., Unsel, M., Brennicke, A., 1999. The mitochondrial genome of *Arabidopsis* is composed of both native and immigrant information. *Trends Plant Sci.* 4, 495–502.
- Mollier, P., Hoffmann, B., Debast, C., Small, I., 2002. The gene encoding *Arabidopsis thaliana* mitochondrial ribosomal protein S13 is a recent duplication of the gene encoding plastid S13. *Curr. Genet.* 40, 405–409.
- Moraes, C.T., 1998. Characteristics of mitochondrial DNA diseases. In: Singh, K.K. (Ed.), *Mitochondrial DNA Mutations in Aging, Disease and Cancer*. Springer, New York, pp. 167–184.
- Mower, J.P., Palmer, J.D., 2006. Patterns of partial RNA editing in mitochondrial genes of *Beta vulgaris*. *Mol. Genet. Genomics* 276, 285–293.
- Nakazono, M., Nishiwaki, S., Tsutsumi, N., Hirai, A., 1996. A chloroplast-derived sequence is utilized as a source of promoter sequences for the gene for subunit 9 of NADH dehydrogenase (*nad9*) in rice mitochondria. *Mol. Gen. Genet.* 252, 371–378.
- Newton, K.J., Mariano, J.M., Gibson, C.M., Kuzmin, E., Gabay-Laughnan, S., 1996. Involvement of S2 episomal sequences in the generation of the NCS4 deletion mutation in maize mitochondria. *Dev. Genet.* 19, 277–286.
- Notsu, Y., Masood, S., Nishikawa, T., Kubo, N., Akiduki, G., Nakazono, M., Hirai, A., Kadowaki, K., 2002. The complete sequence of the rice (*Oryza sativa* L.) mitochondrial genome: frequent DNA sequence acquisition and loss during the evolution of flowering plants. *Mol. Genet. Genomics* 268, 434–445.
- Ogihara, Y., Yamazaki, Y., Murai, K., Kanno, A., Terachi, T., Shiina, T., Miyashita, N., Nasuda, S., Nakamura, C., Mori, N., Takumi, S., Murata, M., Futo, S., Tsunewaki, K., 2005. Structural dynamics of cereal mitochondrial genomes as revealed by complete nucleotide sequencing of the wheat mitochondrial genome. *Nucleic Acids Res.* 33, 6235–6250.
- Ohtani, K., Yamamoto, H., Akimitsu, K., 2002. Sensitivity to *Alternaria alternata* toxin in citrus because of altered mitochondrial RNA processing. *Proc. Natl. Acad. Sci. USA* 99, 2439–2444.
- Oldenburg, D.J., Bendich, A.J., 1996. Size and structure of replicating mitochondrial DNA in cultured tobacco cells. *Plant Cell* 8, 447–461.
- Palmer, J.D., Herbon, L.A., 1987. Unicircular structure of the *Brassica hirta* mitochondrial genome. *Curr. Genet.* 11, 565–570.
- Pereira de Souza, A., Jubier, M.F., Delcher, E., Lancelin, D., Lejeune, B., 1991. A *trans*-splicing model for the expression of the tripartite *nad5* gene in wheat and maize mitochondria. *Plant Cell* 3, 1363–1378.
- Pineau, B., Mathieu, C., Gerard-Hirne, C., De Paepe, R., Chetrit, P., 2005. Targeting the NAD7 subunit to mitochondria restores a functional complex I and a wild type phenotype in the *Nicotiana sylvestris* CMSII mutant lacking *nad7*. *J. Biol. Chem.* 280, 25994–26001.
- Qiu, Y.-L., Palmer, J.D., 2004. Many independent origins of *trans* splicing of a plant mitochondrial group II intron. *J. Mol. Evol.* 59, 80–89.

- Rasmusson, A.G., Geisler, D.A., Moller, I.M., 2007. The multiplicity of dehydrogenases in the electron transport chain of plant mitochondria. *Mitochondrion* 8, 47–60.
- Rhoads, D.M., Subbaiah, C.C., 2007. Mitochondrial retrograde regulation in plants. *Mitochondrion* 7, 177–194.
- Richardson, A.O., Palmer, J.D., 2007. Horizontal gene transfer in plants. *J. Exp. Bot.* 58, 1–9.
- Robinson, M.M., Wolyn, D.J., 2002. Complex organization of the mitochondrial genome of petaloid CMS carrot. *Mol. Genet. Genomics* 268, 232–239.
- Saber, M., Gagliardi, D., Balk, J., Leaver, C.J., 2003. ORFB is a subunit of F1F0-ATPase synthase: insight into the basis of cytoplasmic male sterility in sunflower. *EMBO Rep.* 4, 381–386.
- Salinas, T., Duchene, A.-M., Delage, L., Nilson, S., Glaser, E., Zaepfel, M., Marechal-Drouard, L., 2006. The voltage-dependent anion channel, a major component of the tRNA import machinery in plant mitochondria. *Proc. Natl. Acad. Sci. USA* 103, 18362–18367.
- Sandhu, A.P., Abdelnoor, R.V., Mackenzie, S.A., 2007. Transgenic induction of mitochondrial rearrangements for cytoplasmic male sterility in crop plants. *Proc. Natl. Acad. Sci. USA* 104, 1766–1770.
- Sarria, R., Lyznik, A., Vallejos, C.E., Mackenzie, S.A., 1998. A cytoplasmic male sterility-associated mitochondrial peptide in common bean is post-transcriptionally regulated. *Plant Cell* 10, 1217–1228.
- Satoh, M., Kubo, T., Nishizawa, S., Estiati, A., Itchoda, N., Mikami, T., 2004. The cytoplasmic male-sterile type and normal type mitochondrial genomes of sugar beet share the same complement of genes of known function but differ in the content of expressed ORFs. *Mol. Genet. Genomics* 272, 247–256.
- Satoh, M., Kubo, T., Mikami, T., 2006. The Owen mitochondrial genome in sugar beet (*Beta vulgaris* L): possible mechanisms of extensive rearrangements and the origin of the mitotype-unique regions. *Theor. Appl. Genet.* 113, 477–484.
- Scheffler, I.E., 1999. *Mitochondria*. Wiley-Liss, New York.
- Schnable, P.S., Wise, R.P., 1998. The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends Plant Sci.* 3, 175–180.
- Shedge, V., Arrieta-Montiel, M., Christensen, A.C., Mackenzie, S.A., 2007. Plant mitochondrial recombination surveillance requires unusual *RecA* and *MutS* homologs. *Plant Cell* 19, 1251–1264.
- Shikanai, T., 2006. RNA editing in plant organelles: machinery, physiological function and evolution. *Cell Mol. Life Sci.* 63, 698–708.
- Small, I., Suffolk, R., Leaver, C.J., 1989. Evolution of plant mitochondrial genomes via substoichiometric intermediates. *Cell* 58, 69–76.
- Sugiyama, Y., Watase, Y., Nagase, M., Makita, N., Yagura, S., Hirai, A., Sugiura, M., 2005. The complete nucleotide sequence and multipartite organization of the tobacco mitochondrial genome: comparative analysis of mitochondrial genomes in higher plants. *Mol. Genet. Genomics* 272, 603–615.
- Takenaka, M., Verbitskiy, D., van der Merwe, J.A., Zehrmann, A., Brennicke, A., 2008. The process of RNA editing in plant mitochondria. *Mitochondrion*, this issue.
- Terasawa, K., Odahara, M., Kabeya, Y., Kikugawa, T., Sekine, Y., Fujiwara, M., Sato, N., 2007. The mitochondrial genome of the moss *Physcomitrella patens* sheds new light on mitochondrial evolution in land plants. *Mol. Biol. Evol.* 24, 699–709.
- Tian, X., Zheng, J., Hu, S., Yu, J., 2006. The rice mitochondrial genomes and their variations. *Plant Physiol.* 140, 401–410.
- Timmis, J., Ayliffe, M.A., Huang, C.Y., Martin, W., 2004. Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nat. Rev. Genet.* 5, 123–135.
- Unsold, M., Marienfeld, J.R., Brandt, P., Brennicke, A., 1997. The mitochondrial genome of *Arabidopsis thaliana* contains 57 genes in 366,924 nucleotides. *Nat. Genet.* 15, 57–61.
- Vidal, G., Ribas-Carbo, M., Garmier, M., Dubertret, G., Rasmusson, A.G., Mathieu, C., Foyer, C.H., De Paepe, R., 2007. Lack of respiratory chain complex I impairs alternative oxidase engagement and modulates redox signaling during elicitor-induced cell death in tobacco. *Plant Cell* 19, 640–655.
- Wissinger, B., Schuster, W., Brennicke, A., 1991. *Trans* splicing in *Oenothera* mitochondria: *nad1* mRNAs are edited in exon and *trans*-splicing group II intron sequences. *Cell* 65, 473–482.
- Woloszynska, M., Kieleczawa, J., Ornatowska, M., Wozniak, M., Janska, H., 2001. The origin and maintenance of the small repeat in the bean mitochondrial genome. *Mol. Genet. Genomics* 265, 865–872.
- Yamamoto, M.P., Kubo, T., Mikami, T., 2005. The 5'-leader sequence of sugar beet mitochondrial *atp6* encodes a novel polypeptide that is characteristic of the Owen cytoplasmic male sterility. *Mol. Genet. Genomics* 273, 342–349.
- Yamato, K.T., Newton, K.J., 1999. Heteroplasmy and homoplasmy for maize mitochondrial mutants: a rare homoplasmic *nad4* deletion mutant plant. *J. Heredity* 90, 369–373.
- Zaegel, V., Guermann, B., Le Ret, M., Andre, C., Meyer, D., Erhardt, M., Canaday, J., Gualberto, J.M., Imbault, P., 2006. The plant-specific ssDNA binding protein OSB1 is involved in the stoichiometric transmission of mitochondrial DNA in *Arabidopsis*. *Plant Cell* 18, 3548–3563.