Critical Reviews in Plant Sciences

Title: **Male sterility-inducing mitochondrial genomes: how do they differ?**[1]

Authors: Tomohiko Kubo*, Kazuyoshi Kitazaki, Muneyuki Matsunaga, Hiroyo Kagami and Tetsuo Mikami

Affiliation: Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

Referee: Dr. Françoise Budar, INRA, Station de Genetique et d'Amelioration des Plantes, 78026 Versailles, France

* Corresponding author (E-mail: gelab@abs.agr.hokudai.ac.jp)

[1]This review is dedicated to the memory of Dr. Toshiro Kinoshita

TABLE OF CONTENTS

Abstract

Twenty-nine mitochondrial genomes from 19 angiosperm species have been completely sequenced and have been found to vary in genome size and gene content. Seven of these mitochondrial genomes are known to induce cytoplasmic male sterility (CMS), and thus can be utilized for hybrid seed production or the prevention of pollen dispersal. Genome rearrangement frequently is observed in MS-inducing mitochondria, but it also occurs as part of the normal inter- or intraspecific variation in male fertile (MF) mitochondria. Sequence analyses have revealed that the repertoire of genuine genes is indistinguishable between MS-inducing and MF mitochondria. Deleterious mutations appear to be rare in MS-inducing mitochondria, which may be consistent with the lack of systemic manifestation of CMS. On the other hand, several nucleotide substitutions remain to be investigated for their potential mild effects. Various mitochondrial ORFs are associated with CMS (CMS-ORFs). There are some common but not strict features shared by CMS-ORFs such as their uniqueness to the CMS mitochondrial genome, their association with genes for ATPase subunits, and the hydrophobic nature of their putative translation products. It should be noted that some CMS-ORFs do not satisfy all of these criteria, and ORFs that satisfy these criteria are not necessarily associated with CMS. Therefore, it is difficult to infer the capability of MS induction of mitochondrial genomes solely from their nucleotide sequences. Morphological, physiological, and molecular biological studies suggest that multiple mechanisms cause CMS. Nuclear genes that suppress CMS have been identified. Post-transcriptional suppression of CMS-ORFs mediated by a certain class of RNA binding proteins (pentatrico peptide repeat proteins) is the predominant mechanism of fertility restoration. On the other hand, CMS suppression that is not associated with post-transcriptional suppression of CMS-ORFs has also been reported, suggesting that various types of gene-products are involved in fertility restoration.

KEY WORDS: cytoplasmic male sterility, fertility restorer gene, nuclear–mitochondrial interaction, mitochondrial genome, plant mitochondria

I. Introduction

It is widely accepted that mitochondria originated from endosymbionts resembling α-proteobacteria (Gray, 1999; Scheffler, 1999). According to this Endosymbiotic Theory, the mitochondrial genome is derived from the initial endosymbiont genome. However, mitochondrial genomes have drastically diverged in terms of molecular form, genome size, gene content and arrangement, and organization of intergenic regions (Gray et al., 2004; Bullerwell and Gray, 2004). Among the known mitochondrial genomes, the angiosperm mitochondrial genome has been considered unique in terms of its organization and large size (up to ~3000 kbp, see below) ever since the initial studies of the late 1970s (e. g. Quetier and Vedel, 1977). It was not until 1997 that the first nucleotide sequence of an entire angiosperm mitochondrial genome was published (*Arabidopsis thaliana;* Unseld et al., 1997), whereas the human and bovine mitochondrial genomes had been sequenced entirely in the early 1980s (Anderson et al., 1981; Anderson et al., 1982).

The angiosperm mitochondrial genome attracts interest not only from pure science but also from researchers in the applied sciences including plant breeding. This is because the angiosperm mitochondrial genome can control cytoplasmic male sterility (CMS), an important breeding character for hybrid seed production. In this review, CMS is defined as male sterility (MS) caused by nuclear–mitochondrial interactions without additional defects in the female reproductive organs and vegetative organs (Budar et al., 2006). MS due to a sterilizing factor (S) in the mitochondrial genome can be eliminated by a specific, mostly dominant nuclear gene termed “restorer of fertility” (*Rf*). In the absence of the sterilizing factor in the mitochondrial genome, male fertility (MF) is not affected by the *Rf* genotype. A summary of genotypes and their corresponding phenotypes is shown in Figure 1. Because MS plants cannot self-pollinate, their use as a seed parent can yield large amounts of pure hybrid seeds.

CMS is known from over 140 plant species (Laser and Lersten, 1972), but not all of them are useful in practical applications. Therefore novel mitochondrial genotypes

(mitotypes) that induce CMS are sought for in many crops. For example, CMS that is sensitive to environmental conditions is considered unfavorable for hybrid breeding because incomplete MS allows for self-pollination which reduces the purity of hybrid seeds. In *Brassica*, MS induction by nap-type CMS mitochondria is unstable at high temperatures (Fan and Stefansson, 1986), making nap-type CMS impractical. In wheat, CMS mitochondria introduced from wild relatives does not induce MS under a short photoperiod which enables MS line maintenance by selfing. On the other hand, long photoperiods promote MS and the MS line becomes a suitable parent for hybrid seeds (Murai and Tsunewaki, 1993). Combining multiple CMS types reduces risks emerging from the reliance on a single type; the use of maize T-type CMS in hybrid seed production collapsed in the early 1970s due to the pleiotropic expression of disease sensitivity (Pring and Lonsdale, 1989).

As shown in Figure 1, the establishment of a CMS system requires at least two mitotypes, one of which can cause MS while the other has no effect. Therefore, efforts to discover novel CMS include two questions: first, the identification of new mitotypes in a gene pool, and second, an assessment of their potential to induce MS. The former task is not difficult if sufficient genetic resources are available because molecular techniques such as the detection of polymorphisms of restriction fragment lengths of mitochondrial DNA are well established. Furthermore, advances in nucleotide sequencing technology will enable us to obtain entire nucleotide sequences of all mitoypes in a gene pool. On the other hand, the second task appears formidable because the only way to test the capability of inducing MS is in field trials, including backcrossing. In other words, no general sequence marker is available for the easy identification of mitotypes with an S factor. What makes MS-inducing mitotypes different?

In this review, we first focus on recent progress in mitochondrial genome organization and variation, and then discuss CMS and *Rf*. Because of the definition of CMS in this review, we will not address fascinating mitochondrial mutants such as maize non-chromosomal stripes, tobacco CMSI, CMSII, and cucumber mosaic (Gabay-Laughnan and Newton, 2005; Bartoszewski et al., 2007; Vidal et al., 2007). Crop species are the main subject of this review, however, it should be noted that studies on mitochondrial genome diversity and CMS are also conducted in non-crop plants in population genetics and ecology (Tiffin et al., 2001; Murayama et al., 2004; Delph et al., 2007; McCauley and Olson, 2008; McCauley and Bailey, 2009). We will refrain from

discussing topics that were evaluated in the recent past; readers may refer to previous reviews (Schnable and Wise, 1998; Hanson and Bentolila, 2004; Budar et al., 2004; Knoop, 2004; Budar et al., 2006; Chase, 2007; Kubo and Mikami, 2007; Pelletier and Budar, 2007; Kubo and Newton, 2008; Fujii and Toriyama, 2008b; Woloszynska, 2010; Kitazaki and Kubo, 2010). In this review, unless indicated otherwise, mitochondrial genes are given in italicized lower case letters (e.g. *atp6*) while nuclear genes appear in italicized upper case letters (e.g. *AOX*). The only exception is the nuclear gene *Rf*, for historical reasons and because of the necessity to distinguish dominant (*Rf*) and recessive (*rf*) alleles. Polypeptides are given in roman upper case letters, like ATP6, AOX and RF.

II. Molecular diversity of the angiosperm mitochondrial genome

A. Genome size and organization

The molecular form of the angiosperm mitochondrial genome is controversial and the observation that a mixture of linear, circular, and networked DNA molecules constitutes the mitochondrial genome certainly requires consideration (Andre and Walbot, 1995; Bendich, 1993; Oldenburg and Bendich, 1996; Dai et al., 2005; Manchekar et al., 2009). Nonetheless, the entire set of genetic information in the angiosperm mitochondrial genome can be described by a single circular configuration, the so-called master circle (Palmer and Shields, 1984). A total of 29 master circles from 19 angiosperm species have been completely sequenced to date (Table 1). This number will no doubt increase rapidly due to technical advances in nucleotide sequencing. For example, next-generation sequencing technologies, such as the GS-FLX (also known as '454') system, have been applied recently to sequence two rice mitochondrial genomes (Fujii et al., 2010a). However, certain technical difficulties remain unresolved, such as the discrimination of mitochondrial sequences from contaminating plastid or nuclear DNA. The integrity of the obtained contigs should be verified. Moreover, the assembly of short reads also raises difficulties even though reference sequences exist, because of the variation in organization and unique sequences (F. Budar and T. Terachi, personal communication; see below).

The sizes of master circles vary from 208 to 2936 kbp (Palmer and Herbon, 1987;

Ward et al., 1981; Alverson et al., 2010); the latter value represents the largest mitochondrial genome of a eukaryote reported to date (Bartoszewski et al., 2009). As seen in Table 1, the smallest accessible mitochondrial genome sequence is 222 kbp and the largest is 983 kbp. Some master circles contain a large duplication. For example, the NA-type genome of maize has a 120-kbp duplication (Allen et al., 2007). Apart from large duplications that exceed the capacity of cloning vectors such as phages and cosmids, repeat sequences of various lengths are common in angiosperm mitochondrial genomes (Andre et al., 1992). These repeat sequences are usually classified into two classes. One class contains sequences larger than 1 kbp and active in the recombination of the repeat copies. Recombination between direct repeats in the master circle results in two subgenomic circles (loop out). On the other hand, recombination between inverted repeats results in an isomeric form of the master circle that differs in the orientation of the region between the repeats (flip flop). These recombinations may explain the heterogeneity of mitochondrial DNA molecules. Active recombination between repeat copies may be involved in DNA replication and/or repair in plant mitochondria (Marechal and Brisson, 2010).

Repeat sequences exceeding 1 kbp in length appear to be ubiquitous in angiosperm mitochondria; the only exception known for a long time was black mustard (*Brassica hirta*) (Palmer and Herbon, 1987). Recently, two additional exceptions have been found: grapevine and zucchini (Goremykin et al., 2009; Alverson et al., 2010). Although these two genomes constitute the two largest mitochondrial genomes to be completely sequenced to date, no repeat sequences exceeding 1 kbp have been found. It is not known whether active recombination in these genomes is mediated by sequences of less than 1 kbp, or if any site-specific recombination occurs. It is generally accepted that recombination between the short repeat sequences is infrequent but plays an important role in the evolution of the angiosperm mitochondrial genome (Andre et al., 1992; Arrieta-Montiel et al., 2009).

B. Genes in the genome

The angiosperm mitochondrial genome includes genes for subunits of Complexes I to V of the electron transport chain, ribosomal proteins, genes involved in cytochrome *c*

maturation, protein translocation system subunits, maturase, and genes for rRNAs and tRNAs. The arrangement of angiosperm mitochondrial genes is less conservative than in animal mitochondrial or plastid genomes, except for certain local syntenic linkages which are broken in some plant lineages (Sugiyama et al., 2004; Ogihara et al., 2005).

The number of genes in the plant mitochondrial genome varies from 50 to 69, including 30 to 37 protein-coding genes. Differences are due to the variable repertoire of genes for subunits of Complex II, ribosomal proteins and tRNAs; Table 2 shows a comparison of protein-coding genes between the smallest sequenced mitochondrial genome (rapeseed, 222 kbp) and the largest one (zucchini, 983 kbp). Differences are found for six genes, which account for only 2596 bp in total, including introns (Handa, 2003; Alverson et al., 2010). It thus can be assumed that the total number of nucleotides of genes and introns is rather constant among angiosperms at ~70 kbp, whereas the genome sizes differ more than 14-fold (208 kbp versus 2936 kbp). From these data, the variability of the size of the angiosperm mitochondrial genome can be attributed to expansion or reduction of the intergenic regions. Intergenic regions are less conservative than coding regions or introns. Stretches of such intergenic sequences that are conserved among taxa have been identified, but they are rare and their significance remains unclear (Hazle and Bonen, 2007; Alverson et al., 2010). There is no evidence that the majority of intergenic regions are under any functional constraints.

A systematic survey of protein-coding genes in angiosperm mitochondria showed that many genes for subunits of Complex II and for ribosomal proteins have been lost independently in diverse angiosperm lineages (Adams et al., 2002b). For example, loss of *sdh3* occurred in 40 different angiosperm lineages during the course of angiosperm diversification. Thus, there may be undiscovered mitochondrial genes that have been overlooked so far because they were lost from those mitochondrial genomes that have been extensively studied to date, such as that of *Arabidopsis*. In fact, *rpl10* has recently been added to the list of angiosperm mitochondrial genes (Mower and Bonen, 2009; Kubo and Arimura, 2010), but it is absent from the *Arabidopsis* mitochondrial genome. Genes encoding proteins other than Complex II or ribosomal proteins are generally preserved in the angiosperm mitochondrial genome, but a well-known exception is *cox2* which has been lost from some legume mitochondrial genomes (Nugent and Palmer, 1991; Covello and Gray, 1992).

Some protein-coding genes lost from mitochondrial genomes migrated to the nucleus and became functional in the nuclear genome. Translation products of these migratory genes are imported from the cytoplasm into the mitochondria (Adams and Palmer, 2003; Kleine et al., 2009). In other cases, no migratory copies are found in the nuclear genome. In such cases, the function of the lost genes is taken over by homologous genes that originally functioned in other cellular compartments. For example, the loss of mitochondrial *rps13* is balanced by the nuclear-encoded *RPS13* of plastid origin, and the loss of *rps8* is compensated for by *RPS15A* which originally encoded cytosolic ribosomal proteins (Adams et al., 2002a).

tRNA genes in the angiosperm mitochondrial genome are classified either as 'native' tRNA genes that are descendents of genes of the initial endosymbiont or as tRNA genes that have been transferred to the mitochondria from the plastid genome ('plastid'-like tRNA genes) or from unknown organisms (Marechal-Drouard et al., 1993; Kubo et al., 2000). In general, it is difficult to determine the functionality of a tRNA gene solely on the basis of its nucleotide sequence. For example, although plastid-like *trnW*-CCA is encoded by several angiosperm mitochondrial genes, it is expressed in wheat, maize, *Oenothera*, potato, sunflower, and sugar beet but not in *Arabidopsis* (Marechal et al., 1987; Schuster et al., 1988; Leon et al., 1989; Marechal-Drouard et al., 1990; Kubo et al., 1995; Ceci et al., 1996; Duchene and Marechal-Drouard, 2001). In this context, angiosperm species are rare in which all mitochondrial tRNA genes as well as their activities have been characterized (Joyce and Gray, 1989; Sangare et al., 1990; Duchene and Marechal-Drouard, 2001; Glover et al., 2001; Duchene et al., 2009). In wheat and *Arabidopsis*, three expressed mitochondrial tRNA genes differ in their genetic origin and four have been lost from either genome (Table 3). Lack of tRNA genes in the mitochondrial genome is compensated for by the import of cognate tRNA molecules from the cytoplasm (Salinas et al., 2008; Duchene et al., 2009).

In the angiosperm mitochondrial genomes listed in Table 1, the eleven genes interrupted by an intron(s) include *nad1*, *nad2*, *nad4*, *nad5*, *nad7*, *cox1*, *cox2*, *ccmFC*, *rpl2*, *rps3*, and *rps10*. All these introns are of the group II type except for that in watermelon *cox1*, which belongs to group I. In *nad1*, *nad2*, and *nad5*, *trans*-splicing occurs in which exons are transcribed as separate precursor RNA molecules and then combined into a single mature mRNA (Bonen, 2008). No rRNA gene has been reported to be interrupted by an intron(s) to date. A tRNA gene with an introns is of plastid origin

(Ohtani et al., 2002), and splicing of this intron has not been examined. The total size of the introns in a mitochondrial genome is 28 kbp in sugar beet, 25 kbp in maize and tobacco, 23 kbp in wheat, 32 kbp in watermelon, and 31 kbp in zucchini; the total number of introns varies from 20 (sugar beet) to 25 (grapevine) (Kubo et al., 2000; Clifton et al., 2004; Sugiyama et al., 2004; Ogihara et al., 2005; Goremykin et al., 2009; Alverson et al., 2010). Variation in intron content is well exemplified in *cox1* in which a group I intron was once horizontally transferred from a fungal donor, spread from plant to plant, and lost in some angiosperm lineages (Cusimano et al., 2008; Sanchez-Puerta et al., 2008). Watermelon is the only plant in Table 1 in which *cox1* includes a group I intron. In contrast to the group I intron in *cox1*, group II introns seem to be rather stable, but some variations are known that are due to alterations in the mode of splicing (i. e., *cis* versus *trans*) or loss of the intron (Geiss et al., 1994; Pla et al., 1995; Kubo et al., 2000; Itchoda et al., 2002; Kudla, 2002; Kim and Yoon, 2010). Acquisition of group II introns during angiosperm evolution has not been reported to date.

Plant mitochondrial genes are transcribed by T7-phage type RNA polymerase (Kuhn et al., 2009). It is possible that some accessory factors are involved (Kuhn et al., 2007). In addition to promoters that share a consensus sequence motif, non-consensus promoters are also common in angiosperm mitochondria (Holec et al., 2008). Because of the absence of an efficient transcription terminator, transcription can occur anywhere in the genome (Holec et al., 2008). Accumulation of mRNA seems to be controlled by both transcriptional and post-transcriptional processes including degradation and stabilization of transcripts where nuclear-encoded factors may be involved (Holec et al., 2008).

In many angiosperm mitochondrial transcripts, a number of specific cytidine residues are converted to uridine residues by RNA editing, but the reverse editing (uridine to cytidine) is rare (Takenaka et al., 2008). The majority of RNA editing occurs in the protein-coding regions where some modifications create the initiation and/or termination codons while others convert internal codons to better conserved ones. The number of reported editing sites in the protein-coding regions ranges from 357 to 491, but this may be an underestimation of the total number as RNA editing also occurs in introns and tRNA genes, albeit less frequently. The significance of RNA editing in these regions may be an improvement of the higher-order structure of RNA, which in turn may be associated with RNA processing (Marechal-Drouard et al., 1996; Kunzmann et al., 1998; Li-Pook-Than et al., 2004; Li-Pook-Than et al., 2007). Furthermore, RNA editing may

occur in pseudogenes that share editing sites with genuine genes (Williams et al., 1998). Some editing sites exhibit tissue specificity or nuclear genotype dependency (Bentolila et al., 2008; Zehrmann et al., 2008; Picardi et al., 2010), making it difficult to determine the precise number of editing sites in an angiosperm mitochondrial genome.

The length of the 5′- and 3′-untranslated regions (UTR) is 21–645 and 10–498 bases, respectively, in most mitochondrial genes of *Arabidopsis* (Forner et al., 2007). However, the lack of a 5′ UTR (*rps4*) or 3′ UTR (*ccmC*, *nad6* and *mttB*) also has been observed (Forner et al., 2007). Interestingly, *ccmC* translation products have been detected (Raczynska et al., 2006); the mechanisms by which such genes are expressed are unclear. The translational initiation codon is AUG but utilization of an alternative codon is possible (Bock et al., 1994; Sunkel et al., 1994; Thomson et al., 1994; Siculella et al., 1996; Sakamoto et al., 1997; Dong et al., 1998; Handa 2003).

In general, the structure of angiosperm mitochondrial genes is quite high, but several exceptions exist. One is *atp6*, whose translation product consists of a highly conserved C-terminal core region that corresponds to the mature ATP6 polypeptide (249–252 amino acid residues) and a variable N-terminal extension (5–389 amino acid residues) (Onodera et al., 1999). The N-terminal extension is removed from the precursor polypeptide to generate the mature ATP6 polypeptide (Krishnasamy et al., 1994; Yamamoto et al., 2005). While the roles of the N-terminal extension in angiosperm ATP6 are unclear, the N-terminal extension of yeast ATP6 is necessary for an efficient integration of mature ATP6 into the ATP9 ring of F_0 (Zeng et al., 2007). Sugar beet *ccmC* is another example. Its coding region encodes a conserved core region (239 amino acid residues) and an N-terminal extension (279 amino acid residues) that contains duplicated partial ATP9 and a sequence of unknown origin (Kitazaki et al., 2009). As in angiosperm ATP6, the N-terminal extension of sugar beet CCMC is removed from the precursor to generate the mature CCMC polypeptide. Accumulation of the cleaved N-terminal extension is not observed. Intriguingly, no other angiosperm *ccmC* encoding such an N-terminal extension is known. Another structurally altered gene is *rps2*, which bears a C-terminal extension of unknown origin (Kubo et al., 2005). The cleaved C-terminal extension of maize *rps2* accumulates as a solitary polypeptide as well as a mature RPS2 polypeptide (Perrotta et al., 2002). In carrot, a C-terminal extension is encoded in *atp8* (also known as *orfB*) and *cox1*, but both genes appear to be functional copies (Robison and Wolyn, 2006a, 2006b). In the case of sugar beet *ccmC*, maize *rps2*, carrot *atp8*, and carrot *cox1*, no

association between these altered mitochondrial genes and mitochondrial impairment is evident, suggesting that angiosperm mitochondria have an ability to cope with such genetic alterations. Furthermore, a significant number of probably nonfunctional ORFs are present in angiosperm mitochondrial genomes, and these comprise sequences of unknown origin and/or partially duplicated sequences (Marienfeld et al., 1999). Expression of such ORFs is usually repressed at the post-transcriptional level (Holec et al., 2008).

C. Transferred sequences

The angiosperm mitochondrial genome contains sequences homologous to the DNA of plastids, nuclei, other organisms such as fungi, and viruses. This suggests sequence transfer from other genetic compartments.

Sequences homologous to plastid DNA have been found in all seed plant mitochondrial genomes analyzed to date. Because the organization and nucleotide sequence of the plastid genome is well conserved (Palmer, 1990), the direction of sequence transfer probably is from the plastid to the mitochondrion. The proportion of plastid-derived sequence contents in angiosperm mitochondrial genomes varies from 1.6% to 11.5% (see references in Table 1). Surprisingly, the sequences homologous to plastid DNA in zucchini mitochondria are 113 kbp in length, which corresponds to 79% of the cucumber plastid genome (Alverson et al., 2010). One of the well-known roles of plastid-derived sequences in angiosperm mitochondria is their function as a source of tRNA genes [see section II (B) Genes in the genome]. Recently, chimeric mitochondrial *atp1* and *rrn18* in which parts of the coding regions had been replaced with plastid *atpA* and *rrn16*, respectively, were reported (Hao and Palmer, 2009; Sloan et al., 2010). No evidence for a preferentially transferred region of the plastid genome has been found (Wang et al., 2007). Because plastid-derived sequences are conspicuous in the mitochondrial genome, their evolutionary dynamics have been examined, leading to the conclusion that acquisition and loss of plastid-derived sequences is ongoing on an evolutionary scale. For example, a sequence homologous to *rbcL* has been independently acquired in diverse angiosperm species (Cummings et al., 2003).

Sequences homologous to nuclear DNA constitute 0.1% to 13.4% of angiosperm

mitochondrial genomes (see the references in Table 1). The identification of nucleus-derived sequences is more difficult than that of plastid-derived ones because complete nucleotide sequences of the nuclear DNA are not available for most plants at present. Sequences homologous to nuclear DNA include retrotransposons and protein-coding genes (Marienfeld et al., 1999; Kubo et al., 2000; Notsu et al., 2002; Alverson et al., 2010). The function of nucleus-derived DNA in angiosperm mitochondria is not known, but their ongoing acquisition and loss appears likely. For example, intergenic sequences that have homology to nuclear DNA were found in some mitotypes of sugar beet. These sequences are not always preserved in the other mitotypes (Satoh et al., 2006; Kawanishi et al., 2010).

From an evolutionary viewpoint, it is interesting that plastid-derived sequences have been found in the mitochondrial genomes of the gymnosperm *Cycas taitungensis* and the quillwort *Isoetes engelmannii* (Wang et al., 2007; Grewe et al., 2009). Furthermore, a nucleus-derived sequence was found in the mitochondrial genome of *Isoetes* (Grewe et al., 2009). Because the angiosperm lineage diverged from the *Cycas* and *Isoetes* lineages at least 300 and 350 million years ago, respectively, the first sequence transfers from plastids and nuclei to mitochondria must have occurred before that time. A mitochondrial sequence in *Cycas* of 18 kbp is homologous to plastid DNA and includes tRNA genes. It is possible that some, if not all, of these tRNA genes are functional in mitochondria. Mitochondrial genomes of the liverwort *Marchantia polymorpha* and the moss *Physcomitrella patens* have no plastid- or nucleus-derived sequences (Oda et al., 1992; Terasawa et al., 2007).

Emerging evidence indicates that angiosperm mitochondrial genomes contain sequences that have been horizontally transferred from non-plant organisms as well as plants (Bergthorsson et al., 2003, 2004; Won and Renner, 2003; Davis and Wurdack, 2004; Mower, et al., 2004; Davis et al., 2005; Barkman et al., 2007; Bock, 2010). The group I intron in *cox1* is one such example (Sanchez-Puerta et al., 2008) [see section II (B) Genes in the genome]. A known virus-derived sequence in *Arabidopsis* mitochondria is absent from sugar beet (Marienfeld et al., 1999; T. Kubo, unpublished data), suggesting that either the acquisition of this sequence occurred independently in a predecessor of *Arabidopsis* or that this sequence has not been maintained in all plant species. Various plasmid-derived DNAs, episomal elements that are independent of the mitochondrial genome, are sometimes found in angiosperm mitochondria; their origin may be fungi

(Handa, 2008). Remnants of plasmid-like DNA integration into the mitochondrial genome have been reported (Kubo et al., 2000; Allen et al., 2007; McDermott et al., 2008; Goremykin et al., 2009). Plant-to-plant horizontal transfer has been shown by phylogenetic analyses of mitochondrial gene sequences (Richardson and Palmer, 2007). However, the frequency of horizontal transfers in plant mitochondrial genome evolution remains controversial (Goremykin et al., 2009).

D. Origin of unknown sequences in the intergenic region

The presence of sequences of unknown origin in the intergenic regions is one of the major mysteries of the angiosperm mitochondrial genome (Kubo and Mikami, 2007; Kubo and Newton, 2008). For example, sequences of unknown origin in sugar beet mitochondria – that is, sequences without homologs in the databases – account for 55.6% of the genome (Kubo et al., 2000). Although these sequences have been considered unique to sugar beet mitochondria, it is quite possible that their apparent uniqueness is due to our limited knowledge of other mitochondrial genomes. Moreover, as research progresses, sequence data of plant nuclear DNA or DNA from other organisms will accumulate in the databases, and this new information may help to identify homologies of intergenic regions in the angiosperm mitochondrial genome. Alternatively, sequences without identifiable homologs may be truly unique because of the accumulation of unique mutations (Satoh et al., 2006).

E. How have angiosperm mitochondrial genomes expanded?

Cucurbitacean plants lend themselves to observing genome-level variation in the mitochondrial genome because this taxonomic group includes plants with average-sized (380 kbp, watermelon) as well as the largest known genomes (2936 kbp, maskmelon) (Ward et al., 1981; Alverson et al., 2010). Introns in cucumber and zucchini generally are longer than in other angiosperm mitochondria (Bartoszewski et al., 2009; Alverson et al., 2010), but it is too early to conclude that intronic expansion is involved in genomic expansion, because longer introns are also observed in the average-sized mitochondrial

genome of watermelon. However, it is possible that the long introns are remnants of the common ancestral genome of this family that may have had large mitochondrial genome (Alverson et al., 2010). On the other hand, the number of short (<1 kbp) repeat sequences appears to exhibit a correlation with the size of the mitochondrial genome in this family: large mitochondrial genomes such as those of zucchini and cucumber are rich in short repeated sequences compared to watermelon (Lilly and Havey, 2001; Alverson et al., 2010). No correlation between the mitochondrial and nuclear genome size has been found in the family Cucurbitaceae (Arumuganathan and Earle, 1991; Ren et al., 2009; Alverson et al., 2010).

The size of the grapevine mitochondrial genome is the second largest after zucchini (Table 1). A common feature shared by grapevine and zucchini mitochondria is the relatively large number of plastid-derived sequences (Goremykin et al., 2009; Alverson et al., 2010). This suggests that transferred sequences could contribute to angiosperm mitochondrial genome expansion. Repeated sequences of the grapevine mitochondrial genome have not been investigated in detail so far.

F. How did angiosperm mitochondrial genomes become so varied?

DNA recombination plays an important role in the alteration of the organization of the angiosperm mitochondrial genome. The rate of nucleotide substitutions in the angiosperm mitochondrial genome is low compared with that in animal mitochondrial genomes (Palmer, 1990), with some exceptions (Laroche et al., 1997; Cho et al., 2004; Mower et al., 2007; Barr et al., 2007; Sloan et al., 2008, 2009). Comparative analysis of the mitochondrial genomes between closely related species and within species revealed genome rearrangements as well as deletions and/or insertions, which are based on DNA recombination mediated by short (<1 kbp) repeat sequences (Palmer, 1988; Allen et al., 2007; Nishizawa et al., 2007; Marechal and Brisson, 2010). Considering the large number of short repeat sequences (for example, 33 repeat families of 108–556 bp are found in *Arabidopsis* mitochondria), the integrity of the plant mitochondrial genome would be endangered if recombination occurred freely. Nuclear genes that apparently suppress such recombination have been identified (Abdelnoor et al., 2003; Zaegel et al., 2006; Shedge et al., 2007; Odahara et al., 2009).

A current model of angiosperm mitochondrial genome evolution involving DNA recombination includes the regulation of the copy number of recombinant DNA molecules: a recombinant molecule can either be maintained as a substoichiometric DNA species, become a major DNA species, or become lost (Small et al., 1989). The transition from a substoichiometric to a major species or vice versa is called substoichiometric shifting (SSS). SSS occurs during angiosperm evolution and can be induced by a specific genotype or physiological condition (Small et al., 1989; Kanazawa et al., 1994; Janska et al., 1998; Arrieta-Montiel et al., 2001; Abdelnoor et al., 2003; Kuzmin et al., 2005; Zaegel et al., 2006; Shedge et al., 2007; Bartoszewski et al., 2007; Arrieta-Montiel et al., 2009). This suggests that although DNA recombination is under strict control in angiosperm mitochondria, some DNA molecules with accumulated mutations can 'hide' in the angiosperm mitochondria and can be transmitted to progeny.

Intraspecific variation is common in angiosperm mitochondrial genomes. Examples of such variations include rather small alterations such as deletions (*B. nigra*), inversions (*B. hirta*), or an altered number of tandem repeats (sugar beet) (Palmer, 1988; Cheng et al. 2010). On the other hand, extensive genome rearrangements have also been documented (Fauron and Casper, 1994; Nishizawa et al., 2007). For example, organizational differences between NA- and NB-type mitochondrial genomes (both can be used as the cytoplasmic genetic component of maintainer lines) of maize are characterized by 16 rearrangements and the occurrence of 2% and 5 %, respectively, of mitotype-specific sequences not present in the other genome (Allen et al., 2007). Therefore, diversification of the angiosperm mitochondrial genome by the above-mentioned mechanisms appears to be rapid. On the other hand, coding regions are generally highly conserved; however, a 6-base insertion and a synonymous substitution were found in *atp4* (also known as *orf25*) and *nad4L*, respectively (Allen et al., 2007). Similar results were obtained for rice and wheat when nucleotide sequences of the mitochondrial genomes were compared between varieties (Tian et al., 2006; Cui et al., 2009).

III. The mitochondrial genome and cytoplasmic male sterility (CMS)

A. Potential sources of CMS

Before describing the mitochondrial genomes of CMS plants, it is worth reviewing the sources of CMS. In some cases, natural CMS plants have been found in a crop variety or a breeding line. In other cases, CMS plants have been obtained from crosses between different cultivars, species, and genera (Kaul, 1988). Table 1 contains seven mitochondrial genomes that are known to induce MS on certain nuclear backgrounds. Owen-type CMS in sugar beet, T-type, S-type, and C-type CMS in maize, and nap-type CMS in rapeseed were found in crop varieties or breeding lines (Owen, 1942; Duvik, 1965; Beckett, 1971; Thompson, 1972). Genealogical analysis based on sequence polymorphism of plastid and mitochondrial DNA supported the notion that Owen-type cytoplasm is an intraspecific variant rather than introgressed alien cytoplasm (Fenart et al., 2006; Nishizawa et al., 2007). On the other hand, LD- and CW-type CMS in rice were identified after crossing different cultivar groups (japonica and indica) and different species (*Oryza sativa* and *O. rufipogon*), respectively. In both cases, the cytoplasmic donor parent is MF, but MS was expressed after recurrent backcrossing with a japonica cultivar as the pollen parent (Katsuo and Mizushima, 1958; Watanabe et al., 1968). The plants obtained from the reciprocal crosses were MF (Katsuo and Mizushima, 1958; Watanabe et al., 1968).

Another source of CMS is cell fusion, which can induce a novel type of CMS (Dubreucq et al., 1999; Carlsson et al., 2007). Recently, it was shown that RNA interference directed at a gene involved in mitochondrial genome stability induced MS with an altered mitochondrial genome (Sandhu et al., 2007). The altered mitochondrial genome was transmitted through the female parent and continued to induce MS (i. e. it never reverted to an MF genome after segregation of the transgene). No *Rf* for this MS has been reported to date and this is a future challenge.

B. What do mitochondrial genomes of CMS plants tell us?

Molecular studies of CMS have scrutinized mitochondrial genome organization ever since CMS-associated loci were identified in mitochondria. In addition to the seven CMS mitochondrial genomes shown in Table 1, physical maps of pet1-type CMS (sunflower), Ogura-type CMS (radish), pol-type CMS (rapeseed), G-type CMS (sea beet, a wild relative of sugar beet), common bean CMS, and petunia CMS have been provided

(Makaroff and Palmer, 1988; Siculella and Palmer, 1988; Folkerts and Hanson, 1991; L'Homme and Brown, 1993; Janska and Mackenzie, 1993; Kawanishi et al., 2010). Comparisons of CMS and MF (i.e., maintainer line) mitochondrial genomes suggest that some CMS mitochondrial genomes are highly rearranged and include inversions, insertions and deletions, whereas others preserve colinearity and possess few rearranged regions. The former class includes two sugar beet CMS, three maize CMS, petunia CMS, and two rice CMS, while the latter class comprises sunflower CMS and pol-type CMS in rapeseed. Nucleotide sequences of CMS mitochondria suggest that DNA recombination leading to genome rearrangement is mediated by short repeat sequences (Satoh et al., 2006; Allen et al., 2007). Nucleotide sequences unique to CMS mitochondria contribute 5% to 14% of the genome, whereas those unique to MF mitochondria amount to 3% to 8% (Satoh et al., 2006; Allen et al., 2007). Genomic alterations found in CMS mitochondria reflect the mitochondrial genome diversity in angiosperms, supporting the view that CMS mitochondria have emerged through the common evolutionary mechanisms acting on angiosperm mitochondrial genomes.

It should be emphasized that the portion of the CMS genotypes listed in Table 1 that contains conserved genes of known function (compare Table 2) is indistinguishable from that of MF mitochondria of the same species. In other words, the normal repertoire of mitochondrial genes is present in CMS mitochondria, suggesting that deleterious mutations are unlikely to be responsible for CMS (Satoh et al., 2004; Allen et al., 2007; Fujii et al., 2010a) [see section III (C) CMS-associated loci for the only known exception]. Sequences of tRNA genes are identical in CMS and MF mitochondria. In rRNA- and protein-coding genes, some nucleotide substitutions and small indels exist, but the effects of these mutations, if any, and their phenotypic consequences have not been investigated to date.

C. CMS-associated loci

An apparent lesion of a genuine mitochondrial gene in CMS mitochondria has been found only in G-type CMS in sea beet (*Beta vulgaris* ssp. *maritima*) (Ducos et al., 2001). In this CMS type, a nonsense mutation truncates eight C-terminal amino acid residues of COX2, and another mutation adds 14 residues to the C-terminus of NAD9 (Fig. 2) (Ducos et al.,

2001). *Rf* for the G-type CMS exists in populations of sea beet and sugar beet (Touzet et al., 2004; Kawanishi et al., 2010), but its molecular action and the gene products remain unclear. In other cases where CMS-associated loci have been identified, the loci have included ORFs unique to CMS mitochondria. A well-known example is an ORF of 115 amino acid residues termed *urf13-T* in maize T-type CMS, which consists of duplicated fragments of the coding and flanking regions of the *rrn26* gene (Fig. 2) (Dewey et al., 1986). Like *urf13-T*, unique ORFs found in the CMS-associated loci exhibit a characteristic organization that presumably results from an accumulation of mutations; otherwise, their origin is unknown. In general, homologies between the unique ORFs are rare: CMS genomes from different sources usually include different and unique ORFs. One exception is the involvement of *orf224*-variants in some *Brassica* CMS. *orf224* was first discovered in pol-type CMS (Singh and Brown, 1991) before a related ORF, *orf222*, was found in nap-type CMS (Fig. 2) (L'Homme et al., 1997), and yet another variant exists in stem mustard CMS (Yang et al., 2009). In radish, *orf138* was first identified as a unique ORF in Ogura-type CMS (Fig. 2) (Bonhomme et al., 1992), and at least nine variants have been found subsequently in wild radish accessions and radish cultivars (Iwabuchi et al., 1999; Yamagishi and Terachi, 2001). In rice, *orf79* was initially identified as the ORF associated with BT-type CMS (Fig. 2) (Iwabuchi et al., 1993; Akagi et al., 1994). Later, homologous sequences have been found in other sources of rice CMS (Li et al., 2008; Itabashi et al., 2009). Interestingly, trans-taxonomic homology has also been observed between *orf79* and *orf107* (Fig. 2), which is associated with A3-type CMS in sorghum (Tang et al., 1996).

CMS-associated unique ORFs (hereafter CMS-ORF) occur near the 5′ or 3′ termini of genuine protein-coding genes, and their expression is generally constitutive. CMS-ORFs appear most frequently associated with genes for subunits of ATPases (Fig. 2) (Hanson and Bentolila, 2004). Intriguingly, the 5′ UTR of mitochondrial ATPase subunit genes are known to be highly variable in angiosperms (Hazle and Bonen, 2007). On the other hand, *Brassica orf222* and petunia *pcf* are linked to *nad5* (exon 3) and *nad3*, respectively, both of which encode subunits of Complex I (Young and Hanson, 1987; Rasmussen and Hanson, 1989; L'Homme et al., 1997; Fig. 2). The close linkage between CMS-ORFs and genuine mitochondrial genes may facilitate their co-transcription ('opportunistic' transcription; Touzet and Budar, 2004). The significance of such co-transcription for MS expression can be derived from data indicating that the molecular

action of *Rf* sometimes involves cleavage of the CMS-ORF region from the linked genuine mitochondrial gene (Kazama et al., 2008). In addition, the linkage *orf138-atp8* was broken in a fertility-reverted cybrid of *Brassica*, in which the *orf138* mRNA had become unstable (Bellaoui et al., 1997). On the other hand, monocistronic CMS-ORFs such as sorghum *orf107* and sugar beet *orf129* exist (Fig. 2) (Tang et al., 1996; Yamamoto et al., 2008); it seems possible that their 5′ and 3′ regions are sufficient to ensure expression.

Approaches to identify CMS-associated loci include analyses of revertants or cybrids that have a recombinant mitochondrial genome derived from CMS and MF plants (Schnable and Wise, 1998; Hanson and Bentolila, 2004). Given that CMS-ORFs are often cotranscribed with mitochondrial genes, it also may be worthwhile attempting to find ORFs near genuine mitochondrial genes, especially those for ATPase components. Indeed, this approach has worked in some cases (Singh and Brown, 1991; Dieterich et al., 2003; Shinada et al., 2006; Kim et al., 2007; Ashutosh et al., 2008). On the other hand, the comparative genomics approach has attracted few researchers for the identification of CMS-ORF, because comparisons of mitochondrial genomes required the construction of physical maps or nucleotide sequencing of more than 600 kbp in total (see II. A. Genome size and organization), which seemed impracticable in most circumstances. Additionally, the detected alterations probably would be too numerous to be investigated in detail. Therefore, this approach was preferred only when the organizational difference between the mitochondrial genomes was estimated to be rather small. However, because of technical advances in sequencing entire mitochondrial genomes, this approach may become helpful for identifying CMS-associated loci. Comparative genomics of CMS and MF mitochondria in sugar beet helped to locate the CMS-ORF, *preSatp6*, which is an exceptional N-terminal extension because the cleaved polypeptides accumulate in CMS mitochondria (Fig. 2) (Satoh et al., 2004; Yamamoto et al., 2005; Matsunaga et al., 2010). Comparison of multiple genomic sequences could lead to the identification of a CMS-ORF in maize (Allen et al., 2007), and a similar approach was used in rice (Fujii et al., 2010a).

However, a unique ORF found in CMS mitochondria but absent in MF will not necessarily be associated with MS expression, and CMS likely is independent of the vast majority of unique ORFs. Many unique ORFs unrelated to CMS may be present in CMS mitochondria, and some of them may even be cotranscribed with genuine mitochondrial

genes (Handa, 2003; Satoh et al., 2004; Allen et al., 2007). In conclusion, nucleotide sequences can help but are insufficient for discriminating candidate CMS-ORFs. To identify a CMS-ORF, it is recommended that alterations in the expression of the candidate ORF in response to *Rf* should be investigated as such alterations are observed in many CMS-ORFs (Hanson and Bentolila, 2004) (see below, V. Nuclear genes that suppress CMS).

Polypeptides translated from a CMS-ORF often contain several membrane-spanning domains and/or a hydrophobic N-terminal domain (Schnable and Wise, 1998; Hanson and Bentolila, 2004). Localization studies revealed that the translation products of URF13-T in maize T-type CMS and of radish ORF138 are located in the inner mitochondrial membrane (Dewey et al., 1987; Duroc et al., 2009) where they form oligomers (Rhoads et al., 1998; Duroc et al., 2009). In this context, oligomer formation of preSATP6 may indicate its association with CMS (Yamamoto et al., 2005). On the other hand, some polypeptides including petunia PCF and sugar beet ORF129 (Fig. 2) have been detected in both the membrane and matrix fractions, suggesting that they are loosely associated with the membrane (Nivison et al., 1994; Yamamoto et al., 2008). The common bean ORF239 is unique because it can be detected outside of mitochondria (Abad et al., 1995).

It certainly is reassuring if translation products of the candidate ORF can be detected, but further evidence is needed to conclude that the candidate is the causal agent of CMS. The mitochondrial ORF that is responsible for disease sensitivity in rough lemon is translated but it is not associated with CMS (Ohtani et al., 2002; K. Akimitsu, personal communication). The potential of an ORF to induce MS has been investigated via a transgenic approach based on the introduction of a construct in which an ORF fused with the mitochondrial transit peptide is driven by a constitutive or tissue-specific promoter. The resulting transgenics expressed MS and the phenotype was cosegregated with the transgene in the progeny (He et al., 1996; Wang et al., 2006; Kim et al., 2007; Yamamoto et al., 2008; Nizampatnam et al., 2009). However, it remains puzzling that not all CMS-ORFs induced MS in similar experiments (Chaumont et al., 1995; Wintz et al., 1995; Duroc et al., 2006). Intraorganellar localization of translation products and/or the expression profile of the transgene might be different from those of endogenous CMS-ORFs.

IV. How is CMS expressed?

In sugar beet G-type CMS, which is characterized by a truncated *cox2* and a 3'-terminally extended *nad9*, reduced activity was observed in Complex IV but not in Complex I in young leaves (Ducos et al., 2001). Insufficient Complex IV activity may be associated with CMS, but it cannot be ruled out that Complex I activity is also reduced in anthers (Ducos et al., 2001).

In cases of CMS involving a CMS-ORF, the ORF's translation product may modify mitochondrial function and thereby induce MS. CMS-ORFs have the potential to modify mitochondrial function, according to data from experiments using a heterologous system. Intensive studies using *Escherichia coli*, budding yeast, and insect cells have clarified biochemical aspects of *urf13-T*, which pleiotropically induces sensitivity to southern corn leaf blight and chemicals such as the insecticide methomyl (Dewey et al., 1988; Glab et al., 1990; Huang et al., 1990; Korth and Levings, 1993; Rhoads et al., 1998). It was concluded that *urf13-T* translation products form a tetramer in the membrane which functions as a molecular pore when it binds to the fungal toxin or the insecticide. As a result, ion leakage as well as uncoupling of oxidative phosphorylation occurs. The existence of an anther-specific substrate resembling the fungal toxin or the insecticide would resolve the question why the CMS-ORF causes MS but affects no other organ despite its constitutive expression, as proposed by Flavell (1974). However, no such substrate, the so-called factor X, has been found to date.

On the other hand, some CMS-ORFs seem to be cytotoxic without an additional substrate. Radish *orf138* and sunflower *orf522* have the potential to inhibit growth of *E. coli* (Nakai et al., 1995; Duroc et al., 2005). Furthermore, mitochondrial morphology changed when the radish ORF138 polypeptide accumulated in the mitochondria of yeast, onion, and *Arabidopsis* (Duroc et al., 2006). Cytotoxicity of rice ORF79 has also been reported; expression of transgenic ORF79 interferes with the regeneration of rice callus (Kojima et al., 2010). It should be noted that these effects only occur when the expressed CMS-ORF is intact: truncated ORF138 does not affect growth of *E. coli* (Duroc et al., 2005). Therefore, it seems likely that the amino acid sequence of CMS-ORF is functionally significant and has an unknown physiological effect, although the alternative hypothesis that accumulation of aberrant polypeptides *per se* is stressful for mitochondria

cannot be ruled out.

If the CMS-ORF is more or less cytotoxic, its effects should show in organs/tissues other than the anthers but this is rarely the case. Duvik (1965) noted that maize T-type CMS affected plant height, number of leaves, and grain yield. It is generally assumed that such phenotypic effects are so small that they are hardly detectable. Despite the lack of visible phenotypes, differential gene expression in non-anther organs has been detected in maize. Comparison of the mitochondrial proteome in ears of the NA- and T-type cytoplasm of maize (see Table 1) revealed mitotype-specific expression of several nuclear genes (Hochholdinger et al., 2004), suggesting a cytoplasmic effect.

Emerging evidence suggests that mitochondria in the vegetative organs of CMS lines are physiologically distinct from those of maintainer lines. In sunflower pet1-type CMS, Complex V (F_0F_1-ATPase) activity is reduced in seedlings (Sabar et al., 2003). A competitive effect between genuine *atp8* and *orf522* which consists of a partially duplicated *atp8* and a sequence of unknown origin (Fig. 2), has been postulated (Saber et al., 2003). Decreased Complex V activity has also been reported from rice HL-type CMS seedlings (Zhang et al., 2007) in which *orfH79* was co-transcribed with *atp6*. On the other hand, no reduction of any respiratory complex activity was observed in radish Ogura-type CMS (Duroc et al., 2009). Instead, mitochondrial oxygen consumption was altered, which led Duroc et al. (2009) to hypothesize that the ORF138 polypeptide exerts a mild uncoupling effect by forming oligomers. The terms “dominant defective mutation,” which refers to CMS-ORFs such as sunflower *orf522*, and “gain of function,” which refers to CMS-ORFs such as radish *orf138*, have been proposed for CMS-ORFs with different effects (Duroc et al., 2009).

To date, it remains obscure why mitochondrial function is changed in the entire CMS plant whereas phenotypic effects above the level of the mitochondria are restricted to anthers. The common bean is an exception to the rule because an accumulation of ORF239 occurs specifically in the mitochondria of anthers and involves mitochondrial protease (Abad et al., 1995; Sarria et al., 1998). A hypothesis currently applied to many CMS plants assumes differential requirements for mitochondrial activity between organs: the effects of CMS-ORF are so small that most organs remain phenotypically normal. Since highest mitochondrial activity is required in anthers, the small effects are sufficient to cause MS. Many studies including morphological and molecular analyses support this differential mitochondrial activity hypothesis (Warmke and Lee, 1977; Majewskasawka

et al., 1993; Huang et al., 1994; Lalanne et al., 1998).

Research into the mechanisms of CMS has focused on the anther tapetum, a nursing tissue that surrounds male meiocytes, because it is often impaired in association with CMS (Laser and Lersten, 1972; Warmke and Lee, 1977; Kaul, 1988; Majewskasawka et al., 1993). It appears likely that the mitochondrial function that is modified by CMS-ORF affects the function and/or integrity of the tapetum. Programmed cell death (PCD) of the tapetum is a key process in pollen development (Skibbe et al., 2008; Wilson and Zhang, 2009; Parish and Li, 2010). In sunflower pet1-type CMS, premature cell death is associated with MS expression, including the advanced release of cytochrome *c*, which triggers PCD in animal systems (Balk and Leaver, 2001). On the other hand, the tapetum morphology of some CMS lines appears indistinguishable from that of their maintainer lines. In maize S-type CMS, only pollen grains after the first or second pollen mitosis exhibited developmental abnormalities (Laughnan and Gabay-Laughnan, 1983) which suggests that the male gametophyte itself is most affected by S-type CMS.

Increased levels of reactive oxygen species (ROS), which are associated with the onset of PCD (Gamaley and Klyubin, 1999; Jabs, 1999), have been reported in rice HL-type CMS and cotton CMS anthers (Wan et al., 2007; Jiang et al., 2007). On the other hand, no apparent increase in ROS was detected in maize T-type CMS anthers (Liu and Schnable, 2002). Although ROS involvement is uncertain, anther tissues of CMS plants appear to be under stress, at least in maize, sorghum, and rice, because *AOX*, a nuclear gene encoding alternative oxidase, is more active in CMS plants than in maintainer strains (Pring et al., 2006; Karpova et al., 2002; Fujii and Toriyama, 2008a). AOX is a terminal oxidase in the mitochondrial electron transport chain which is a functional alternative to Complex IV (cytochrome *c* oxidase). *AOX* is known to be a marker of different types of stress. Activation of AOX is triggered by high respiratory substrate availability or high endogenous or exogenous levels of ROS (Polidoros et al., 2009). Increased *AOX* expression was observed in the tassel but not in the ear of maize CMS (Karpova et al., 2002). The degree of gene-induction differs among T-, S- and C-type CMS (Karpova et al., 2002), suggesting a differential cytoplasmic effect. On the other hand, no increased enzymatic activity of AOX was seen in the anthers of common bean and sorghum CMS (Johns et al., 1993; Sane et al., 1997), and reduced AOX activity was reported from immature anthers of petunia (Connett and Hanson, 1990).

Altered expression of nuclear genes in CMS anthers other than *AOX* have also been

reported (Pring and Tang, 2004; Geddy et al., 2005; Matsuhira et al., 2007; Yang et al., 2008; Fujii and Toriyama, 2008a; Fujii et al., 2009; Fujii et al., 2010b). The mechanism by which the expression of these genes is affected is unknown, but it is possible that some of the genes, if not all, are under the regulation of retrograde signaling, which senses the mitochondrial condition and regulates nuclear gene expression in response.

V. Nuclear genes that suppress CMS

In most cases, dominant alleles of *Rf*s restore MF, whereas fertility restoration by the recessive allele is rare. Fertility restoration by *Rf* is either sporophytic and all pollen grains are viable in heterozygous (*Rfrf*) as well as in homozygous plants (*RfRf*), or it is gametophytic and only pollen grains that inherit the *Rf* allele are viable.

The finding that maize *Rf1* altered the mRNA profile of *urf13-T* and reduced the amount of URF13-T polypeptides (Dewey et al., 1987) raised the question whether *Rf* generally controls the expression of CMS-ORF. In many cases, it does; RNA gel blot analyses using CMS-ORF as a probe detected different mRNA profiles in CMS and fertility-restored plants (Hanson and Bentolila, 2004). The underlying mechanism included the loss of a specific size class of RNA molecules and/or altered processing of RNA molecules. Transcriptional alteration by *Rf* may occur in the entire plant or in a specific organ, e.g. in anthers. Simultaneously, CMS-ORF translation products are decreased. In the radish Ogura-type CMS, the mRNA profile is unchanged but accumulation of the ORF138 polypeptide is decreased by *Rfo* (Krishnasamy and Makaroff, 1994; Bellaoui et al., 1999; Uyttewaal et al., 2008).

The effects of *Rf*s seem confined to the locus containing the CMS-ORF but some *Rf*s such as *Rfn* in *Brassica* (for nap-type CMS), *Rf3* and *rfl1* in maize (for S-type CMS), and sorghum *Rf3* (for A3-type CMS) have been reported to pleiotropically alter the transcription profile of additional mitochondrial loci (Li et al., 1998; Tang et al., 1998; Wen and Chase, 1999; Wen et al., 2003). However, the possibility cannot be ruled out that apparent pleiotropic effects of *Rf* in fact are due to other genes because the *Rf* locus often exhibits a clustering of similar genes (Touzet and Budar, 2004). On the other hand, maize *rfl1* appears to have certain pleiotropic effect. Maize *rfl1* is an exceptional *rf* as the recessive allele restores MF in a gametophytic manner (Wen et al., 2003). The recessive

allele reduces the accumulation of *orf355-orf77* (Fig. 2) as well as *atp1* transcripts, even though the two genes are 9 kbp apart (Wen et al., 2003; Allen et al., 2007). Since the functional dominant allele of *rfl1* seems necessary for *orf355-orf77* and *atp1* expression (Wen et al., 2003), the homozygous recessive (*rfl1rfl1*) zygote is embryonic lethal because of an ATP1 deficiency. However, *rfl1* pollen can germinate and participate in fertilization. It is worth noting that in pollen less mitochondrial activity is required because energy production in pollen relies on a unique pathway (Mellema et al., 2002; Gass et al., 2005). The *rfl1* gene product still is unknown.

*Rf*s of petunia, radish, and rice reduce the amount of CMS-ORF translation products. Details of how this is achieved differ; for example, the mRNA profiles of rice *orf79* are altered whereas that of petunia *pcf* and radish *orf138* remains unchanged (Rasmussen and Hanson, 1989; Nivison et al., 1994; Iwabuchi et al., 1993; Krishnasamy and Makaroff, 1994; Bellaoui et al., 1999; Uyttewaal et al., 2008). Molecular cloning of *Rf*s from the three species revealed that all three *Rf*s encode proteins containing a common degenerate motif called a pentatrico peptide repeat (PPR) (Bentolila et al., 2002; Koizuka et al., 2003; Kazama and Toriyama, 2003; Desloire et al., 2003; Brown et al., 2003; Komori et al., 2004; Akagi et al., 2004). Genes encoding PPR proteins constitute a large gene family in land plants and are involved in a range of transcriptional and post-transcriptional processes including transcription, splicing, RNA cleavage, RNA editing, and translation (Schmitz-Linneweber and Small, 2008). PPR proteins encoded by *Rf*s seem to have no catalytic activity, and therefore are likely to be 'molecular adaptors.' (Schmitz-Linneweber and Small, 2008). Provided that RF proteins bind RNA in a sequence-specific manner and recruit catalytic proteins to the site of action, the *Rf*s could affect the expression of specific genes such as CMS-ORF. Obviously, these *Rf*s could affect several genes that share their binding sites. It has been shown that petunia RF, rice RF1, and radish RFO bind to transcripts containing *pcf*, *orf79* and *orf138*, respectively (Gillman et al., 2007; Kazama et al., 2008; Uyttewaal et al., 2008). On the basis of these results, it is plausible to hypothesize that (some) *Rf* genes encode PPR proteins that control the activity of mitochondrial genes including those that function in CMS. In some plant species, a genetic linkage between *Rf* and PPR-protein coding genes has been found (Klein et al., 2005; Jordan et al., 2010; Barr and Fishman, 2010). It would be interesting to see whether any unique ORFs responding to the *Rf* can be identified in the mitochondrial genomes of such plants.

*Rf*s may encode several types of proteins other than PPRs. Common bean *Fr* (the genetic symbol for *Rf* in this species) is a unique *Rf* because it acts as if it deleted the mitochondrial (mt) DNA region termed *pvs* which contains the CMS-ORF, *orf239* (Fig. 2) (Janska and Mackenzie, 1993; Janska et al., 1998). This phenomenon involves regulation of the copy number of specific mtDNA molecules, that is, SSS. The copy number of mtDNA molecules that uniquely contain *pvs* but are otherwise colinear with other molecules is decreased by an unknown mechanism, presumably during oogenesis and/or transmission to zygotes. A similar phenomenon was discovered in perl millet (Feng et al., 2009) and rapeseed (J. Imamura, personal communication). These reports clearly indicate that SSS is under genetic control and is one of the mechanisms for eliminating CMS-associated regions.

In these examples, *Rf* appears to reduce the amount of CMS-ORF translation products by modifying gene expression or eliminating the mtDNA molecule that encodes CMS-ORF. In these cases, the relationship between CMS-ORF and *Rf* is clear and consistent with a genetic model featuring specific interactions between MS-inducing mitochondria and *Rf*. In contrast, other *Rf*s are more difficult to place in a direct relationship with CMS-ORF. Maize *Rf2a* for T-type CMS was the first cloned *Rf* and encodes mitochondrial aldehyde dehydrogenase (Cui et al., 1996). Although progress has been made in understanding functional aspects of the RF2A protein (Liu et al., 2001; Liu and Schnable, 2002), it remains unclear how RF2A interacts with *urf13-T,* which has raised doubts about the specificity of *Rf2a* for T-type CMS (Touzet, 2002).

Molecular cloning of *Rf17* which functions to restore rice CW-type CMS in a gametophytic manner, has been successful but the precise function of its translation product (RMS) is unclear (Fujii and Toriyama, 2009). Interestingly, rice CW-type CMS plants express more *RMS* than fertility-restored rice. Transgenic rice over-expressing *RMS* exhibited MS, indicating that an excess amount of RMS is harmful for pollen production (Fujii and Toriyama, 2009). It was concluded that CW-type mitochondria induce over-expression of *RMS* via a retrograde signaling pathway, thereby causing MS, whereas a non-inducible allele blocks MS expression resulting in fertility restoration. If so, the role of *Rf17* is not the elimination of the causal agents of CMS, but the obstruction of the execution of MS. Identification of the mitochondrial locus that is associated with CW-type CMS is necessary for further insights into this process.

VI. Concluding remarks and perspectives

Because of its mode of inheritance, CMS provides a unique means of preventing pollen dispersal. For CMS utilization, it is necessary to find an MS-inducing mitotype. If multiple mitotypes are found in a gene pool and if their nucleotide sequences are available (it will be much easier to obtain complete sequences in the future), will it be possible to predict the potential of each mitotype? At present, it is difficult to answer this questions but the goal is worth the challenge. For example, although the entire nucleotide sequences for mitochondrial genomes of maize C-type CMS and rice CW-type CMS are available, the causal agents of these CMS remain obscure (Allen et al., 2007; Fujii et al., 2010a). The two mitotypes are known to be capable of inducing MS, because MS is expressed under certain nuclear genotypes. One of the characteristics of CMS mitochondria is extensive genome rearrangement, as seen in maize and sugar beet (Fauron and Havlik, 1989; Kubo et al., 1999), but this is not always the case as exemplified by sunflower pet1-type CMS (Siculella and Palmer, 1988).

Functionally unassigned ORFs are ubiquitous and abundant in the angiosperm mitochondrial genome; consequently, CMS-ORFs may “hide” among a number of candidate ORFs. Plant mitochondria have a regulatory system to repress expression of such useless or potentially harmful ORFs (Holec et al., 2008). From the viewpoint of CMS-ORF evolution, cotranscription with a genuine mitochondrial gene may be one of the solutions for CMS-ORFs to overcome the mitochondrial defense that represses the expression of aberrant ORFs (Holec et al., 2008). This arrangement may also help to avoid elimination by recombination (F. Budar, personal communication). CMS-ORF translation products are likely to be subject to mitochondrial proteolytic activity, as is seen in the tissue-specific accumulation of common bean ORF239 and in the N-terminal trimming of petunia PCF (Sarria et al., 1998; Nivison et al., 1994). Genes involved in the quality control system of mitochondrial proteins have been identified (Kolodziejczak et al., 2007). However, translation of a unique ORF and accumulation of its translation products may not always cause MS; for example, maize *rps2* and sugar beet *ccmC* are structurally aberrant functional genes that do not appear to be associated with mitochondrial dysfunction (Perrotta et al., 2002; Kitazaki et al., 2009). Perhaps MS induction requires an additional feature such as oligomer formation as in the cases of

maize URF13-T, radish ORF138 and sugar beet preSATP6 (Rhoads et al., 1998; Yamamoto et al., 2005; Duroc et al., 2009).

There is increasing evidence indicating that a CMS-ORF is necessary for CMS expression. For example, maize fertility revertants have lost *urf13-T* or *orf355-orf77* (Zabala et al., 1997; Schnable and Wise, 1998), and their cognate *Rf* decreases the amount of translation products (Hanson and Bentolila, 2004). However, researchers mostly have overlooked the fact that a few non-synonymous substitutions in genuine mitochondrial genes have been found in all CMS mitochondria that have been sequenced to date (Satoh et al., 2004; Allen et al., 2007; Fujii et al., 2010a). It is likely that these genes merely represent evolutionary polymorphism and are functionally non-significant, but it also is possible that amino acid substitutions in mitochondrial proteins are involved in MS expression. In eukaryotes other than plants, the occurrence of nuclear-mitochondrial incompatibility includes amino acid substitutions or altered UTRs of genuine mitochondrial genes, some of which are associated with MS (St John et al., 2005; Harrison and Burton, 2006; Lee et al., 2008).

Although CMS-related morphological variation has not been discussed in detail in this review, it is worth noting that tapetum degeneration prior to pollen abortion is not always associated with CMS. Laser and Lersten (1972) summarized the stages of morphological deviation from microspore mother cells to mature pollen in various CMS plants. In addition, some CMS plants are known to develop homeotic conversions of stamina to other floral organs (Linke and Borner, 2005). The mechanisms underlying these phenotypic variations of CMS plants remain unknown, but transcriptomic analysis may be an approach for solving this problem.

It has been reported that multiple *Rf*s exist for a single CMS in some cases, which suggests that a CMS is not necessarily regulated by a single mechanism. In fact, rice *orf79* expression can be suppressed by either of two genes, *Rf1a* that cleaves *atp6-orf79* mRNA, or *Rf1b* that degrades *atp6-orf79* mRNA (Wang et al., 2006). *Rf1a* and *Rf1b* are linked in the *Rf-1* locus (Wang et al., 2006). In maize T-type CMS, the function of *Rf1* that alters the processing of *urf13-T-atp4* transcripts can be partially replaced by *Rf8* or *Rf** (Dill et al., 1997). In common bean CMS, a nuclear gene that alters *orf239* expression exists in addition to *Fr* that is associated with SSS (Mackenzie, 1991). In wild radish, a novel *Rf* that alters the transcription pattern of *orf138*, unlike the previously cloned *Rfo*, has been found (Bett and Lydiate, 2004; Yasumoto et al., 2008; Yasumoto et al., 2009).

Furthermore, more than two *Rf*s are involved not only in maize T-type CMS (which requires *Rf1* and *Rf2* for fertility restoration), but also in other species such as sugar beet (Owen, 1942; Owen, 1945; Kaul, 1988). Molecular characterization of these *Rf*s will be of great help in understanding the mechanisms and evolutionary aspects of the interactions between the nucleus and mitochondria.

Acknowledgements The authors wish to thank Dr. Françoise Budar for reviewing the manuscript, and Dr. Sally Mackenzie, Dr. Kinya Toriyama and Dr. Tomohiko Kazama for providing valuable information. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology and the Program for Promotion of Basic and Applied Research for Innovation in Bio-oriented Industry (BRAIN).

VII. References

Abad, A.R., Mehrtens, B.J. and Mackenzie, S.A. 1995. Specific expression in reproductive tissues and fate of a mitochondrial sterility-associated protein in cytoplasmic male-sterile bean. *Plant Cell.* 7: 271-285.

Abdelnoor, R.V., Yule, R., Elo, A., Christensen, A.C., Meyer-Gauren, G. and Mackenzie, S.A. 2003. Substiochiometric 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., Daley, D.O., Whelan, J. and Palmer, J.D. 2002a. Genes for two mitochondrial ribosomal proteins in flowering plants are derived from their chloroplast or cytosolic counterparts. *Plant Cell*. 14: 931-943.

Adams, K.L. and Palmer. J.D. 2003. Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. *Mol. Phylogenet. Evol.* 29: 380-395.

Adams, K.L., Qiu, Y.L., Stoutemyer, M. and Palmer, J.D. 2002b. Punctuated evolution of mitochondrial gene content: High and variable rates of mitochondrial gene loss and transfer to the nucleus during angiosperm evolution. *Proc*. *Natl. Acad. Sci.*

USA. 99: 9905-9912.

Akagi, H., Nakamura, A., Yokozeki-Misono, Y., Inagaki, A., Takahashi, H., Mori, K. and Fujimura, T. 2004. Positional cloning of the rice *Rf-1* gene, a restorer of BT-type cytoplasmic male sterility that encodes a mitochondria-targeting PPR protein. *Theor. Appl. Genet.* 108: 1449-1457.

Akagi, H., Sakamoto, M., Shinjyo, C., Shimada, H. and Fujimura, T. 1994. A unique sequence located downstream from the rice mitochondrial *atp6* may cause male sterility. *Curr. Genet.* 25: 52-58.

Allen, J.O., Fauron, C.M., Minx, P.R., 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. and Newton, K.J. 2007. Comparisons among two fertile and three male-sterile mitochondrial genomes of maize. *Genetics*, 177: 1173-1192.

Alverson, A.J., Wei, X.X., Rice, D.W., Stern, D.B., Barry, K. and Palmer, J.D. 2010. Insights into the evolution of mitochondrial genome size from complete sequences of *Citrullus lanatus* and *Cucurbita pepo* (Cucurbitaceae). *Mol. Biol. Evol.* 27: 1436-1448.

Anderson, S., Bankier, A.T., Barrell, B.G., de Bruijn, M.H., Coulson, A.R., Drouin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J., Staden, R. and Young, I.G. 1981. Sequence and organization of human mitochondrial genome. *Nature*. 290: 457-465.

Anderson, S., de Bruijn, M.H., Coulson, A.R., Eperon, I.C., Sanger, F. and Young, I.G. 1982. Complete nucleotide sequence of bovine mitochondrial DNA: conserved features of the mammalian mitochondrial genomes. *J. Mol. Biol.* 156: 683-717.

Andre, C., Levy, A. and Walbot, V. 1992. Small repeated sequences and the structure of plant mitochondrial genomes. *Trends Genet.* 8: 128-132.

Andre, C.P. and Walbot, V. 1995. Pulsed field gel mapping of maize mitochondrial chromosomes. *Mol. Gen. Genet.* 247: 255-263.

Arrieta-Montiel, M., Lyznik, A., Woloszynska, M., Janska, H., Tohme, J. and Mackenzie, S. 2001. Tracing evolutionary and developmental implications of mitochondrial stoichiometric shifting in the common bean. *Genetics*. 158: 851-864.

Arrieta-Montiel, M.P., Shedge, V., Davila, J., Christensen, A.C. and Mackenzie, S.A. 2009. Diversity of the Arabidopsis mitochondrial genome occurs via nuclear-controlled recombination activity. *Genetics*. 183: 1261-1268.

Arumuganathan, K. and Earle, E.D. 1991. Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rep.* 9: 208-218.

Ashutosh, Kumar, P., Kumar, V.D., Sharma, P.C., Prakash, S. and Bhat, S.R. 2008. A novel *orf108* co-transcribed with the *atpA* gene is associated with cytoplasmic male sterility in *Brassica juncea* carrying *Moricandia arvensis* cytoplasm. *Plant Cell Physiol.* 49: 284-289.

Balk, J. and Leaver, C.J. 2001. The PET1-CMS mitochondrial mutation in sunflower is associated with premature programmed cell death and cytochrome c release. *Plant Cell*. 13: 1803-1818.

Barkman, T.J., McNeal, J.R., Lim, S.H., Coat, G., Croom, H.B., Young, N.D. dePamphilis, C.W. 2007. Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. *BMC Evol. Biol.* 7: 248.

Barr, C.M. and Fishman, L. 2010. The nuclear component of a cytonuclear hybrid incompatibility in *Mimulus* maps to a cluster of pentatricopeptide repeat genes. *Genetics*. 184: 455-465.

Barr, C.M., Keller, S.R., Ingvarsson, P.K., Sloan, D.B. and Taylor, D.R. 2007. Variation in mutation rate and polymorphism among mitochondrial genes of *Silene vulgaris.* *Mol. Biol. Evol.* 24: 1783-1791.

Bartoszewski, G., Gawronski, P., Szklarczyk, M., Verbakel, H. and Havey, M.J. 2009. A one-megabase physical map provides insights on gene organization in the enormous mitochondrial genome of cucumber. *Genome*. 52: 299-307.

Bartoszewski, G., Havey, M.J., Ziolkowska, A., Dlugosz, M. and Malepszy, S. 2007. The selection of mosaic (MSC) phenotype after passage of cucumber (*Cucumis sativus* L.) through cell culture: a method to obtain plant mitochondrial mutants. *J. Appl. Genet.* 48: 1-9.

Beckett, J.B. 1971. Classification of male-sterile cytoplasms in maize (*Zea mays* L.). *Crop Sci.* 11: 724-727.

Bellaoui, M., Pelletier, G. and Budar, F. 1997. The steady-state level of mRNA from the Ogura cytoplasmic male sterility locus in *Brassica* cybrids is determined post-transcriptionally by its 3' region. *EMBO J.* 16: 5057-5068.

Bellaoui, M., Grelon, M. and Budar, F. 1999. The restorer *Rfo* gene acts post-transcriptionally on the stability of the ORF138 Ogura CMS-associated

protein in reproductive tissues of rapeseed cybrids. *Plant Mol. Biol.* 40: 893-902.

Bendich, A.J. 1993. Reaching for the ring: the study of mitochondrial genome structure. *Curr. Genet.* 24: 279-290.

Bentolila, S., Alfonso, A.A. and Hanson, M.R. 2002. A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. *Proc. Natl. Acad. Sci. USA.* 99: 10887-10892.

Bentolila, S., Elliott, L.E. and Hanson, M.R. 2008. Genetic architecture of mitochondrial editing in *Arabidopsis thaliana*. *Genetics*. 178: 1693-1708.

Bergthorsson, U., Adams, K.L., Thomason, B. and Palmer, J.D. 2003. Widespread horizontal transfer of mitochondrial genes in flowering plants. *Nature*. 424: 197-201.

Bergthorsson, U., Richardson, A.O., Young, G.J., Goertzen, L.R. and 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.

Bett, K.E. and Lydiate, D.J. 2004. Mapping and genetic characterization of loci controlling the restoration of male fertility in Ogura CMS radish. *Mol. Breed.* 13: 125-133.

Bock, H., Brennicke, A. and Schuster, W. 1994. *Rps3* and *rpl16* genes do not overlap in *Oenothera* mitochondria: GTG as a potential translation initiation codon in plant mitochondria? *Plant Mol. Biol.* 24: 811-818.

Bock, R. 2010. The give-and-take of DNA: horizontal gene transfer in plants. *Trends Plant Sci.* 15: 11-22.

Bonen, L. 2008. *Cis*- and *trans*-splicing of group II introns in plant mitochondria. *Mitochondrion*. 8: 26-34.

Bonhomme, S., Budar, F., Lancelin, D., Small, I., Defrance, M.C. and Pelletier, G. 1992. Sequence and transcript analysis of the *Nco2.5* Ogura-specific fragment correlated with cytoplasmic male-sterility in *Brassica* cybrids. *Mol. Gen. Genet.* 235: 340-348.

Brown, G.G., Formanova, N., Jin, H., Wargachuk, R., Dendy, C., Patil, P., Laforest, M., Zhang, J.F., Cheung, W.Y. and Landry, B.S. 2003. The radish *Rfo* restorer gene of Ogura cytoplasmic male sterility encodes a protein with multiple pentatricopeptide repeats. *Plant J.* 35: 262-272.

Budar, F., Delourme, R. and Pelletier, G. 2004. Male sterility. In *Biotechnology in agriculture and forestry: Brassica* (C., P.E. and Douglas, C.J. eds). Berlin: Springer-Verlag, pp. 43-64.

Budar, F., Touzet, P. and Pelletier, G. 2006. Cytoplasmic male sterility. In *Flowering and its manipulation*. Oxford: Blackwell Publishing, pp. 147-180.

Bullerwell, C.E. and Gray, M.W. 2004. Evolution of the mitochondrial genome: protist connections to animals, fungi and plants. *Curr. Opin. Microbiol.* 7: 528-534.

Carlsson, J., Leino, M. and Glimelius, K. 2007. Mitochondrial genotypes with variable parts of *Arabidopsis thaliana* DNA affect development in *Brassica napus* lines. *Theor. Appl. Genet.* 115: 627-641.

Ceci, L.R., Veronico, P. and Gallerani, R. 1996. Identification and mapping of tRNA genes on the *Helianthus annuus* mitochondrial genome. *DNA Seq.* 6: 159-166.

Chase, C.D. 2007. Cytoplasmic male sterility: a window to the world of plant mitochondrial-nuclear interactions. *Trends Genet.* 23: 81-90.

Chaumont, F., Bernier, B., Buxant, R., Williams, M.E., Levings, C.S. and Boutry, M. 1995. Targeting the maize *T-urf13* product into tobacco mitochondria confers methomyl sensitivity to mitochondrial respiration. *Proc. Natl. Acad. Sci. USA.* 92: 1167-1171.

Cheng, D., Yoshida, Y., Kitazaki, K., Negoro, S., Takahashi, H., Xu, D., Mikami, T. and Kubo, T. 2010. Mitochondrial genome diversity in *Beta vulgaris* L. ssp *vulgaris* (Leaf and Garden Beet Groups) and its implications concerning the dissemination of the crop. *Genet. Res. Crop Evol.* doi: 10.1007/s10722-010-9598-9.

Cho, Y., Mower, J.P., Qiu, Y.L. Palmer, J.D. 2004. Mitochondrial substitution rates are extraordinarily elevated and variable in a genus of flowering plants. *Proc. Natl. Acad. Sci. USA.* 101: 17741-17746.

Clifton, S.W., Minx, P., Fauron, C.M.R., Gibson, M., Allen, J.O., Sun, H., Thompson, M., Barbazuk, W.B., Kanuganti, S., Tayloe, C., Meyer, L., Wilson, R.K. and Newton, K.J. 2004. Sequence and comparative analysis of the maize NB mitochondrial genome. *Plant Physiol.* 136: 3486-3503.

Connett, M.B. and 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.

Covello, P.S. and Gray, M.W. 1992. Silent mitochondrial and active nuclear genes for subunit 2 of cytochrome *c* oxidase (*cox2*) in soybean: evidence for RNA-mediated gene transfer. *EMBO J.* 11: 3815-3820.

Cui, P., Liu, H.T., Lin, Q., Ding, F., Zhuo, G.Y., Hu, S.N., Liu, D.C., Yang, W.L., Zhan, K.H., Zhang, A.M. and Yu, J. 2009. A complete mitochondrial genome of wheat (*Triticum aestivum* cv. Chinese Yumai), and fast evolving mitochondrial genes in higher plants. *J. Genet.* 88: 299-307.

Cui, X.Q., Wise, R.P. and Schnable, P.S. 1996. The *rf2* nuclear restorer gene of male-sterile T-cytoplasm maize. *Science*. 272: 1334-1336.

Cummings, M.P., Nugent, J.M., Olmstead, R.G. and Palmer, J.D. 2003. Phylogenetic analysis reveals five independent transfers of the chloroplast gene *rbcL* to the mitochondrial genome in angiosperms. *Curr. Genet.* 43: 131-138.

Cusimano, N., Zhang, L.B. Renner, S.S. 2008. Reevaluation of the *cox1* intron in Araceae and angiosperms indicates a history dominated by loss rather than horizontal transfer. *Mol. Biol. Evol.* 25: 265-276.

Dai, H., Lo, Y.-S., Litvinchuk, A., Wang, Y.-T., Jane, W.-N., Hsiao, L.-J. and Chiang, K.-S. 2005: Structural and functional characterizations of mung bean mitochondrial nucleoids. *Nucl. Acids Res.* 33: 4725-4739.

Davis, C. C., Anderson, W. R. and Wurdack, K. J. 2005. Gene transfer from a parasitic flowering plant to a fern. *Proc. R. Soc. B.* 272: 2237-2242.

Davis, C. C. and Wurdack, K. J. 2004. Host-to-parasite gene transfer in flowering plants: phylogenetic evidence from Malpighiales. *Science.* 305: 676-678.

Delph, L.F., Touzet, P. and Bailey, M.F. 2007. Merging theory and mechanism in studies of gynodioecy. *Trends Ecol. Evol.* 22: 17-24.

Desloire, S., Gherbi, H., Laloui, W., Marhadour, S., Clouet, V., Cattolico, L., Falentin, C., Giancola, S., Renard, M., Budar, F., Small, I., Caboche, M., Delourme, R. and Bendahmane, A. 2003. Identification of the fertility restoration locus, *Rfo*, in radish, as a member of the pentatricopeptide-repeat protein family. *EMBO Rep.* 4: 588-594.

Dewey, R.E., Levings, C.S.III and Timothy, D.H. 1986. Novel recombination in the maize mitochondrial genome produce a unique transcriptional unit in the Texas male-sterile cytoplasm. *Cell*. 44: 439-449.

Dewey, R. E., Timothy, D. H. and Levings, C. S. III. 1987. A mitochondrial protein

associated with cytoplasmic male sterility in the T cytoplasm of maize. *Proc. Natl. Acad. Sci. USA.* 84: 5374-5378.

Dewey, R.E., Siedow, J.N., Timothy, D.H. and Levings, C.S. III 1988. A 13-kilodalton maize mitochondrial protein in *Escherichia coli* confers sensitivity to *Bipolaris maydis* toxin. *Science*. 239: 293-295.

Dieterich, J.H., Braun, H.P. and Schmitz, U.K. 2003. Alloplasmic male sterility in *Brassica napus* (CMS 'Tournefortii-Stiewe') is associated with a special gene arrangement around a novel *atp9* gene. *Mol. Genet. Genomics.* 269: 723-731.

Dill, C.L., Wise, R.P. and Schnable, P.S. 1997. *Rf8* and *Rf** mediate unique *T-urf13*-transcript accumulation, revealing a conserved motif associated with RNA processing and restoration of pollen fertility in T-cytoplasm maize. *Genetics*. 147: 1367-1379.

Dong, F.G., Wilson, K.G. and Makaroff, C.A. 1998. The radish (*Raphanus sativus* L.) mitochondrial *cox2* gene contains an ACG at the predicted translation initiation site. *Curr. Genet.* 34: 79-87.

Dubreucq, A., Berthe, B., Asset, J.F., Boulidard, L., Budar, F., Vasseur, J. and Rambaud, C. 1999. Analyses of mitochondrial DNA structure and expression in three cytoplasmic male-sterile chicories originating from somatic hybridisation between fertile chicory and CMS sunflower protoplasts. *Theor. Appl. Genet.* 99: 1094-1105.

Duchene, A.M. and Marechal-Drouard, L. 2001. The chloroplast-derived *trnW* and *trnM-e* genes are not expressed in *Arabidopsis* mitochondria. *Biochem. Biophys. Res. Comm.* 285: 1213-1216.

Duchene, A.M., Pujol, C. and Marechal-Drouard, L. 2009. Import of tRNAs and aminoacyl-tRNA synthetases into mitochondria. *Curr. Genet.* 55: 1-18.

Ducos, E., Touzet, P. and Boutry, M. 2001. The male sterility G cytoplasm of wild beet displays modified mitochondrial respiratory complexes. *Plant J.* 26: 171-180.

Duroc, Y., Gaillard, C., Hiard, S., Defrance, M.C., Pelletier, G. and Budar, F. 2005. Biochemical and functional characterization of ORF138, a mitochondrial protein responsible for Ogura cytoplasmic male sterility in Brassiceae. *Biochimie*. 87: 1089-1100.

Duroc, Y., Gaillard, C., Hiard, S., Tinchant, C., Berthome, R., Pelletier, G., and Budar, F. 2006. Nuclear expression of a cytoplasmic male sterility gene modifies

mitochondrial morphology in yeast and plant cells. *Plant Sci.* 170: 755-767.

Duroc, Y., Hiard, S., Vrielynck, N., Ragu, S. and Budar, F. 2009. The Ogura sterility-inducing protein forms a large complex without interfering with the oxidative phosphorylation components in rapeseed mitochondria. *Plant Mol. Biol.* 70: 123-137.

Duvick, D.N. 1965. Cytoplasmic pollen sterility in corn. *Advances Genet.* 13: 1-56.

Fan, Z. and Stefansson, B.R. 1986. Influence of temperature on sterility of two cytoplasmic male-sterility systems in rape (*Brassica napus* L.). *Can. J. Plant Sci*. 66: 221-227.

Fauron, C.M.R. and Casper, M. 1994. A second type pf normal maize mitochondrial genome: an evolutionary link. *Genetics*. 137: 875-882.

Fauron, C. and Havlik, M. 1989. The maize mitochondrial genome of the normal type and the cytoplasmic male sterile type-T have very different organization. *Curr. Genet.* 15: 149-154.

Fenart, S., Touzet, P., Arnaud, J.-F. and Cuguen, J. 2006. Emergence of gynodioecy in wild beet (*Beta vulgaris* ssp. *maritima* L.): a genealogical approach using chloroplastic nucleotide sequences. *Proc. Roy. Soc.* B. 273: 1391-1398.

Feng, X., Kaur, A.P., Mackenzie, S.A. and Dweikat, I.M. 2009. Substoichiometric shifting in the fertility reversion of cytoplasmic male sterile pearl millet. *Theor. Appl. Genet.* 118: 1361-1370.

Flavell, R. 1974. A model for the mechanism of cytoplasmic male sterility in plants, with special reference to maize. *Plant Sci. Lett.* 3: 259-263.

Folkerts, O. and Hanson, M.R. 1991. The male sterility-associated *pcf* gene and the normal *atp9-1* gene in petunia are located on different mitochondrial DNA molecules. *Genetics*, 129: 885-895.

Forner, J., Weber, B., Thuss, S., Wildum, S. and Binder, S. 2007. Mapping of mitochondrial mRNA termini in *Arabidopsis thaliana*: t-elements contribute to 5' and 3' end formation. *Nucl. Acids Res.* 35: 3676-3692.

Fujii, S., Kazama, T., Yamada, M. and Toriyama, K. 2010a Discovery of global genomic re-organization based on comparison of two newly sequenced rice mitochondrial genomes with cytoplasmic male sterility-related genes. *BMC Genom.* 11: 15.

Fujii, S. and Toriyama, K. 2008a. DCW11, Down-Regulated gene 11 in CW-type cytoplasmic male sterile rice, encoding mitochondrial protein phosphatase 2C is

related to cytoplasmic male sterility. *Plant Cell Physiol.* 49: 633-640.

Fujii, S. and Toriyama, K. 2008b. Genome barriers between nuclei and mitochondria exemplified by cytoplasmic male sterility. *Plant Cell Physiol.* 49: 1484-1494.

Fujii, S. and Toriyama, K. 2009. Suppressed expression of *RETROGRADE-REGULATED MALE STERILITY* restores pollen fertility in cytoplasmic male sterile rice plants. *Proc. Natl. Acad. Sci. USA.* 106: 9513-9518.

Fujii, S., Yamada, M., Fujita, M., Itabashi, E., Hamada, K., Yano, K., Kurata, N. and Toriyama, K. 2010b. Cytoplasmic-nuclear genomic barriers in rice pollen development revealed by comparison of global gene expression profiles among five independent cytoplasmic male sterile lines. *Plant Cell Physiol.* 51: 610-620.

Fujii, S., Yamada, M. and Toriyama, K. 2009. Cytoplasmic male sterility-related protein kinase, OsNek3, is regulated downstream of mitochondrial protein phosphatase 2C, DCW11. *Plant Cell Physiol.* 50: 828-837.

Gabay-Laughnan, S. and Newton, K.J. 2005. Mitochondrial mutations in maize. *Maydica*. 50: 349-359.

Gamaley, I.A. and Klyubin, I.V. 1999. Roles of reactive oxygen species: Signaling and regulation of cellular functions. *Int. Rev. Cyt.* 188: 203-255.

Gass, N., Glagotskaia, T., Mellema, S., Stuurman, J., Barone, M., Mandel, T., Roessner-Tunali, U. and Kuhlemeier, C. 2005. Pyruvate decarboxylase provides growing pollen tubes with a competitive advantage in petunia. *Plant Cell*, 17: 2355-2368.

Geddy, R., Mahe, L. and Brown, G.G. 2005. Cell-specific regulation of a *Brassica napus* CMS-associated gene by a nuclear restorer with related effects on a floral homeotic gene promoter. *Plant J.* 41: 333-345.

Geiss, K.T., Abbas, G.M. and Makaroff, C.A. 1994. Intron loss from the NADH dehydrogenase subunit 4 gene of lettuce mitochondrial DNA: evidence for homologous recombination of a cDNA intermediate. *Mol. Gen. Genet.* 243: 97-105.

Gillman, J.D., Bentolila, S. and Hanson, M.R. 2007. The petunia restorer of fertility protein is part of a large mitochondrial complex that interacts with transcripts of the CMS-associated locus. *Plant J.* 49: 217-227.

Glab, N., Wise, R.P., Pring, D.R., Jacq, C. and Slonimski, P. 1990. Expression in *Saccharomyces cerevisiae* of a gene associated with cytoplasmic male-sterility

from maize: respiratory dysfunction and uncoupling of yeast mitochondria. *Mol. Gen. Genet.* 223: 24-32.

Glover, K.E., Spencer, D.F. and Gray, M.W. 2001. Identification and structural characterization of nucleus-encoded transfer RNAs imported into wheat mitochondria. *J. Biol. Chem.* 276: 639-648.

Goremykin, V.V., Salamini, F., Velasco, R. and Viola, R. 2009. Mitochondrial DNA of *Vitis vinifera* and the issue of rampant horizontal gene transfer. *Mol. Biol. Evol.* 26: 99-110.

Gray, M.W. 1999. Evolution of organellar genomes. *Curr. Opin. Genet. Dev.* 9: 678-687.

Gray, M.W., Lang, B.F. and Burger, G. 2004. Mitochondria of protists. *Ann. Rev. Genet.* 38: 477-524.

Grewe, F., Viehoever, P., Weisshaar, B. and Knoop, V. 2009. A *trans*-splicing group I intron and tRNA-hyperediting in the mitochondrial genome of the lycophyte *Isoetes engelmannii*. *Nucl. Acids Res.* 37: 5093-5104.

Handa, H. 2003. The complete nucleotide sequence and RNA editing content of the mitochondrial genome of rapeseed (*Brassica napu*s L.): comparative analysis of the mitochondrial genomes of rapeseed and *Arabidopsis thaliana*. *Nucl. Acids Res.* 31: 5907-5916.

Handa, H. 2008. Linear plasmids in plant mitochondria: peaceful coexistences or malicious invasions? *Mitochondrion*. 8: 15-25.

Hanson, M.R. and Bentolila, S. 2004. Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell*. 16: S154-S169.

Hao, W. and Palmer, J.D. 2009. Fine-scale mergers of chloroplast and mitochondrial genes create functional, transcompartmentally chimeric mitochondrial genes. *Proc. Natl. Acad. Sci. USA.* 106: 16728-16733.

Harrison, J.S. and Burton, R.S. 2006. Tracing hybrid incompatibilities to single amino acid substitutions. *Mol. Biol. Evol.* 23: 559-564.

Hazle, T. and Bonen, L. 2007. Comparative analysis of sequences preceding protein-coding mitochondrial genes in flowering plants. *Mol. Biol. Evol.* 24: 1101-1112.

He, S.C., Abad, A.R., Gelvin, S.B., and Mackenzie, S.A. 1996. A cytoplasmic male sterility-associated mitochondrial protein causes pollen disruption in transgenic tobacco. *Proc. Natl. Acad. Sci. USA.* 93: 11763-11768.

Hochholdinger, F., Guo, L. and Schnable, P.S. 2004. Cytoplasmic regulation of the accumulation of nuclear-encoded proteins in the mitochondrial proteome of maize. *Plant J.* 37: 199-208.

Holec, S., Lange, H., Canaday, J. and Gagliardi, D. 2008. Coping with cryptic and defective transcripts in plant mitochondria. *Biochim. Biophys. Acta*. 1779: 566-573.

Huang, J., Lee, S.H., Lin, C., Medici, R., Hack, E. and Myers, A.M. 1990. Expression in yeast of the T-URF13 protein from Texas male-sterile maize mitochondria confers sensitivity to methomyl and to Texas-cytoplasm-specific fungal toxins. *EMBO J.* 9: 339-347.

Huang, J., Struck, F., Matzinger, D.F. and Levings, C.S. 1994. Flower-enhanced expression of a nuclear-encoded mitochondrial respiratory protein is associated with changes in mitochondrial number. *Plant Cell*. 6: 439-448.

Itabashi, E., Kazama, T. and Toriyama, K. 2009. Characterization of cytoplasmic male sterility of rice with Lead Rice cytoplasm in comparison with that with Chinsurah Boro II cytoplasm. *Plant Cell Rep.* 28: 233-239.

Itchoda, N., Nishizawa, S., Nagano, H., Kubo, T. and Mikami, T. 2002. The sugar beet mitochondrial *nad4* gene: an intron loss and its phylogenetic implication in the Caryophyllales. *Theor. Appl. Genet.* 104: 209-213.

Iwabuchi, M., Kyozuka, J. and Shimamoto, K. 1993. Processing followed by complete editing of an altered mitochondrial *atp6* RNA restores fertility of cytoplasmic male sterile rice. *EMBO J.* 12: 1437-1446.

Iwabuchi, M., Koizuka, N., Fujimoto, H., Sakai, T. and Imamura, J. 1999. Identification and expression of the kosena radish (*Raphanus sativus* cv. Kosena) homologue of the ogura radish CMS-associated gene, *orf138*. *Plant Mol. Biol.* 39: 183-188.

Jabs, T. 1999. Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. *Biochem. Pharmacol.* 57: 231-245.

Janska, H. and Mackenzie, S.A. 1993. Unusual mitochondrial genome organization in cytoplasmic male-sterile common bean and the nature of cytoplasmic reversion to fertility. *Genetics*, 135: 869-879.

Janska, H., Sarria, R., Woloszynska, M., Arrieta-Montiel, M. and Mackenzie, S.A. 1998. Stoichiometric shifts in the common bean mitochondrial genome leading to male sterility and spontaneous reversion to fertility. *Plant Cell*. 10: 1163-1180.

Jiang, P.D., Zhang, X.Q., Zhu, Y.G., Zhu, W., Xie, H.Y. and Wang, X.D. 2007. Metabolism of reactive oxygen species in cotton cytoplasmic male sterility and its restoration. *Plant Cell Rep.* 26: 1627-1634.

Johns, C., Nickels, R., McIntosh, L. and Mackenzie, S. 1993. The expression of alternative oxidase and alternative respiratory capacity in cytoplasmic male-sterile common bean. *Sex. Plant Reprod.* 6: 257-265.

Jordan, D.R., Mace, E.S., Henzell, R.G., Klein, P.E. and Klein, R.R. 2010. Molecular mapping and candidate gene identification of the *Rf2* gene for pollen fertility restoration in sorghum *Sorghum bicolor* (L.) Moench. *Theor. Appl. Genet.* 120: 1279-1287.

Joyce, P.B.M. and Gray, M.W. 1989. Chloroplast-like transfer RNA genes expressed in wheat mitochondria. *Nucl. Acids Res.* 17: 5461-5476.

Kanazawa, A., Tsutsumi, N. and 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. and Newton, K.J. 2002. Differential expression of alternative oxidase genes in maize mitochondrial mutants. *Plant Cell*. 14: 3271-3284.

Katsuo, K. and Mizushima, U. 1958. Studies on the cytoplasmic difference among rice varieties, *Oryza sativa* L. I. On the fertility of hybrids obtained reciprocally between cultivated and wild varieties. *Japan. Journal Breed.* 8: 1-5.

Kaul, M.L.H. 1988. *Male sterility in higher plants.* Berlin: Springer-Verlag.

Kawanishi, Y., Shinada, H., Matsunaga, M., Masaki, Y., Mikami, T. and Kubo, T. 2010. A new source of cytoplasmic male sterility found in wild beet and its relationship to other CMS types. *Genome*. 53: 251-256.

Kazama, T., Nakamura, T., Watanabe, M., Sugita, M. and Toriyama, K. 2008. Suppression mechanism of mitochondrial ORF79 accumulation by *Rf1* protein in BT-type cytoplasmic male sterile rice. *Plant J.* 55: 619-628.

Kazama, T. and Toriyama, K. 2003. A pentatricopeptide repeat-containing gene that promotes the processing of aberrant *atp6* RNA of cytoplasmic male-sterile rice. *FEBS Lett.* 544: 99-102.

Kim, D.H., Kang, J.G. and Kim, B.D. 2007. Isolation and characterization of the cytoplasmic male sterility-associated *orf456* gene of chili pepper (*Capsicum*

annuum L.). *Plant Mol. Biol.* 63: 519-532.

Kim, S. and Yoon, M.K. 2010. Comparison of mitochondrial and chloroplast genome segments from three onion (*Allium cepa* L.) cytoplasm types and identification of a trans-splicing intron of *cox2*. *Curr. Genet.* 56: 177-188.

Kitazaki, K. and Kubo, T. 2010. Cost of having the largest mitochondrial genome: evolutionary mechanism of plant mitochondrial genome. *J. Bot.* 2010: Article ID: 620137.

Kitazaki, K., Nomoto, Y., Aoshima, A., Mikami, T. and Kubo, T. 2009. A mitochondrial gene involved in cytochrome c maturation (*ccmC*) is expressed as a precursor with a long NH_2-terminal extension in sugar beet. *J. Plant Physiol.* 166: 775-780.

Klein, R.R., Klein, P.E., Mullet, J.E., Minx, P., Rooney, W.L. and Schertz, K.F. 2005. Fertility restorer locus *Rf1* of sorghum (*Sorghum bicolor* L.) encodes a pentatricopeptide repeat protein not present in the colinear region of rice chromosome 12. *Theor. Appl. Genet.* 111: 994-1012.

Kleine, T., Maier, U.G. and Leister, D. 2009. DNA Transfer from organelles to the nucleus: the idiosyncratic genetics of endosymbiosis. *Annu. Rev. Plant Biol.* 60: 115-138.

Knoop, V. 2004. The mitochondrial DNA of land plants: peculiarities in phylogenetic perspective. *Curr. Genet.* 46: 123-139.

Koizuka, N., Imai, R., Fujimoto, H., Hayakawa, T., Kimura, Y., Kohno-Murase, J., Sakai, T., Kawasaki, S. and Imamura, J. 2003. Genetic characterization of a pentatricopeptide repeat protein gene, *orf687*, that restores fertility in the cytoplasmic male-sterile Kosena radish. *Plant J.* 34: 407-415.

Kojima, H., Kazama, T., Fujii, S. and Toriyama, K. 2010. Cytoplasmic male sterility-associated ORF79 is toxic to plant regeneration when expressed with mitochondrial targeting sequence of ATPase gamma subunit. *Plant Biotech.* 27: 111-114.

Kolodziejczak, M., Gibala, M., Urantowka, A. and Janska, H. 2007. The significance of *Arabidopsis* AAA proteases for activity and assembly/stability of mitochondrial OXPHOS complexes. *Physiol. Plant.* 129: 135-142.

Komori, T., Ohta, S., Murai, N., Takakura, Y., Kuraya, Y., Suzuki, S., Hiei, Y., Imaseki, H. and Nitta, N. 2004. Map-based cloning of a fertility restorer gene, *Rf-1*, in rice (*Oryza sativa* L.). *Plant J.* 37: 315-325.

Korth, K.L. and Levings, C.S. 1993. Bacurovirus expression of the maize mitochondrial protein URF13 confers insecticidal activity in cell-cultures and larvae. *Proc*. *Natl. Acad. Sci. USA.* 90: 3388-3392.

Krishnasamy, S., Grant, R.A. and Makaroff, C.A. 1994. Subunit 6 of the F_0-ATP synthase complex from cytoplasmic male-sterile radish: RNA editing and NH_2-terminal protein sequencing. *Plant Mol. Biol.* 24: 129-141.

Krishnasamy, S. and Makaroff, C.A. 1994. Organ-specific reduction in the abundance of a mitochondrial protein accompanies fertility restoration in cytoplasmic male-sterile radish. *Plant Mol. Biol.* 26: 935-946.

Kubo, N. and Arimura, S. 2010. Discovery of the *rpl10* gene in diverse plant mitochondrial genomes and its probable replacement by the nuclear gene for chloroplast RPL10 in two lineages of angiosperms. *DNA Res.* 17: 1-9.

Kubo, N., Salomon, B., Komatsuda, T., von Bothmer, R. and Kadowaki, K. 2005. Structural and distributional variation of mitochondrial *rps2* genes in the tribe Triticeae (Poaceae). *Theor. Appl. Genet.* 110: 995-1002.

Kubo, T. and Mikami, T. 2007. Organization and variation of angiosperm mitochondrial genome. *Physiol. Plant.* 129: 6-13.

Kubo, T. and Newton, K.J. 2008. Angiosperm mitochondrial genomes and mutaitons. *Mitochondrion*, 8: 5-14.

Kubo, T., Nishizawa, S. and 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. and Mikami, T. 2000. The complete nucleotide sequence of the mitochondrial genome of sugar beet (*Beta vulgaris* L.) reveals a novel gene for $tRNA^{Cys}$(GCA). *Nucl. Acids Res.* 28: 2571-2576.

Kubo, T., Yanai, Y., Kinoshita, T. and Mikami, T. 1995. The chloroplast *trnP-trnW-petG* gene cluster in the mitochondrial genomes of *Beta vulgaris*, *B. trigyna* and *B. webbiana*: evolutionary aspects. *Curr. Genet.* 27: 285-289.

Kudla, J., Albertazzi, F.J., Blazevic, D., Hermann, M. and Bock, R. 2002. Loss of the mitochondrial *cox2* intron 1 in a family of monocotyledonous plants and utilization of mitochondrial intron sequences for the construction of a nuclear intron. *Mol. Genet. Genomics.* 267: 223-230.

Kuhn, K., Bohne, A.V., Liere, K., Weihe, A. and Borner, T. 2007. Arabidopsis phage-type RNA polymerases: accurate in vitro transcription of organellar genes. *Plant Cell*. 19: 959-971.

Kuhn, K., Richter, U., Meyer, E.H., Delannoy, E., de Longevialle, A.F., O'Toole, N., Borner, T., Millar, A.H., Small, I.D. and Whelan, J. 2009. Phage-type RNA polymerase RPOTmp performs gene-specific transcription in mitochondria of *Arabidopsis thaliana*. *Plant Cell*. 21: 2762-2779.

Kunzmann, A., Brennicke, A. and Marchfelder, A. 1998. 5' end maturation and RNA editing have to precede tRNA 3' processing in plant mitochondria. *Proc. Natl. Acad. Sci. USA*. 95: 108-113.

Kuzmin, E.V., Duvick, D.N. and Newton, K.J. 2005. A mitochondrial mutator system in maize. *Plant Physiol.* 137: 779-789.

L'Homme, Y. and Brown, G.G. 1993. Organizational differences between cytoplasmic male-sterile and male fertile *Brassica* mitochondrial genomes are confined to a single transposed locus. *Nucl. Acids Res.* 21: 1903-1909.

L'Homme, Y., Stahl, R.J., Li, X.Q., Hameed, A. and Brown, G.G. 1997. Brassica *nap* cytoplasmic male sterility is associated with expression of a mtDNA region containing a chimeric gene similar to the *pol* CMS-associated *orf224* gene. *Curr. Genet.* 31: 325-335.

Lalanne, E., Mathieu, C., Vedel, F. and De Paepe, R. 1998. Tissue-specific expression of genes encoding isoforms of the mitochondrial ATPase beta subunit in *Nicotiana sylvestris*. *Plant Mol. Biol.* 38: 885-888.

Laroche, J., Li, P., Maggia, L. and Bousquet, J. 1997. Molecular evolution of angiosperm mitochondrial introns and exons. *Proc. Natl. Acad. Sci. USA*. 94: 5722-5727.

Laser, K.D. and Lersten, N.R. 1972. Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. *Bot. Rev.* 38: 425-454.

Laughnan, J.R. and Gabay-Laughnan, S. 1983. Cytoplasmic male sterility in maize. *Annu. Rev. Genet.* 17: 27-48.

Lee, H.Y., Chou, J.Y., Cheong, L., Chang, N.H., Yang, S.Y. and Leu, J.Y. 2008. Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species. *Cell*. 135: 1065-1073.

Leon, P., Walbot, V. and Bedinger, P. 1989. Molecular analysis of the linear 2.3-kb plasmid of maize mitochondria: apparent capture of tRNA genes. *Nucl. Acids Res.*

17: 4089-4099.

Li, S.Q., Tan, Y.P., Wang, K., Wan, C.X. and Zhu, Y.G. 2008. Gametophytically alloplasmic CMS line of rice (*Oryza sativa* L.) with variant *orfH79* haplotype corresponds to specific fertility restorer. *Theor. Appl. Genet.* 117: 1389-1397.

Li, X.Q., Jean, M., Landry, B.S. and Brown, G.G. 1998. Restorer genes for different forms of *Brassica* cytoplasmic male sterility map to a single nuclear locus that modifies transcripts of several mitochondrial genes. *Proc. Natl. Acad. Sci. USA*. 95: 10032-10037.

Lilly, J.W. and Havey, M.J. 2001. Small, repetitive DNAs contribute significantly to the expanded mitochondrial genome of cucumber. *Genetics*: 159: 317-328.

Li-Pook-Than, J., Carrillo, C. and Bonen, L. 2004. Variation in mitochondrial transcript profiles of protein-coding genes during early germination and seedling development in wheat. *Curr. Genet.* 46: 374-380.

Li-Pook-Than, J., Carrillo, C., Niknejad, N., Calixte, S., Crosthwait, J. and Bonen, L. 2007. Relationship between RNA splicing and exon editing near intron junctions in wheat mitochondria. *Physiol. Plant.* 129: 23-33.

Linke, B. and Borner, T. 2005 Mitochondrial effects on flower and pollen development. *Mitochondrion*, 5: 389-402.

Liu, F., Cui, X.Q., Horner, H.T., Weiner, H. and Schnable, P.S. 2001. Mitochondrial aldehyde dehydrogenase activity is required for male fertility in maize. *Plant Cell*. 13: 1063-1078.

Liu, F. and Schnable, P.S. 2002. Functional specialization of maize mitochondrial aldehyde dehydrogenases. *Plant Physiol.* 130: 1657-1674.

Mackenzie, S.A. 1991. Identification of a sterility-inducing cytoplasm in a fertile accession line of *Phaseolus vulgaris* L. *Genetics*. 127: 411-416.

Majewskasawka, A., Rodriguezgarcia, M.I., Nakashima, H. and Jassen, B. 1993. Ultrastructural expression of cytoplasmic male-sterility in sugar beet (*Beta vulgaris* L.). *Sex. Plant Reprod.* 6: 22-32.

Makaroff, C.A. and Palmer, J.D. 1988. Mitochondrial DNA rearrangements and transcriptional alterations in the male-sterile cytoplasm of Ogura radish. *Mol. Cell. Biol.* 8: 1474-1480.

Manchekar, M., Scissum-Gunn, K.D., Hammett, L.A., Backert, S. and Nielsen, B.L. 2009. Mitochondrial DNA recombination in *Brassica campestris*. *Plant Sci.* 177:

629-635.

Marechal, A. and Brisson, N. 2010. Recombination and the maintenance of plant organelle genome stability. *New Phytol.* 186: 299-317.

Marechal, L., Runebergroos, P., Grienenberger, J.M., Colin, J., Weil, J.H., Lejeune, B., Quetier, F. and Lonsdale, D.M. 1987. Homology in the region containing a $tRNA^{Trp}$ gene and a (complete or partial) $tRNA^{Pro}$ gene in wheat mitochondrial and chloroplast genomes. *Curr. Genet.* 12: 91-98.

Marechal-Drouard, L., Guillemaut, P., Cosset, A., Arbogast, M., Weber, F., Weil, J.H. and Dietrich, A. 1990. Transfer RNAs of potato (*Solanum tuberosum*) mitochondria have different genetic origins. *Nucl. Acids Res.* 18: 3689-3696.

Marechal-Drouard, L., Kumar, R., Remacle, C. and Small, I. 1996. RNA editing of larch mitochondrial $tRNA^{His}$ precursors is a prerequisite for processing. *Nucl. Acids Res.* 24: 3229-3234.

Marechal-Drouard, L., Weil, J.-H. and Dietrich, A. 1993. Transfer RNAs and transfer RNA genes in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44: 13-32.

Marienfeld, J., Unseld, M. and Brennicke, A. 1999. The mitochondrial genome of *Arabidopsis* is composed of both native and immigrant information. *Trends Plant Sci.* 4: 495-502.

Matsuhira, H., Shinada, H., Yui-Kurino, R., Hamato, N., Umeda, M., Mikami, T. and Kubo, T. 2007. An anther-specific lipid transfer protein gene in sugar beet: its expression is strongly reduced in male-sterile plants with Owen cytoplasm. *Physiol. Plant.* 129: 407-414.

Matsunaga, M., Nagano, H., Mikami, T. Kubo, T. 2010. Large 3′ UTR of sugar beet *rps3* is truncated in cytoplasmic male-sterile mitochondria. *Plant Cell Rep.* doi: 10.1007/s00299-010-0912-y.

McCauley, D.E. and Bailey, M.F. 2009. Recent advances in the study of gynodioecy: the interface of theory and empiricism. *Annal. Bot.* 104: 611-620.

McCauley, D.E. and Olson, M.S. 2008. Do recent findings in plant mitochondrial molecular and population genetics have implications for the study of gynodioecy and cytonuclear conflict ? *Evolution*. 62: 1013-1025.

McDermott, P., Connolly, V. and Kavanagh, T.A. 2008. The mitochondrial genome of a cytoplasmic male sterile line of perennial rygrass (*Lolium perenne* L.) contains an integrated linear plasmid-like element. *Theor. Appl. Genet.* 117: 459-470.

Mellema, S., Eichenberger, W., Rawyler, A., Suter, M., Tadege, M. and Kuhlemeier, C. 2002. The ethanolic fermentation pathway supports respiration and lipid biosynthesis in tobacco pollen. *Plant J.* 30: 329-336.

Mower, J.P. and Bonen, L. 2009. Ribosomal protein L10 is encoded in the mitochondrial genome of many land plants and green algae. *BMC Evol. Biol.* 9: 265.

Mower, J.P., Stefanovic, S., Young, G.J. and Palmer, J.D. 2004. Gene transfer from parasitic to host plants. *Nature*. 432: 165-166.

Mower, J.P., Touzet, P., Gummow, J.S., Delph, L.F., Palmer, J.D. 2007. Extensive variation in synonymous substitution rates in mitochondrial genes of seed plants. *BMC Evol. Biol.* 7: 135.

Murai, K. and Tsunewaki, K. 1993. Photoperiod-sensitive male sterility in wheat with *Aegilops crassa* cytoplasm. *Euphytica*. 67: 41-48.

Murayama, K., Yahara, T. and Terachi, T. 2004. Variation of female frequency and cytoplasmic male-sterility gene frequency among natural gynodioecious populations of wild radish (*Raphanus sativus* L.). *Mol. Ecol.* 13: 2459-2464.

Nakai, S., Noda, D., Kondo, M. and Terachi, T. 1995. High-level expression of a mitochondrial *orf522* gene from the male-sterile sunflower is lethal to *Escherichia coli*. *Breeding Sci.* 45: 233-236.

Nishizawa, S., Mikami, T. and Kubo, T. 2007. Mitochondrial DNA phylogeny of cultivated and wild beets: relationships among cytoplasmic male-sterility-inducing and nonsterilizing cytoplasms. *Genetics*. 177: 1703-1712.

Nivison, H.T., Sutton, C.A., Wilson, R.K. and Hanson, M.R. 1994. Sequencing, processing, and localization of the petunia CMS-associated mitochondrial protein. *Plant J.* 5: 613-623.

Nizampatnam, N.R., Doodhi, H., Kalinati Narasimhan, Y., Mulpuri, S. and Viswanathaswamy, D.K. 2009. Expression of sunflower cytoplasmic male sterility-associated open reading frame, *orfH522* induces male sterility in transgenic plants. *Planta*. 229: 987-1001.

Notsu, Y., Masood, S., Nishikawa, T., Kubo, N., Akiduki, G., Nakazono, M., Hirai, A. and 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.

Nugent, J.M. and Palmer, J.D. 1991. RNA-mediated transfer of the gene *coxII* from the

mitochondrion to the nucleus during flowering plant evolution. *Cell*. 66: 473-481.

Oda, K., Yamato, K., Ohta, E., Nakamura, Y., Takemura, M., Nozato, N., Akashi, K., Kanegae, T., Ogura, Y., Kohchi, T. and Ohyama, K. 1992. Gene organization deduced from the complete sequence of liverwort *Marchantia polymorpha* mitochondrial DNA: a primitive form of plant mitochondrial genome. *J. Mol. Biol.* 223: 1-7.

Odahara, M., Kuroiwa, H., Kuroiwa, T. and Sekine, Y. 2009. Suppression of repeat-mediated gross mitochondrial genome rearrangements by *RecA* in the moss *Physcomitrella patens*. *Plant Cell*. 21: 1182-1194.

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. and Tsunewaki, K. 2005. Structural dynamics of cereal mitochondrial genomes as revealed by complete nucleotide sequencing of the wheat mitochondrial genome. *Nucl. Acids Res.* 33: 6235-6250.

Ohtani, K., Yamamoto, H. and 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. and Bendich, A.J. 1996. Size and structure of replicating mitochondrial DNA in cultured tobacco cells. *Plant Cell*. 8: 447-461.

Onodera, Y., Yamamoto, M.P., Kubo, T., and Mikami, T. 1999. Heterogeneity of the *atp6* presequences in normal and different sources of male-sterile cytoplasms of sugar beet. *J. Plant Physiol.* 155: 656-660.

Owen, F.V. 1942. Male sterility in sugar beets produced by complementary effects of cytoplasmic and mendelian inheritance. *Am J. Bot.* 29: 692.

Owen, F.V. 1945. Cytoplasmically inherited male-sterility in sugar beets. *J. Agr. Res.* 71: 423-440.

Palmer, J.D. 1988. Intraspecific variation and multicircularity in *Brassica* mitochondrial DNAs. *Genetics*. 118: 341-351.

Palmer, J.D. 1990. Contrasting modes and tempos of genome evolution in land plant organelles. *Trends Genet.* 6: 115-120.

Palmer, J.D. and Herbon, L.A. 1987. Unicircular structure of the *Brassica hirta* mitochondrial genome. *Curr. Genet.* 11: 565-570.

Palmer, J.D. and Shields, C.R. 1984. Tripartite structure of the *Brassica campestris*

mitochondrial genome. *Nature*. 307: 437-440.

Parish, R.W. and Li, S.F. 2010. Death of a tapetum: A programme of developmental altruism. *Plant Sci.* 178: 73-89.

Pelletier, G. and Budar, F. 2007. The molecular biology of cytoplasmically inherited male sterility and prospects for its engineering. *Curr. Opin. Biotech.* 18: 121-125.

Perrotta, G., Grienenberger, J.M. and Gualberto, J.M. 2002. Plant mitochondrial *rps2* genes code for proteins with a C-terminal extension that is processed. *Plant Mol. Biol.* 50: 523-533.

Picardi, E., Horner, D.S., Chiara, M., Schiavon, R., Valle, G. and Pesole, G. 2010. Large scale detection and analysis of RNA editing in grape mtDNA by RNA deep-sequencing. *Nucl. Acids Res.* doi:10.1093/nar/gkq202

Pla, M., Mathieu, C., De Paepe, R., Chetrit, P. and Vedel, F. 1995. Deletion of the last two exons of the mitochondrial *nad7* gene results in lack of the NAD7 polypeptide in a *Nicotiana sylvestris* CMS mutant. *Mol. Gen. Genet.* 248: 79-88.

Polidoros, A.N., Mylona, P.V. and Arnholdt-Schmitt, B. 2009. *Aox* gene structure, transcript variation and expression in plants. *Physiol. Plant.* 137: 342-353.

Pring, D.R. and Lonsdale, D.M. 1989. Cytoplasmic male-sterility and maternal inheritance of disease susceptibility in maize. *Ann. Rev. Phytopathol.* 27: 483-502.

Pring, D.R. and Tang, H.V. 2004. Transcript profiling of male-fertile and male-sterile sorghum indicates extensive alterations in gene expression during microgametogenesis. *Sex. Plant Reprod.* 16: 289-297.

Pring, D.R., Tang, H.V., Chase, C.D. and Siripant, M.N. 2006. Microspore gene expression associated with cytoplasmic male sterility and fertility restoration in sorghum. *Sex. Plant Reprod.* 19: 25-35.

Quetier, F. and Vedel, F. 1977. Heterogeneous population of mitochondrial DNA molecules in higher plants. *Nature*. 268: 365-368.

Raczynska, K.D., Le Ret, M., Rurek, M., Bonnard, G., Augustyniak, H. and Gualberto, J.M. 2006. Plant mitochondrial genes can be expressed from mRNAs lacking stop codons. *FEBS Lett.* 580: 5641-5646.

Rasmussen, J. and Hanson, M.R. 1989. A NADH dehydrogenase subunit gene is co-transcribed with the abnormal *Petunia* mitochondrial gene associated with cytoplasmic male sterility. *Mol. Gen. Genet.* 215: 332-336.

Ren, Y., Zhang, Z.H., Liu, J.H., Staub, J.E., Han, Y.H., Cheng, Z.C., Li, X.F., Lu, J.Y., Miao, H., Kang, H.X., Xie, B.Y., Gu, X.F., Wang, X.W., Du, Y.C., Jin, W.W. and Huang, S.W. 2009. Integrated genetic and cytogenetic map of the cucumber genome. *Plos One*, 4: 8.

Rhoads, D.M., Brunner-Neuenschwander, B., Levings, C.S.III. and Siedow, J.N. 1998. Cross-linking and disulfide bond formation of introduced cysteine residues suggest a modified model for the tertiary structure of URF13 in the pore-forming oligomers. *Arch. Biochem. Biophys.* 354: 158-164.

Richardson, A.O. and Palmer, J.D. 2007. Horizontal gene transfer in plants. *J. Exp. Bot.* 58: 1-9.

Robison, M.M. and Wolyn, D.J. 2006a. A 60 kDa COX1 protein in mitochondria of carrot irrespective of the presence of C-terminal extensions in the *cox1* reading frame. *Mol. Genet. Genomics.* 275: 68-73.

Robison, M.M. and Wolyn, D.J. 2006b. Petaloid-type cms in carrot is not associated with expression of *atp8* (*orfB*). *Theor. Appl. Genet.* 112: 1496-1502.

Sabar, M., Gagliardi, D., Balk, J. and Leaver, C.J. 2003. ORFB is a subunit of F_1F_O-ATP synthase: insight into the basis of cytoplasmic male sterility in sunflower. *EMBO Rep.* 4: 381-386.

Sakamoto, W., Tan, S.H., Murata, M. and Motoyoshi, F. 1997. An unusual mitochondrial *atp9-rpl16* cotranscript found in the maternal distorted leaf mutant of *Arabidopsis thaliana*: implication of GUG as an initiation codon in plant mitochondria. *Plant Cell Physiol.* 38: 975-979.

Salinas, T., Duchene, A.M. and Marechal-Drouard, L. 2008. Recent advances in tRNA mitochondrial import. *Trends Biochem. Sci.* 33: 320-329.

Sanchez-Puerta, M.V., Cho, Y., Mower, J.P., Alverson, A.J. and Palmer, J.D. 2008. Frequent, phylogenetically local horizontal transfer of the *cox1* group I intron in flowering plant mitochondria. *Mol. Bio. Evol.* 25: 1762-1777.

Sandhu, A.P.S., Abdelnoor, R.V. and Mackenzie, S.A. 2007. Transgenic induction of mitochondrial rearrangements for cytoplasmic male sterility in crop plants. *Proc. Natl. Acad. Sci. USA.* 104: 1766-1770.

Sane, A.P., Nath, P. and Sane, P.V. 1997. Differences in kinetics of F_1-ATPases of cytoplasmic male sterile, maintainer and fertility restored lines of sorghum. *Plant Sci.* 130: 19-25.

Sangare, A., Weil, J.-H., Grienenberger, J.-M., Fauron, C. and Lonsdale, D. 1990. Localization and organization of tRNA genes on the mitochondrial genomes of fertile and male sterile lines of maize. *Mol. Gen. Genet.* 223: 224-232.

Sarria, R., Lyznik, A., Vallejos, C.E. and Mackenzie, S.A. 1998. A cytoplasmic male sterility-associated mitochondrial peptide in common bean is post-translationally regulated. *Plant Cell*. 10: 1217-1228.

Satoh, M., Kubo, T. and 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.

Satoh, M., Kubo, T., Nishizawa, S., Estiati, A., Itchoda, N. and 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.

Scheffler, I.E. 1999. *Mitochondria*. New York: Wiley-Liss.

Schmitz-Linneweber, C. and Small, I. 2008. Pentatricopeptide repeat proteins: a socket set for organelle gene expression. *Trends Plant Sci.* 13: 663-670.

Schnable, P.S. and Wise, R.P. 1998. The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends Plant Sci.* 3: 175-180.

Schuster, W., Grienenberger, J.M., Weil, J.H. and Brennicke, A. 1988. Comparison of $tRNA^{Trp}$ and $tRNA^{Pro}$ gene sequences in the chloroplast and mitochondrial genomes of *Oenothera*. *Nucl. Acids Res.* 16: 7737.

Shedge, V., Arrieta-Montiel, M.P., Christensen, A.C. and Mackenzie, S.A. 2007. Plant mitochondrial recombination surveillance requires unusual *RecA* and *MutS* homologs. *Plant Cell*. 19: 1251-1264.

Shinada, T., Kikuchi, Y., Fujimoto, R. and Kishitani, S. 2006. An alloplasmic male-sterile line of *Brassica oleracea* harboring the mitochondria from *Diplotaxis muralis* expresses a novel chimeric open reading frame, *orf72*. *Plant Cell Physiol.* 47: 549-553.

Siculella, L., Pacoda, D., Treglia, S., Gallerani, R. and Ceci, L.R. 1996. GTG as translation initiation codon in the apocytochrome b gene of sunflower mitochondria. *DNA Seq.* 6: 365-369.

Siculella, L. and Palmer, J.D. 1988. Physical and gene organization of mitochondrial DNA in fertile and male sterile sunflower: CMS-associated alterations in

structure and transcription of the *atpA* gene. *Nucl. Acids Res.* 16: 3787-3799.

Singh, M. and Brown, G.G. 1991. Suppression of cytoplasmic male-sterility by nuclear genes alters expression of a novel mitochondrial gene region. *Plant Cell*. 3: 1349-1362.

Skibbe, D.S., Wang, X.J., Borsuk, L.A., Ashlock, D.A., Nettleton, D. and Schnable, P.S. 2008. Floret-specific differences in gene expression and support for the hypothesis that tapetal degeneration of *Zea mays* L. occurs via programmed cell death. *J. Genet. Genom.* 35: 603-616.

Sloan, D.B., Alverson, A.J., Storchova, H., Palmer, J.D. and Taylor, D.R. 2010. Extensive loss of translational genes in the structurally dynamic mitochondrial genome of the angiosperm *Silene latifolia. BMC Evol. Biol.* 10: 274.

Sloan, D.B., Barr, C.M., Olson, M.S., Keller, S.R. Taylor, D.R. 2008. Evolutionary rate variation at multiple levels of biological organization in plant mitochondrial DNA. *Mol. Biol. Evol.* 25: 243-246.

Sloan, D.B., Oxelman, B., Rautenberg, A. Taylor, D.R. 2009. Phylogenetic analysis of mitochondrial substitution rate variation in the angiosperm tribe Sileneae. *BMC Evol. Biol.* 9: 260.

Small, I., Suffolk, R. and Leaver, C.J. 1989. Evolution of plant mitochondrial genomes via substoichiometric intermediates. *Cell*. 58, 69-76.

St John, J.C., Jokhi, R.P. and Barratt, C.L.R. 2005. The impact of mitochondrial genetics on male infertility. *Int. J. Androl.* 28: 65-73.

Sugiyama, Y., Watase, Y., Nagase, M., Makita, N., Yagura, S., Hirai, A. and 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.

Sunkel, S., Brennicke, A. and Knoop, V. 1994. RNA editing of a conserved reading frame in plant mitochondria increases its similarity to two overlapping reading frames in *Escherichia coli*. *Mol. Gen. Genet.* 242: 65-72.

Takenaka, M., Verbitskiy, D., van der Merwe, J.A., Zehrmann, A. and Brennicke, A. 2008. The process of RNA editing in plant mitochondria. *Mitochondrion*. 8: 35-46.

Tang, H.V., Chang, R.Y. and Pring, D.R. 1998. Cosegregation of single genes associated with fertility restoration and transcript processing of sorghum mitochondrial

orf107 and *urf209*. *Genetics*. 150: 383-391.

Tang, H.V., Pring, D.R., Shaw, L.C., Salazar, R.A., Muza, F.R., Yan, B. and Schertz, K.F. 1996. Transcript processing internal to a mitochondrial open reading frame is correlated with fertility restoration in male-sterile sorghum. *Plant J.* 10: 123-133.

Terasawa, K., Odahara, M., Kabeya, Y., Kikugawa, T., Sekine, Y., Fujiwara, M. and Sato, N. 2007. The mitochondrial genome of the moss *Physcomitrella patens* sheds new light on mitochondrial evolution in land plants. *Mol. Bio. Evol.* 24: 699-709.

Thompson, K.F. 1972. Cytoplasmic male sterility in oil-seed rape. *Heredity*. 29: 253-257.

Thomson, M.C., Macfarlane, J.L., Beagley, C.T. and Wolstenholme, D.R. 1994. RNA editing of *mat-r* transcripts in maize and soybean increases similarity of the encoded protein to fungal and bryophyte group II intron maturases: evidence that *mat-r* encodes a functional protein. *Nucl. Acids Res.* 22: 5745-5752.

Tian, X.J., Zheng, J., Hu, S.N. and Yu, J. 2006. The rice mitochondrial genomes and their variations. *Plant Physiol.* 140: 401-410.

Tiffin, P., Olson, M.S. and Moyle, L.C. 2001. Asymmetrical crossing barriers in angiosperms. *Proc. R. Soc. London B.* 268: 861-867.

Touzet, P. 2002. Is *rf2* a restorer gene of CMS-T in maize? *Trends Plant Sci.* 7: 434-434.

Touzet, P. and Budar, F. 2004. Unveiling the molecular arms race between two conflicting genomes in cytoplasmic male sterility? *Trends Plant Sci.* 9: 568-570.

Touzet, P., Hueber, N., Burkholz, A., Barnes, S. and Cuguen, J. 2004 Genetic analysis of male fertility restoration in wild cytoplasmic male sterility G of beet. *Theor. Appl. Genet.* 109: 240-247.

Unseld, M., Marienfeld, J.R., Brandt, P. and Brennicke, A. 1997. The mitochondrial genome of *Arabidopsis thaliana* contains 57 genes in 366,924 nucleotides, *Nat. Genet.* 15: 57-61.

Uyttewaal, M., Arnal, N., Quadrado, M., Martin-Canadell, A., Vrielynck, N., Hiard, S., Gherbi, H., Bendahmane, A., Budar, F. and Mireau, H. 2008. Characterization of *Raphanus sativus* pentatricopeptide repeat proteins encoded by the fertility restorer locus for Ogura cytoplasmic male sterility. *Plant Cell.* 20: 3331-3345.

Vidal, G., Ribas-Carbo, M., Garmier, M., Dubertret, G., Rasmusson, A.G., Mathieu, C., Foyer, C.H. and 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.

Wan, C.X., Li, S.Q., Wen, L., Kong, J., Wang, K. and Zhu, Y.G. 2007. Damage of oxidative stress on mitochondria during microspores development in Honglian CMS line of rice. *Plant Cell Rep.* 26: 373-382.

Wang, D., Wu, Y.-W., Shih, A.C.-C., Wu, C.-S., Wang, Y.-N. and Chaw, S.-M. 2007. Transfer of chloroplast genomic DNA to mitochondrial genome occurred at least 300 MYA. *Mol. Biol. Evol.* 24: 2040-2048.

Wang, Z., Zou, Y., Li, X., Zhang, Q., Chen, L., Wu, H., Su, D., Chen, Y., Guo, J., Luo, D., Long, Y., Zhong, Y. and Liu, Y.G. 2006. Cytoplasmic male sterility of rice with boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing. *Plant Cell*. 18: 676-687.

Ward, B.L., Anderson, R.S. and Bendich, A.J. 1981. The mitochondrial genome is large and variable in a family of plants (Cucurbitaceae). *Cell*. 25: 793-803.

Warmke, H.E. and Lee, S.-L.J. 1977. Mitochondrial degeneration in Texas cytoplasmic male-sterile corn anthers. *J. Heredity*. 68: 213-222.

Watanabe, Y., Sakaguchi, S. and Kudo, M. 1968. On the male sterile rice plant possessing the cytoplasm of Burmese variety "Lead Rice.". *Japan. Journal Breed.* 18 (Supple. 2): 77-78.

Wen, L.Y. and Chase, C.D. 1999. Pleiotropic effects of a nuclear restorer-of-fertility locus on mitochondrial transcripts in male-fertile and S male-sterile maize. *Curr. Genet.* 35: 521-526.

Wen, L.Y., Ruesch, K.L., Ortega, V.M., Kamps, T.L., Gabay-Laughnan, S. and Chase, C.D. 2003. A nuclear restorer-of-fertility mutation disrupts accumulation of mitochondrial ATP synthase subunit alpha in developing pollen of S male-sterile maize. *Genetics*. 165: 771-779.

Williams, M.A., Kutcher, B.M. and Mulligan, R.M. 1998. Editing site recognition in plant mitochondria: the importance of 5′-flanking sequences. *Plant Mol. Biol.* 36: 229-237.

Wilson, Z.A. and Zhang, D.B. 2009. From *Arabidopsis* to rice: pathways in pollen development. *J. Exp. Bot.* 60: 1479-1492.

Wintz, H., Chen, H.C., Sutton, C.A., Conley, C.A., Cobb, A., Ruth, D. and Hanson, M.R. 1995. Expression of the CMS-associated *urfS* sequence in transgenic petunia and tobacco. *Plant Mol. Biol.* 28: 83-92.

Woloszynska, M. 2010. Heteroplasmy and stoichiometric complexity of plant

mitochondrial genomes - though this be madness, yet there's method in't. *J. Exp. Bot.* 61: 657-671.

Won, H. and Renner, S. S. 2003. Horizontal gene transfer from flowering plants to *Gnetum*. *Proc. Natl. Acad. Sci. USA*. 100: 10824-10829.

Yamagishi, H. and Terachi, T. 2001. Intra- and inter-specific variations in the mitochondrial gene *orf138* of Ogura-type male-sterile cytoplasm from *Raphanus sativus* and *Raphanus raphanistrum*. *Theor. Appl. Genet.* 103: 725-732.

Yamamoto, M.P., Kubo, T., and Mikami, T. 2005. The 5'-leader sequence of sugar beet mitochondrial *atp6* encodes a novel polypeptide that is characteristic of Owen cytoplasmic male sterility. *Mol. Genet. Genomics*. 273: 342-349.

Yamamoto, M.P., Shinada, H., Onodera, Y., Komaki, C., Mikami, T. and Kubo, T. 2008. A male sterility-associated mitochondrial protein in wild beets causes pollen disruption in transgenic plants. *Plant J.* 54: 1027-1036.

Yang, J.H., Huai, Y. and Zhang, M.F. 2009. Mitochondrial *atpA* gene is altered in a new *orf220*-type cytoplasmic male-sterile line of stem mustard (*Brassica juncea*). *Mol. Biol. Rep.* 36: 273-280.

Yang, S., Terachi, T. and Yamagishi, H. 2008. Inhibition of chalcone synthase expression in anthers of *Raphanus sativus* with Ogura male sterile cytoplasm. *Annal Bot.* 102: 483-489.

Yasumoto, K., Nagashima, T., Umeda, T., Yoshimi, M., Yamagishi, H. and Terachi, T. 2008. Genetic and molecular analysis of the restoration of fertility (*Rf*) genes for Ogura male-sterility from a Japanese wild radish (*Raphanus sativus* var. *hortensis* f. *raphanistroides* Makino). *Euphytica*. 164: 395-404.

Yasumoto, K., Terachi, T. and Yamagishi, H. 2009. A novel *Rf* gene controlling fertility restoration of Ogura male sterility by RNA processing of *orf138* found in Japanese wild radish and its STS markers. *Genome*. 52: 495-504.

Young, E.G. and Hanson, M.R. 1987. A fused mitochondrial gene associated with cytoplasmic male-sterility is developmentally regulated. *Cell*. 50: 41-49.

Zabala, G., Gabay-Laughnan, S. and Laughnan, J.R. 1997. The nuclear gene *Rf3* affects the expression of the mitochondrial chimeric sequence R implicated in S-type male sterility in maize. *Genetics*. 147: 847-860.

Zaegel, V., Guermann, B., Le Ret, M., Andres, C., Meyer, D., Erhardt, M., Canaday, J., Gualberto, J.M. and 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.

Zehrmann, A., van der Merwe, J.A., Verbitskiy, D., Brennicke, A. and Takenaka, M. 2008. Seven large variations in the extent of RNA editing in plant mitochondria between three ecotypes of *Arabidopsis thaliana*. *Mitochondrion*. 8: 319-327.

Zeng, X., Neupert, W. and Tzagoloff, A. 2007. The metalloprotease encoded by *ATP23* has a dual function in processing and assembly of subunit 6 of mitochondrial ATPase. *Mol. Biol. Cell*. 18: 617-626.

Zhang, H., Li, S.Q., Yi, P., Wan, C.X., Chen, Z.Y. and Zhu, Y.G. 2007. A Honglian CMS line of rice displays aberrant F_0 of F_0F_1-ATPase. *Plant Cell Rep.* 26: 1065-1071.

Figure legends

Figure 1 Cytoplasmic male sterility (CMS) is expressed as a result of nuclear-mitochondrial interactions. When a plant possesses an MS-inducing mitochondrial genome and a non-restoring allele *rf*, it expresses MS (MS line). A plant with the same nuclear genotype but no sterilizing factor (N) in its mitochondrial genome is male fertile (MF), and this plant is used for the propagation of the MS line (maintainer line). Because the MS line is unable to self-pollinate, a different pollen parent is required and seeds set on the MS line are hybrid. If the hybrids are required to be MF, the pollen parent should possess a restoring allele *Rf*. If the yield of the crop is derived from a vegetative organ, the restorer allele may not be necessary.

Figure 2 Organization of CMS-associated loci mentioned in the text. Yellow parts indicate sequences of unknown origin. Codons including two nucleotide substitutions, one truncates *Gcox2* and the other extends *Gnad9-1* ORFs, are boxed.

Table 1 Available sequences for plant mitochondrial master circles (MC).

Scientific name	Common name	Cultivar/ accession	Size of MC	Accession no.	References
Arabidopsis thaliana	Thale cress	Columbia	366924 bp	Y08501	Unseld et al., 1997
Beta vulgaris subsp. *vulgaris*	Sugar beet	TK81-MS	501020 bp	BA000024	Satoh et al., 2004
Beta vulgaris subsp. *vulgaris*	Sugar beet	TK81-O	368801 bp	BA000009	Kubo et al., 2000
Silene latifolia			253413 bp	HM562727	Sloan et al., 2010
Brassica napus	Rapeseed	Westar	221853 bp	AP006444	Handa, 2003
Carica papaya	Papaya	SunUp	476890 bp	EU431224	Direct submission to DDBJ/EMBL/GenBank data base
Citrullus lanatus	Watermelon	Florida giant	379236 bp	GQ856147	Alverson et al., 2010
Cucurbita pepo	Zucchini	Dark green zucchini	982833 bp	GQ856148	Alverson et al., 2010
Solanum tabacum (syn *Nicotiana tabacum*)	Tobacco	Bright Yellow 4	430597 bp	BA000042	Sugiyama et al., 2005
Vitis vinifera	Grapevine	Pinot noir clone ENTAV115	773279 bp	FM179380	Goremykin et al., 2009
Bambusa oldhamii	Green bamboo		509941 bp	EU365401	Direct submission to DDBJ/EMBL/GenBank data base
Oryza rufipogon		Chinese strain W1	559045 bp	AP011076	Fujii et al., 2010a
Oryza sativa Indica Group	Rice	93-11	491515 bp	DQ167399	Tian et al., 2006
Oryza sativa Indica Group	Rice	Lead rice	434735 bp	AP011077	Fujii et al., 2010a
Oryza sativa Japonica Group	Rice	PA64S	490673 bp	DQ167807	Tian et al., 2006
Oryza sativa Japonica Group	Rice	Nipponbare	490520 bp	BA000029	Notsu et al., 2002
Oryza sativa Japonica Group	Rice	Nipponbare	490669 bp	DQ167400	Tian et al., 2006
Sorghum bicolor	Sorghum	BTx623	468628 bp	DQ984518	Direct submission to DDBJ/EMBL/GenBank data base
Tripsacum dactyloides	Gama grass	Pete	704100 bp	DQ984517	Direct submission to DDBJ/EMBL/GenBank data base
Triticum aestivum	Wheat	Chinese Spring	452528 bp	AP008982	Ogihara et al., 2005
Triticum aestivum	Wheat	Chinese Yumai	452526 bp	EU534409	Cui et al., 2009
Zea mays subsp. *mays*	Maize	A-188, NA type mitochondria	701046 bp	DQ490952	Allen et al., 2007

Zea mays subsp. *mays*	Maize	B37, NB type mitochondria	569630 bp	AY506529	Clifton et al., 2004
Zea mays subsp. *mays*	Maize	B37, CMS-C	739719 bp	DQ645536	Allen et al., 2007
Zea mays subsp. *mays*	Maize	B37, CMS-S	557162 bp	DQ490951	Allen et al., 2007
Zea mays subsp. *mays*	Maize	B37, CMS-T	535825 bp	DQ490953	Allen et al., 2007
Zea mays subsp. *parviglumis*			680603 bp	DQ645539	Direct submission to DDBJ/EMBL/GenBank data base
Zea perennis			570354 bp	DQ645538	Direct submission to DDBJ/EMBL/GenBank data base
Zea luxurians			539368 bp	DQ645537	Direct submission to DDBJ/EMBL/GenBank data base

Table 2 Comparison of gene[*1] content between the largest and smallest mitochondrial genomes in Table 1.

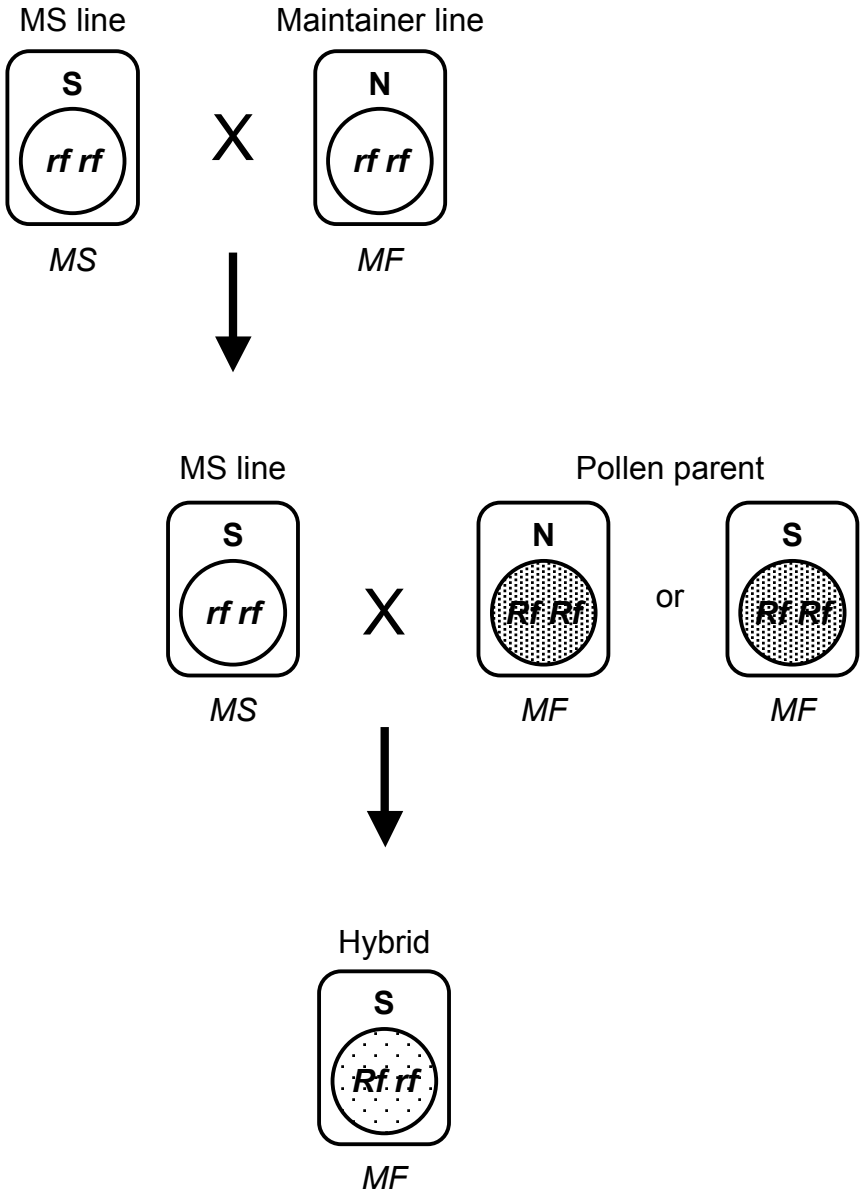
	Name of plant			Name of plant	
Genes	Zucchini	Rapeseed	**Genes**	Zucchini	Rapeseed
Complex I			Cytochrome c maturation		
nad1	+[*2]	+	*ccmB*	+	+
nad2	+	+	*ccmC*	+	+
nad3	+	+	*ccmFC*	+	+
nad4	+	+	*ccmFN*	+	+
nad4L	+	+	Protein translocation system subunit		
nad5	+	+	*mttB*	+	+
nad6	+	+	Maturase		
nad7	+	+	*matR*	+	+
nad9	+	+	Ribosomal protein		
Complex II			*rpl2*	+	+
sdh3	+	–[*3]	*rpl5*	+	+
Complex III			*rpl16*	+	+
cob	+	+	*rps1*	+	–
Complex IV			*rps3*	+	+
cox1	+	+	*rps4*	+	+
cox2	+	+	*rps7*	+	+
cox3	+	+	*rps10*	+	–
Complex V			*rps12*	+	+
atp1	+	+	*rps13*	+	–
atp4	+	+	*rps14*	ψ[*4]	+
atp6	+	+	*rps19*	+	–
atp8	+	+	Ribosomal RNA		
atp9	+	+	*rrn5*	+	+
			rrn18	+	+
			rrn26	+	+

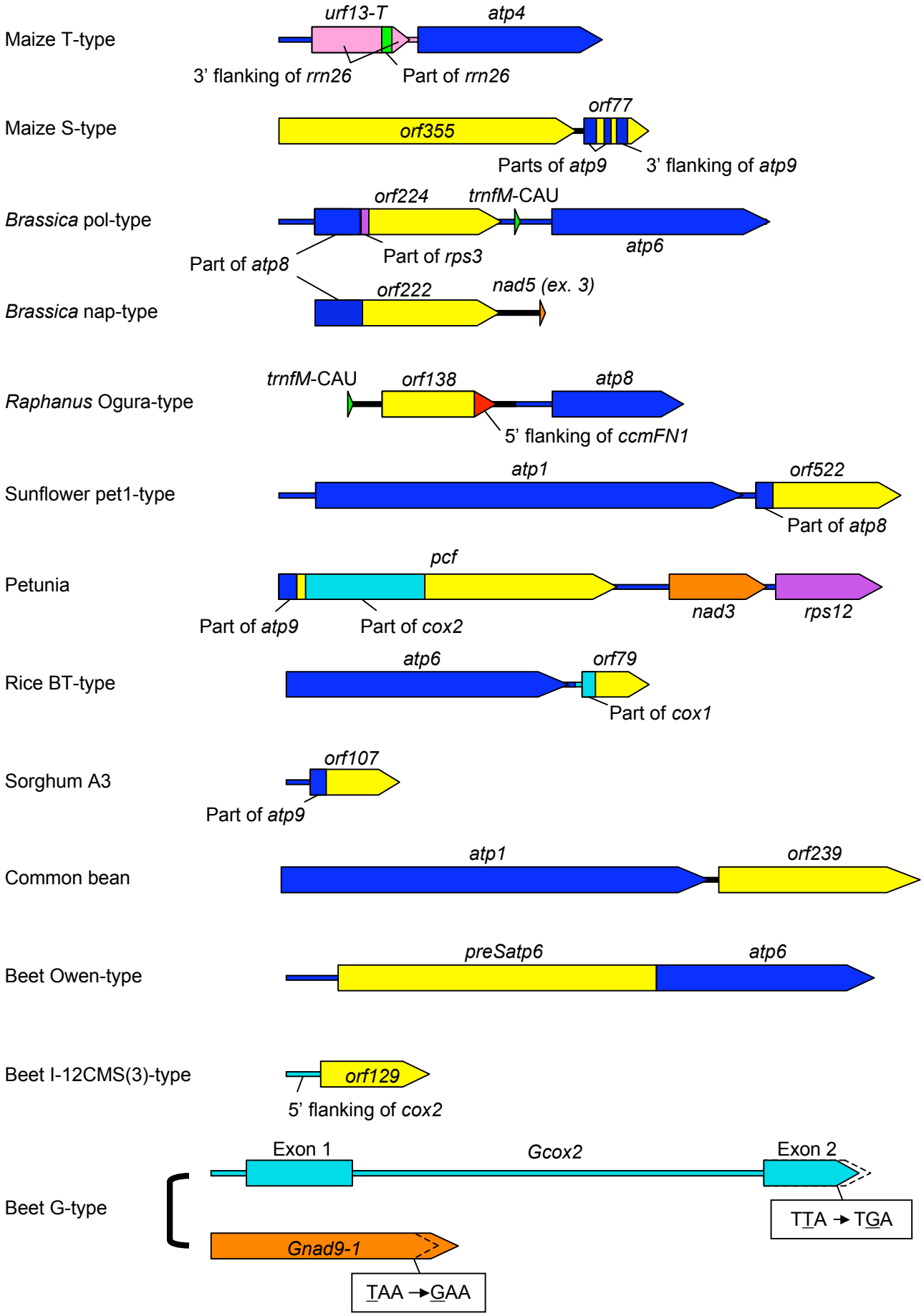
[*1]CMS-ORFs are not considered, [*2]present, [*3], absent, and [*4]pseudogene

Table 3 Origin of tRNA genes encoded in the mitochondrial genomes of *Arabidopsis* and wheat.

	Arabidopsis	**Wheat**
trnC-GCA	N[*1]	P[*2]
trnD-GUC	P	N
trnE-UUC	N	N
trnF-GAA	N	P
trnG-GCC	N	–[*3]
trnH-GUG	P	–
trnI-LAU	N	N
trnK-UUU	N	N
trnfM-CAU	N	N
trnM-CAU	–	P
trnN-GUU	P	P
trnP-UGG	N	N
trnQ-UUG	N	N
trnS-GCU	N	N
trnS-GGA	P	P
trnS-UGA	N	N
trnW-CCA	–	P
trnY-GUA	N	N

[*1]native class, [*2]plastid origin, and [*3]absent from mitochondria


MS line
Maintainer line
S
rf rf
X
N
rf rf
MS
MF
MS line
Pollen parent
S
rf rf
X
N
Rf Rf
or
S
Rf Rf
MS
MF
MF
Hybrid
S
Rf rf
MF


Maize T-type
urf13-T
atp4
3' flanking of rrn26
Part of rrn26
Maize S-type
orf355
orf77
Parts of atp9
3' flanking of atp9
Brassica pol-type
orf224
trnfM-CAU
atp6
Part of atp8
Part of rps3
Brassica nap-type
orf222
nad5 (ex. 3)
Raphanus Ogura-type
trnfM-CAU
orf138
atp8
5' flanking of ccmFN1
Sunflower pet1-type
atp1
orf522
Part of atp8
Petunia
pcf
nad3
rps12
Part of atp9
Part of cox2
Rice BT-type
atp6
orf79
Part of cox1
Sorghum A3
orf107
Part of atp9
Common bean
atp1
orf239
Beet Owen-type
preSatp6
atp6
Beet I-12CMS(3)-type
orf129
5' flanking of cox2
Beet G-type
Exon 1
Gcox2
Exon 2
TTA → TGA
Gnad9-1
TAA → GAA