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Cytoplasmic male sterility and mitochondrial metabolism in plants

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ABSTRACT

Cytoplasmic male sterility (CMS) is a common feature encountered in plant species. It is the result of a genomic conflict between the mitochondrial and the nuclear genomes. CMS is caused by mitochondrial encoded factors which can be counteracted by nuclear encoded factors restoring male fertility. Despite extensive work, the molecular mechanism of male sterility still remains unknown. Several studies have suggested the involvement of respiration on the disruption of pollen production through an energy deficiency. By comparing recent works on CMS and respiratory mutants, we suggest that the "ATP hypothesis" might not be as obvious as previously suggested.

1. Introduction on cytoplasmic male sterility

The occurrence of plants that lost their ability to produce viable pollen grains is frequent in hermaphroditic angiosperm species. As this is usually due to factors coded by the mitochondrial genome, this phenomenon is called cytoplasmic male sterility (CMS). CMS has been extensively exploited in hybrid breeding of crop species. Male sterility is in fact the product of the interaction between the mitochondrial genome and the nuclear genome that can encode for male fertility restorers, which specifically counteract the effect of the mitochondrial sterilizing factors. The link between the mitochondrial molecular phenotype, mitochondrial physiology and pollen sterility remains unknown. In this review, we are comparing recent works on the characterisation of respiratory mutants and CMS lines and their restorers to identify potential mechanisms leading to male sterility.

2. Evolutionary aspects

The evolutionary dynamics happening in species can be understood in light of the concept of genetic conflict between two genomes that do not share the same heredity, maternal for the mitochondrial genome, bi-parental for the nuclear genome (Cosmides and Tooby, 1981). Any mitochondrial gene that favors its own transmission will thus be selected, even at the expense of the nucleus. The evolutionary forces that enable the maintenance of such sexual polymorphism in populations have been investigated in theoretical and empirical studies. Since CMS has gone through the sieve of natural selection, female (male-sterile) plants carrying a sterilizing mitochondrial genome are expected to have a selective advantage that has been called female advantage: more and/or better seeds than her hermaphroditic counterparts. It can be due to the reallocation of the energy saved from pollen production or to the avoidance of inbreeding depression by selfing since females are obligate outcrossers (Dufay and Billard, 2012; Shykoff et al., 2003). Many species exhibit mitochondrial genome diversity with sterilizing and non-sterilizing ("normal") genomes. Theoretical work shows that the maintenance of CMS and non CMS genomes in populations is possible if the sterilizing genome is costly for restored hermaphrodite i.e. if they produce less or lower quality seeds than hermaphrodites carrying a "normal" cytoplasm (Dufay et al., 2007). Last, restorer alleles are expected to bear a cost when they are on the "wrong" cytoplasm i.e. a non-sterilizing cytoplasm or CMS that they cannot restore (Delph et al., 2007). Given these conditions, CMS is predicted to be under a form of selection called balancing selection, under which sterilizing mitochondrial genomes and restorer loci are favored when they are rare, enabling their maintenance for a long period of time (Charlesworth, 2002; Delph and Kelly, 2014; Lahiani et al., 2013).

In conclusion, the mitochondrial dysfunction generated by CMS must be overcome at the seed level. Sterile genes must favor mitochondrial transmission (seed production) and thus affect only pollen production. However as a CMS-associated cost is expected, it can theoretically have mild effects on the overall plant physiology but it should, in any cases, not cause growth retardation.

3. Molecular mechanism of CMS

Studies aiming to identify mitochondrial and nuclear genes involved in CMS have revealed the diversity of mitochondrial sterilizing genes as well as the mechanisms by which restorer genes act (Table 1). This

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Table 1
Mitochondrial male-sterile genes and nuclear male fertility restoration genes.

Species	CMS	CMS gene (sequences from mt genes when chimeric)	Co-transcribed mt gene	CMS gene action	Restorer gene(s)	Restorer effect on CMS factor	References
Bean		*pvs*	–	–	unknown	mt genome rearrangement (Fr), posttranslationnal (Fr2)	(Mackenzie and Chase, 1990)
Beet	CMS-Owen	*preSatp6*?	*atp6*	unknown	Oma1 like (Rf1/X)	Protein–protein interaction?	(Yamamoto et al., 2005) (Matsuhira et al., 2012)
	CMS-E/I-12CMS(3)	*orf129* (*cox2*)	–	unknown	unknown	–	(Yamamoto et al., 2008) (Darracq et al., 2011)
	CMS-G	*G-cox2*?	–	Complex IV dysfunction?	unknown	–	(Darracq et al., 2011; Ducos et al., 2001)
Brassica	Nap	*orf222* (*atp8*)	*nad5c*, *orf139*	–	unknown	Transcript level control	(L'Homme et al., 1997)
	Pol	*orf224* (*atp8*)	*atp6*	–	unknown	Transcript level control	(L'Homme et al., 1997)
Chili pepper		*orf456*	*cox2*	–	–	–	(Kim et al., 2007)
Maize	CMS-C	unknown	–	ROS accumulation, PCD[a]	unknown	–	(Huang et al., 2012)
	CMS-S	*orf355*/*orf77* (*atp9*, *atp4*)	*atp9*		unknown	RNA degradation	(Zabala et al., 1997) (Xiao et al., 2006)
	CMS-T	*T-urf13*	*atp4*	Forming a pore in the inner mitochondrial membrane	ALDH (Rf2)	Detoxification? (Rf2) T-urf13 mRNA control (Rf1)	(Rhoads et al., 1995) (Cui et al., 1996)
Petunia		*Pcf* (*atp9*, *cox2*)	*nad3*	–	PPR (RF-PPR592)	Interaction with *pcf* RNA in a large protein complex	(Bentolila et al., 2002) (Gillman et al., 2007)
Radish	Ogura	*orf138*	*atp8*	Forming a pore in the inner mitochondrial membrane	PPR (Rfo)	Interaction with *orf138* mRNA	(Bellaoui et al., 1999; Duroc et al., 2009) (Brown et al., 2003) (Desloire et al., 2003) (Koizuka et al., 2003) (Uyttewaal et al., 2008)
Rice	BT	*orf79* (*cox1*, *cox2*)	*atp6*	cytotoxic	PPRs (RF1a and Rf1b)	Processing of *orf79-atp6* transcript	(Akagi et al., 1994; Kazama et al., 2008)
	WA	*WA352* (*orf284*, *orf288*)	*rpl5*	Interaction with Complex IV	unknown	Post-transcriptionally (Rf4) and post-translationally (Rf3)	(Luo et al., 2013)
	HL	*orfH79* (*cox1*, *cox2*)	*atp6*	Interaction with complex III	PPR (Rf5)	*atp6-orfH79* RNA processing through the binding of a glycin-rich protein	(Wang et al., 2013) (Hu et al., 2012)
	CW	unknown	–	–	Retrograde Male Sterility gene (Rf17)	Loss of function allele of RMS restores male fertility	(Fujii and Toriyama, 2009)
	LD	unknown	–	–	Glycin Rich Protein (Rf2)	CMS–protein–protein interaction?	(Itabashi et al., 2011)
Sorghum	A3	*orf107* (*atp9*, *BT-orf79*)	–	–	unknown	Transcript processing	(Tang et al., 1996)
Sunflower	PET1	*orf522* (*atp8*)	*atpA*	ATPase activity reduction, PCD	unknown	*atpA-orf522* transcript degradation through polyadenylation	(Balk and Leaver, 2001) (Sabar et al., 2003) (Gagliardi and Leaver, 1999)
Wheat	*timopheevi*	*orf256* (*cox1*)	*cox1*	–	unknown	Transcript processing?	(Hedgcoth et al., 2002)

[a] PCD: Programmed Cell Death.

diversity can be seen not only when we compare species but already at the species level like in maize, beet or rice. Despite this diversity, illustrating the "tinkering" way of evolution, some general features can be given: most sterile genes are *de novo* genes, most probably created via recombination, as attested by their chimerical nature. They are usually in physical proximity of essential genes that enable their co-transcription (Budar et al., 2003). Most restorer loci (Rf) have been recruited in the large family of the Pentatrico Peptide Repeat (PPR) proteins (Schmitz-Linneweber and Small, 2008), involved in organelle gene expression and whose diversification through tandem duplication might have provided the adequate answer to mitochondrial innovation (Fujii et al., 2011; Touzet and Budar, 2004). The mechanisms by which these Rf-PPR restore male fertility are diverse: protein–RNA interaction with the CMS gene transcript (Rf1 in rice BT-CMS (Kazama et al., 2008), Rf in petunia (Gillman et al., 2007), Rfo in radish Ogura CMS, (Uyttewaal et al., 2008), and protein–protein interaction with another protein that acts on CMS transcript (Rf5 in rice HL-CMS (Hu et al., 2012). Recent works have demonstrated that the Rf genes have also been recruited outside the PPR family. In beet Owen CMS, Rf1/X would code for an OMA1-like protein, a protein known from yeast and mammals to be involved in mitochondrial protein quality control (Matsuhira et al., 2012). In rice CW-CMS, the restorer gene is a loss of function allele of a gene that is regulated by mitochondrial retrograde signaling (Fujii and Toriyama, 2009).

The way mitochondrial sterilizing factors cause male sterility is still unknown for most CMSs. Two non-exclusive hypotheses have been proposed to explain the fact that only pollen production is affected by the expression of mitochondrial sterilizing factors. The first hypothesis assumes that normal anther development is interrupted by an interaction between a substance present only in anthers and organelles with altered structures (Flavell, 1974). This "pollen hypothesis" is supported by the cases of *Phaseolus vulgaris* CMS where the CMS-associated protein is degraded by a protease in the mitochondria of vegetative tissues (Sarria et al., 1998) and the rice WA-CMS where the sterilizing factor preferentially accumulates in anthers (Luo et al., 2013). However, most sterilizing factors are constitutively expressed while the subsequent phenotype is restricted to the male gametophyte. The second hypothesis postulates that the mitochondrial dysfunction caused by sterilizing factors will have only visible consequences on pollen production in a developmental step that is highly energy demanding, as suggested by an increase of the number of mitochondria per cell in tapetum or sporogenous cells in maize (Warmke and Lee, 1978). This "ATP hypothesis" has received a large echo as sterilizing genes are often co-transcribed with *atp* genes encoding subunits of the ATP synthase. The expression of sterilizing gene could therefore disturb the expression of the ATP synthase subunits and subsequently affect ATP production (Hanson and Bentolila, 2004). The decrease of ATP has been documented in PET1-CMS in sunflower leading to premature Program Cell Death (PCD) in tapetal cells (Balk and Leaver, 2001; Sabar et al., 2003).

4. Main functions of plant mitochondria

ATP production is the main function of mitochondria. This is achieved through respiration, a metabolic pathway involving glycolysis, the TCA cycle and the Oxidative Phosphorylation (OXPHOS) system. In plants, the OXPHOS system recycles cofactors for the TCA cycle and the glycolysis and transfers electrons to molecular oxygen through a series of complexes (complexes I to IV). During the electron transfer, protons are pumped from the matrix to the intermembrane space, creating a gradient across the inner membrane. This proton gradient will be used by the ATP synthase (also called complex V) to synthesize ATP. In plants, the electron transfer chain contains alternative dehydrogenases and oxidases that offer by-passes of different complexes for electrons (Millar et al., 2011). In addition to their role during cellular respiration, plant mitochondria play important roles in other metabolic pathways such as photorespiration and the metabolism of several amino acids and cofactors (Mackenzie and McIntosh, 1999). They are also involved in PCD (Reape and McCabe, 2010). One of the preliminary steps of PCD in plants involves a Reactive Oxygen Species (ROS) burst followed by the release of cytochrome *c* from the mitochondrial intermembrane space into the cytoplasm (Sun et al., 1999; Vacca et al., 2006).

In plants, the mitochondrial genome encodes about 30 proteins including some subunits of complexes I, III, IV and V as well as assembly factors for *c*-type cytochromes and ribosomal components. Therefore polymorphisms in the mitochondrial genome, as those found in CMS lines, are likely to affect the OXPHOS system. The other subunits of the complexes and proteins involved in the other mitochondrial functions are encoded in the nucleus, synthesized in the cytoplasm and imported into the mitochondria. This suggests a tight coordination between the expressions of the two genomes. Another level of coordination between mitochondria and nucleus involves the reporting of the mitochondrial status to the nucleus. For example, after the application of a respiratory inhibitor, the expression of stress responsive mitochondrial proteins that offer alternative routes for electrons through the respiratory chain is stimulated (Clifton et al., 2005). This phenomenon is called retrograde signaling. This retrograde signaling has been extensively studied for chloroplast (Chan et al., 2010; Leister, 2012) but the pathway(s) originating from mitochondria remain(s) unclear (Schwarzlander and Finkemeier, 2013).

5. Mutants in subunits of OXPHOS complexes

Several respiratory mutants affecting the abundance and function of the OXPHOS complexes have been characterized in plants. Mutants with reduced or non-detectable levels of complex I are the most numerous. Mutants in genes encoding complex I subunit have been identified in Arabidopsis (Han et al., 2010; Lee et al., 2002; Meyer et al., 2009; Wang et al., 2012), tobacco (Gutierres et al., 1999), maize (Marienfeld and Newton, 1994) and cucumber (Juszczuk et al., 2007). Knock-out mutants in complex II subunits are lethal (Leon et al., 2007) but a point mutation mutant reducing complex II activity has been identified (Gleason et al., 2011). No mutants in complex III and complex IV subunits have been described so far. Cytochrome *c* is encoded by two genes in Arabidopsis; the double mutant is not viable but a knock down mutant has been characterized (Welchen et al., 2012). Finally, the F_1F_O ATP synthase or complex V can only be studied using knock-down mutants in Arabidopsis (Geisler et al., 2012; Robison et al., 2009). In addition to these mutants in genes encoding subunits of the OXPHOS complexes, other respiratory mutants have been described, they include mutants in assembly factors (Huang et al., 2013; Meyer et al., 2005; Steinebrunner et al., 2011; Wydro et al., 2013) and mutants in proteins involved in the expression of the mitochondrial genome. The latter are generally knock-down mutants and can affect one or several complexes (Colas des Francs-Small and Small, 2014). To briefly summarize all the mutants studied, knock out mutations of complexes II, III, IV and V lead to lethality whereas complex I is not essential in plants. Overall mutants showing lower levels in one or more complexes show a reduced growth phenotype and an induction of the alternative pathways. The severity of these phenotype correlates with the intensity of the reduction of the complex abundance (E.H. Meyer, unpublished results). Because of their availability, complex I mutants have been extensively characterised; they show altered photosynthesis, strongly modified metabolome and transcriptome as well as accumulation of ROS and higher resistance to mild stresses (for example (Meyer et al., 2009). In *Nicotiana sylvestris*, two male-sterile lines were obtained after regeneration of a callus culture (Li et al., 1988). The CMSII line has been extensively studied. A deletion of Nad7 is the molecular origin of the phenotype (Pla et al., 1995), leading to an impairment of complex I and a respiratory defect (Sabar et al., 2000). CMSII plants show a growth retardation compared to wild type plants. Therefore it cannot be considered as a real CMS line and should be discussed as a complex I mutant.

6. Effect of respiratory mutants on pollen synthesis

Although several respiratory mutants have been extensively studied, very little data regarding the effect of these mutations on pollen synthesis and viability have been produced. Recently, the effects on reproductive tissues of a T-DNA insertion mutant in the gene encoding the δ-subunit of complex V were evaluated in *Arabidopsis*. The male and female transmission efficiencies of the T-DNA are very low (<26%). In addition, the ovules of the mutant develop slower than wild type ovules. This has for consequence the presence of 25% of shrivelled and brown seeds in developing siliques of heterozygous plants (Geisler et al., 2012). Similar observations have been made for the homozygous-lethal restoring allele of maize CMS-S, Rfl1. In haploid rfl1 pollen, ATPA accumulation is impaired but pollen development is not affected (Wen et al., 2003). Mutants in complex I also show severe growth retardation and reduced pollen viability but no pollen lethality (Meyer et al., 2009; Pla et al., 1995). The *indh* mutant is the only complex I mutant in which the reproduction capacity was analysed. Sporophytic defects in both male and female gamete development have been observed but the formation of a homozygous embryo is possible (Wydro et al., 2013). In addition, several homozygous T-DNA mutants for respiratory complexes are embryo lethal (Leon et al., 2007; Meyer et al., 2005; Steinebrunner et al., 2011), suggesting that pollen carrying the mutation is produced and able to fertilise the ovule. As mutants in complexes I and V contain reduced ATP levels (Geisler et al., 2012; Meyer et al., 2009), these observations indicate that, in respiratory mutants, reduced mitochondrial ATP production affects the fitness of both gametes but does not abolish pollen formation. Indeed, pollen containing the mutation is produced and able to fertilise the ovule in order to form an embryo in most known respiratory mutants. To date, the only mutation that cannot be transmitted through the pollen is a mutation in complex II (Leon et al., 2007). Complex II being part of the TCA cycle and the OXPHOS system, a complex II defect might have more severe consequences on mitochondrial functions than an OXPHOS-only problem. In summary, none of the characterised respiratory mutants can be considered as male sterile plants. In other words, reduced mitochondrial ATP production cannot explain the complete and only male sterility observed in CMS lines.

7. Examples of modes of action of the sterilising factors and links with respiratory chain components

In most CMS cases, sterility is caused by the expression of a chimeric protein resulting from a rearrangement of the mitochondrial genome. This protein often contains fragments of OXPHOS complexes subunits (Hanson and Bentolila, 2004). Although the mode of action of the sterilizing factors is generally not known, in few examples data on the role of the chimeric protein in the mitochondria exist. For example, some sterilizing factors such as the maize CMS-T URF13 protein (Rhoads et al., 1995) or Ogura CMS ORF138 (Duroc et al., 2005) create a pore in the mitochondrial inner membrane. However, the precise consequences of this pore are not known. It has been suggested that protons could leak through this pore and thus the respiratory chain and the ATP synthase would be uncoupled leading to reduced ATP synthesis (Duroc et al., 2009; Rhoads et al., 1995).

In some CMS lines, an interaction of the sterilisation factor with the respiratory chain has been evidenced. The sunflower CMS is caused by the expression of the ORF522 protein. ORF522 interacts with the ATP synthase, lowering its activity (Sabar et al., 2003). In the HL-CMS of rice, the sterilizing factor ORFH79 is located in the mitochondrial membrane and interacts with P61, a protein homologous to the QCR10 subunit of complex III. This interaction inhibits the activity of complex III, leading to reduced ATP level and increased ROS content (Wang et al., 2013). In the CMS-WA of rice, the sterilising factor is WA352, a chimeric protein composed of fragments of three mitochondrial ORFs. Several lines of evidence indicate that WA352 interacts with COX11 (Luo et al., 2013). In yeast, COX11 is a cupper chaperon required for complex IV assembly (Hiser et al., 2000) but COX11 was also recently shown to have an important role in peroxide degradation (Veniamin et al., 2011). The interaction WA352–COX11 is believed to inhibit the function of COX11, leading to a higher accumulation of ROS and premature PCD (Luo et al., 2013). However it is not known whether the higher ROS accumulation originates from a misassembled complex IV or is due to the inhibition of peroxide degradation by COX11. The CMS-G of *Beta vulgaris ssp maritima* is intriguing as the sterilising factor has still not been identified. The sequencing of the mitochondrial genome of this CMS line did not allow the identification of any chimeric ORF susceptible to be responsible for the sterility but highlighted the presence of several non-synonymous mutations in genes encoding OXPHOS components (Darracq et al., 2011). Genes encoding complex IV subunits are particularly affected; *cox1* start codon and *cox2* stop codons are mutated. As a consequence complex IV is not detected on a BN-PAGE and its activity is severely impaired (Ducos et al., 2001). This defect in complex IV seems to be the origin of CMS.

These examples illustrate well the intricate link between CMS and OXPHOS complexes and the fact that CMS lines could represent variants of the OXPHOS system, increasing the toolbox for studying this metabolic pathway. Unfortunately, in depth studies of CMS lines are scarce and more biochemical and physiological data would be required to fully understand the mechanisms that lead to pollen abortion in CMS plants.

8. What could be the metabolic cause of male sterility?

CMS is caused by the expression by the mitochondrial genome of an unusual protein called the sterilizing factor. This protein is interfering with mitochondrial functions. Two hypotheses have been proposed to explain why the phenotype is only observed in the pollen (see above). The “ATP hypothesis” is in contradiction with the recent characterisation of the respiratory mutants. Indeed, these mutants are able to produce some viable pollen even when mitochondrial ATP production is significantly reduced (see above). It seems then impossible that CMS is caused by a defect in the mitochondrial ATP production. The “ATP hypothesis” has been experimentally tested. Inducible knock down mutants for two ATP Synthase subunits have been constructed. Upon induction, ATP levels are decreased but no effect on pollen fitness was observed (Robison et al., 2009). In light of these data, the “ATP hypothesis” appears unlikely to explain the male-sterile phenotype of CMS lines; thus another mitochondrial function should be affected in CMS plants. One hypothesis would be that mitochondria fulfil a specific role in the anthers during pollen maturation. For example, the synthesis of a compound of the outer layer of the pollen grain could depend on precursors produced in the mitochondria. Reduced precursor availability in CMS plants would result in abnormal pollen maturation. To date, such a male specific function of mitochondria has not been discovered. Another possible explanation for the cause of the sterility is that mitochondria are targeted by a pollen specific component (Flavell, 1974). This interaction results in pollen lethality when a sterilizing factor is produced in the mitochondria. If this hypothesis is true, all the CMS should result in a common metabolic signature. Such feature has not yet been found, either because it does not exist or due to the reduced amount of physiological studies of CMS lines. However, CMS evolved independently in different species and is caused by a plethora of unrelated sterilising factors. This suggests that the abortion of the pollen is likely to be caused by a single metabolic defect, impairing a major function of the mitochondria during pollen formation. The variety of sterilising factors described so far indicate that this function can be impaired by many ways. Finding a physiological parameter that is altered in the anthers in several CMS lines would contribute greatly to the elucidation of the mechanism of pollen abortion. A few studies of CMS lines point toward a role of mitochondrial ROS in the sterility. In particular, a ROS burst has been observed in the anthers of the PET1-CMS of sunflower

(Balk and Leaver, 2001), the cotton CMS (Jiang et al., 2007) and the HL-CMS (Wang et al., 2013) and the CMS-WA (Luo et al., 2013) of rice. A suggested mechanism involves increased ROS production triggering a premature PCD in the tapetum (Ma, 2013). This deregulated PCD would cause pollen abortion as PCD has to be tightly controlled during pollen maturation (Diamond and McCabe, 2011; Papini et al., 1999). However tempting this hypothesis is, the cause of CMS might be more complex. Indeed respiratory mutants are known to accumulate ROS (Dutilleul et al., 2003; Liu et al., 2010; Meyer et al., 2009) but this accumulation never abolishes pollen production. A more thorough analysis of ROS production in the anthers of CMS lines and respiratory mutants is required to infirm this hypothesis.

9. Restoration and mechanistic

Another approach to elucidating the physiology of sterility in CMS lines is to understand the restoration mechanisms. Indeed restoration of fertility can theoretically be obtained by two mechanisms: repair or compensation. The repair mechanism involves the removal or the inactivation of the sterilising factor. This repair could occur at the expression level (inhibition of transcription or translation) or at the protein level (degradation) but also it could theoretically involve an inhibition of the function of the sterilizing factor that does not involve its removal. Restoration through a repair mechanism is very common and has been described in many cases of CMS (Table 1). Compensation would involve a modification of the cellular metabolism that counteracts the action of the sterilising factor without affecting it. Only one of the described restorers is unlikely to be involved in a repair mechanism: the Rf2 restorer of the maize CMS-T. The restoration of CMS-T requires two genes: Rf1 and Rf2 (Laughnan and Gabay-Laughnan, 1983). The action of Rf1, but not Rf2, reduces the levels of the sterilising factor (Liu et al., 2001). Rf2 has been identified as a putative aldehyde dehydrogenase, an enzyme involved in metabolic reactions (Cui et al., 1996). The mode of action of Rf2 during restoration is still unknown. However, Rf2 is required for anther development in lines lacking the sterilising factor (Liu et al., 2001); this observation questions the role of Rf2 as a restorer (Touzet, 2002). The identification of restorer genes that are not involved in the inactivation of the sterilizing factor will greatly improve our knowledge on the physiological origin of the sterility in CMS lines.

10. Conclusion

In this review, we compared the phenotype of respiratory mutants and CMS line. Our conclusion is that CMS cannot be caused by a reduction in mitochondrial ATP production because respiratory mutant with altered ATP levels are not sterile. The overall defect causing the sterility remains unknown but we propose that it should affect a major mitochondrial function because CMS has a very diverse molecular origin but similar physiological phenotype. We are reporting the lack of in depth physiological studies of CMS lines and suggest that comparative studies of several CMS lines should be undertaken. Identification and characterisation of restorer genes and their functions will also greatly improve our knowledge of the molecular mechanism of male sterility and male fertility restoration.

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