Genetica **117:** 3–16, 2003.

The nucleo-mitochondrial conflict in cytoplasmic male sterilities revisited

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Key words: cytoplasmic male sterility, mitochondrial mutation, natural population, nucleo-cytoplasmic conflict, respiration, sex determinism, stress tolerance

Abstract

Cytoplasmic male sterility (CMS) in plants is a classical example of genomic conflict, opposing maternally-inherited cytoplasmic genes (mitochondrial genes in most cases), which induce male sterility, and nuclear genes, which restore male fertility. In natural populations, this type of sex control leads to gynodioecy, that is, the co-occurrence of female and hermaphroditic individuals within a population. According to theoretical models, two conditions may maintain male sterility in a natural population: (1) female advantage (female plants are reproductively more successful than hermaphrodites on account of their global seed production); (2) the counter-selection of nuclear fertility restorers when the corresponding male-sterility-inducing cytoplasm is lacking. In this review, we re-examine the model of nuclear-mitochondrial conflict in the light of recent experimental results from naturally occurring CMS, alloplasmic CMS (appearing after interspecific crosses resulting from the association of nuclear and cytoplasmic genomes from different species), and CMS plants obtained in the laboratory and carrying mitochondrial mutations. We raise new hypotheses and discuss experimental models that would take physiological interactions between cytoplasmic and nuclear genomes into account.

Introduction

Cytoplasmic male sterility (CMS) in plants is defined as the maternally-inherited inability to produce functional pollen (male gametes) in individuals from an otherwise hermaphroditic species. In all documented examples, the male sterility-inducing cytoplasm effect can be counteracted by nuclear gene(s), therefore called restorer(s) of fertility (Rf).

In wild species, the occurrence of CMS is revealed by gynodioecy, that is, the coexistence of hermaphrodite and female (male sterile) individuals in natural populations. Over the last decades, studies on gynodioecious natural populations led to the emergence of theoretical models based on the conflict between cytoplasmic and nuclear genomes in the production of male gametes (Gouyon & Couvet, 1987; Frank, 1989). According to these models, gynodioecy may appear and be maintained in natural populations under two conditions: (1) female advantage (female plants are reproductively more successful than hermaphrodites on account of their global seed production); (2) the counter-selection of nuclear fertility restorers when the corresponding male-sterility inducing-cytoplasm is lacking. The first strong condition of female advantage may cause one to view CMS as being selectively advantageous for cytoplasmic genes (providing no restriction on pollen), whereas the associated nuclear genes are severely disadvantaged. In a number of cases, CMS has been directly

correlated to mitochondrial variability, pointing to the mitochondrial (mt) genome as the cytoplasmic element of the phenomenon. However, the mt sequences responsible for the CMS phenotype have not yet been identified.

On the other hand, male sterility is very useful for the production of hybrid seeds in cultivated species (Van der Kley, 1955). Consequently, plant breeders have gathered a great amount of experimental data on obtaining and maintaining CMS in cultivated varieties. In a limited number of cases, cytoplasmic genetic determinants have been identified. In all cases of CMS except one (Lefebvre, Scalla & Pfeiffer, 1990) these putative determinants of CMS were found to be mitochondrial (Schnable & Wise, 1998). The phenomenon is, therefore, regarded as a mitochondrial 'mutation' or 'deficiency' causing the normal developmental program of male gamete production to fail.

At first glance, both types of situations (in natural populations v.s. in crop varieties) seem to lead to opposite views on the mechanism of CMS, although mitochondrial genomes are clearly in cause in both cases. Recently, studies on wild accessions carrying identified mitochondrial CMS genes (Arrieta-Montiel et al., 2001; Yamagishi & Terachi, 2001), as well as molecular studies on CMS occurring in wild populations (Ducos, Touzet & Boutry, 2001) have been reported, opening up venues for a more unified view of CMS in plants. We believe a confrontation of experimental results with hypotheses from both approaches may provide ideas on how both points of view may be useful for a comprehensive approach.

In this review, we will attempt to confront population studies and evolutionary models with molecular evidence on mitochondrial CMS-associated genes as well as physiological experiments on mitochondrial metabolism. This should raise and address relevant questions in both areas, all of which attempt to provide a better understanding of the relationship between mitochondrial and nuclear genomes in plants.

CMS in natural populations

Gynodioecy, that is, the coexistence of hermaphrodite and female (male sterile) individuals in natural populations, is relatively frequent in angiosperm species (Delannay, 1978). In a majority of gynodioecious species, gender is determined by the interaction of cytoplasmic determinants of male-sterility (mitochondrial genes), which prevent successful pollen production, with nuclear genes, which can restore male function in individuals that have male-sterile cytoplasm. The dynamics of this nucleo-cytoplasmic interaction can be understood in the light of the theory of genomic conflict (Cosmides & Tooby, 1981; Saumitou-Laprade, Cuguen & Vernet, 1994; Hurst, Atlan & Bengtsson, 1996; Werren & Beukeboom, 1998; Frank, 2000). For the inheritance modes between cytoplasmic factors (uniparental) and nuclear genes (biparental) generate a situation where selective interests are in opposition. Thus, any mitochondrial genetic variant that will reduce allocation to pollen in exchange for an increased seed production will be favoured, since the mitochondrial genes are transmitted only through seeds. Reallocation of resources from pollen to seeds reduces the transmission of nuclear genes because biparental transmission depends on success through both seeds and pollen. Consistent with this idea of conflict, nuclear genes often restore male fertility by overcoming the male-sterility effects of the cytoplasm. In many gynodioecious populations, female individuals produce significantly more viable seeds than hermaphrodites (for reviews see Gouyon & Couvet, 1987; Thompson & Tarayre, 2000). In such cases, the female fertility advantage is obvious. But the overproduction of seeds by female individuals in gynodioecious populations is not always observed (e.g., in beet, Boutin et al., 1988, and review in Alonso & Herrera, 2001). This female fertility advantage is a key parameter in evolutionary models of gynodioecy. It can be caused by:

(i) Maternal sex effects through resource compensation, that is, the reallocation of unused resources in male function, which increases female fecundity or viability in male-sterile individuals;

(ii) Inbreeding effects: females that cannot self-pollinate may produce more viable seeds than hermaphrodites by avoiding inbreeding depression. In this regards, this phenomenon could happen in gynodioecious selfing-species as well as in out-crossing species, where out crossing ratio might be less than one (see review in Thompson & Tarayre, 2000). On the other hand, the expected effect might be limited because of the concomitant reduction of effective population size due to male sterility (Laporte et al., 2000).

In theoretical models, apart from the necessary gain in female fertility conveyed by the sterile cytoplasm, additional alternative constraints must

be invoked to explain the persistence of CMS in populations:

(i) Deleterious effects of the restorer alleles in their alien cytoplasmic type in panmictic population, causing a restoration cost. This notion can be paralleled with host–parasite, resistance/virulence co-evolution. In the case of a cost of restoration, the restorer alleles specific to a given male sterile cytoplasm are expected to be counter-selected when the cytoplasm is rare (Charlesworth, 1981; Delannay, Gouyon & Valdeyron, 1981; Frank, 1989; Gouyon, Vichot & Van Damme, 1991).

(ii) Population structure in non-equilibrium metapopulations via founder effects and recurrent introduction of cytoplasmic male steriles (Frank, 1989; McCauley & Taylor, 1997; Pannell, 1997; Couvet, Ronce & Gliddon, 1998; McCauley et al., 2000).

Experimental studies on thyme, plantain and beet

Given this theoretical framework, we present experimental studies taken from three gynodioecious species to illustrate different features such as multiple CMS occurrence, sex ratio variation between populations, female advantage and cost of restoration.

Thyme

In different species of thyme, all the studied populations are gynodioecious but there is a high inter-population variability in female frequency: from 5 to 95% in *Thymus vulgaris* with a approximate mean of 60% (Thompson et al., 1998), from 41 to 99% in *Thymus mastichina* and from 17 to 87% in *T. zygis* (Manicacci et al., 1996). In *T. vulgaris*, there are at least four different CMSs in populations (Manicacci, Atlan & Couvet, 1997). Female thyme plants usually produce 1.5–3.5 times as many viable seeds as hermaphrodites. This female advantage may vary among populations and in time. On the contrary, no differential survival has been observed between the two morphs (Thompson et al., 1998). In this species, inbreeding depression may partially explain the persistence of gynodioecy as well as the reallocation of resources to seed production and provisioning in females. Both of these parameters are variable among populations and with female frequency (Thompson & Tarayre, 2000). Regarding male fertility restoration, in *Thymus vulgaris*, CMSs seem to differ by the ease with which they can be restored. Furthermore, the genetic determinism of restoration for one type of CMS appears to imply more than one locus (Charlesworth & Laporte, 1998). For three of the five cytoplasms studied (Manicacci, Atlan & Couvet, 1997) revealed that higher restoration rates were obtained for females reintroduced into their original populations, which might suggest a selection of the restorer alleles in the populations with the corresponding cytoplasm. Conversely, on a larger regional scale, no local adaptation between cytoplasmic and nuclear restorer genes could be detected as it would be expected under the hypothesis of a restoration cost (Gigord et al., 1998).

Plantain

Plantago lanceolata

Plantago lanceolata L. is a self-incompatible, mainly wind pollinated common species. In natural populations, the frequencies of male sterile plants range from 0 to 22% (Van Damme & Van Delden, 1982). Two male sterile phenotypes (MS1 and MS2), which do not produce pollen, two intermediate types (IN1 and IN2) producing some pollen, and hermaphrodites can be distinguished. MS1 and MS2 can be identified on the basis of their flower morphology (Van Damme & Van Delden, 1982). Both types can be found in the field but MS1 occurs with a relatively higher frequency (10% v.s. 2%) and in most populations (18 out of 20) compared to MS2 (De Haan, Hundscheid & van Hinsberg, 1997a). These two male sterile phenotypes correspond to three different CMSs (CMI to MS1 and CMIIa and b for MS2) (De Haan et al., 1997c). The genetic determinism of male fertility restoration appears to be under the control of several loci, whether independent or epistatically interacting. Restoration levels vary between the CMS types (De Haan et al., 1997b).

MS1 female plants produced a higher vegetative and reproductive dry mass and a higher reproductive output than hermaphrodite plants, suggesting that compensation, that is, reallocation of resources to female reproduction, might be the main underlying cause for female advantage (Poot, 1997). Additionally, MS1 female plants exhibit better survival at the adult stage than hermaphrodite plants (Van Damme & Van Delden, 1984). A cost of restoration (negative effect of CMSI restorer alleles) was detected on seed biomass (De Haan, Hundscheid & van Hinsberg, 1997a).

Plantago coronopus

In the Netherlands, *P. coronopus* is an annual of short-lived perennial, mainly growing in dune grasslands along the coast. It is a wind-pollinated and self-

compatible species, with an average out-crossing rate of 75%. Reciprocal crossing studies have revealed the occurrence of several cytoplasmic types (CMS), each with their own set of restorer genes (Koelewijn & Van Damme, 1995). In contrast with *P. lanceolata*, the different CMSs cannot be identified morphologically. Koelewijn and Van Damme (1995) suggested the presence of at least five restorer genes for each cytoplasmic type. The gene action could be either recessive or dominant, with putative epistasis.

Beet

Wild beet, *Beta vulgaris* ssp. *maritima*, is a wind-pollinated, short-lived perennial and gynodioecious species. A survey of 93 natural populations along the French coasts revealed that 42% of the populations were gynodioecious, with a variation of the occurrence of male sterility among regions (Cuguen et al., 1994). More than 20 mitotypes have been revealed through RFLP analysis (Saumitou-Laprade et al., 1993; Cuguen et al., 1994; Desplanque et al., 2000). Among them, four haplotypes have been recurrently correlated to male, in order of decreasing frequency in natural populations: *E*, *G*, *H* and *Svulg*. *Svulg* is associated with the Owen CMS, which has been widely used in sugar beet breeding for decades. This mitotype is therefore a signature of the cultivated compartment in Europe (Boudry et al., 1993). The other mitotypes correspond to either non-sterilising cytoplasms or to CMSs for which the restorer loci are fixed in populations. Concerning the occurrence of male sterile cytoplasms, it appears that the mitotypes are generally found in several geographically remote populations, and that a strong linkage disequilibrium is observed between the mitochondrial and chloroplastic haplotypes, which are co-transmitted by maternal lineage. Therefore, migration rather than recurrent mutation might explain the occurrence of mitotypes in populations of wild beets (Desplanque et al., 2000).

In beet, no clear female advantage has been documented so far. A previous study on CMS *E* failed to reveal enhanced female fertility in male sterile plants compared with hermaphrodites (Boutin et al., 1988). When comparing the *in situ* level of restoration of the male-sterilising mitotypes, the ratio of plants which are hermaphrodite on a sterilising cytoplasm varies among populations (Boutin-Stadler et al., 1989) and CMS systems. In particular, *E* CMS appears to be more restored than *G* (Cuguen et al., 1994; Laporte et al., 2001). In a recent study, a cytonuclear disequilibrium might suggest a cost associated with restorer alleles of CMS *E* (Laporte et al., 2001).

A recent molecular study showed that CMS *G* exhibits two mutated mitochondrial genes, each of which results in the production of a respiratory chain complex subunit with an altered molecular weight: the NAD9 subunit has a C-terminal extension while the COX2 subunit has a truncated C-terminus. NADH dehydrogenase activity was unchanged in leaves, but cytochrome *c* oxidase activity was reduced by 50%. Moreover, Western blot analyses revealed that alternative oxidase was more abundant in male sterile *G* plants than in a fertile control (*Nv*) (Ducos, Touzet & Boutry, 2001). These results provide the first experimental clues for the physiological characterisation of a natural CMS. Even though no direct link can be ascertained between the *G* modified respiratory system and male sterility (but see hypotheses in Ducos, Touzet & Boutry, 2001), the characterisation of this peculiar respiratory metabolism will be discussed below (see respiratory mutants) with regards to a possible female advantage through a better survival of male sterile plants.

'Alloplasmic male sterilities'

For many years, breeders seeking out male sterilities for hybrid seed production have observed that CMS frequently occurs in interspecific or intergeneric crosses. In 1988, Kaul defined alloplasmy as 'the phenomenon wherein cells of biological organisms have the cytoplasm of the one and the nucleus of the other species'. (Kaul, 1988). He numbered 175 cases of alloplasmic situations giving rise to male sterility. These CMSs are often said to result from the alloplasmic state, that is, an impaired mitochondrial activity due to a bad cooperation between cytoplasmic and nuclear genomes either at the protein level (assembly of subunits encoded by both genomes into respiratory chain complexes) or at the RNA level (alterations in the expression of mitochondrial genes, Hakansson & Glimelius, 1991). The combination of cytoplasm and nucleus donor species may dramatically influence flower morphology in such lines (Bonnett et al., 1991). Indeed, recent studies on alloplasmic CMS tobacco carrying a *N. repanda* cytoplasm strongly suggest a deficient energy metabolism during flower formation (Bergman et al., 2000; Farbos et al., 2001). These observations seem to argue in favour of the 'loss-of-function' model for CMS,

where the deficient mitochondrial metabolism may be explained by a lack of co-adaptation between nuclear and mitochondrially-encoded subunits of the respiratory complexes. Actually, the same situation may sometimes be observed for the chloroplastic function, when alloplasmic situations lead to deficient photosynthesis (Bannerot, Boulidard & Chupeau, 1977). Nevertheless, there are cases where original chimeric mitochondrial genes are silent in the cytoplasm donor species but are expressed in an alloplasmic situation (Landgren et al., 1996). These can be considered as 'silent' or 'cryptic' CMS genes because of the presence of fixed restorer genes in their species of origin, which become active again in front of new 'naive' nuclear genomes. If one assumes that mt genomes of plants can carry silent CMS genes because of fixed restoration, the frequent occurrence of male sterility in interspecific crosses is not surprising at all. Actually, Ogura male sterility-inducing cytoplasm has been detected in *Raphanus raphanistrum*, where restoration seems to be fixed (Yamagishi & Terachi, 1997). Furthermore, a recent paper reported the occurrence of a male-sterile cytoplasm in a hermaphrodite population of *Lobelia* (Dudle, Mutikainen & Delph, 2001). According to such a hypothesis, the 'gain-of-function' model for CMS may also be valid for some alloplasmic CMSs, assuming that chimeric CMS-associated genes reflect this gain of function. In any case, alloplasmic lines can be considered as cumulating every reason to be male sterile.

Molecular characterisation of CMS genes

Most mt genes which have been identified as being correlated with or responsible for CMS, are unrelated in sequence, with two remarkable exceptions: (1) in *Brassica napus*, *orf222* and *orf224*, from *nap* and *polima* CMS, respectively, exhibit 79% of sequence similarity (L'Homme et al., 1997); (2) *orf79* from *Bo* CMS in rice and *orf107* from A3 cytoplasm of *Sorghum bicolor* share similar sequences (Tang et al., 1996). Despite their apparent unrelatedness, most CMS associated mitochondrial genes share some features (including in the mechanisms of fertility restoration) which may be relevant for a comprehensive approach of the phenomenon. (Reviewed in Schnable & Wise, 1998 and Budar & Pelletier, 2001).

All these genes can be considered as 'chimeric' orfs produced by recombination. Most often, at least a part of the CMS-associated gene can be related to pieces of other known mitochondrial genes (but not necessarily coding sequences). These 'recognisable' parts may constitute most of the CMS gene, like in Texas maize T-*urf13* (Dewey, Timothy & Levings, 1987) or can be reduced to a mere portion of it, like in Ogura *orf138* (Bellaoui et al., 1998). The CMS genes therefore appear as the products of the recombinational activity of plant mitochondrial genomes, which is probably an important mechanism of their evolution mode and is also thought to contribute to their highly complex structure (reviewed in Wolstenholme & Fauron, 1995). As a matter of fact, the *Arabidopsis thaliana* mt genome is filled with orfs which likely result from recombinational activity (Marienfeld et al., 1997) and among which potential CMS genes could be hidden. Furthermore, the mitochondrial genome of thyme has been shown to be highly polymorphic and this polymorphism is thought to be related to the frequent occurrence of CMS in thyme populations (Belhassen et al., 1993). One might wonder why hermaphroditic animals (such as snails for instance), for which evolutionary mechanisms in sex determinism could be the same as for plants, never exhibit mitochondrially determined male sterilities. One answer could be: because their mitochondrial genome, as those of animals in general, is too compact. The striking difference between the evolution modes of plant and animal mitochondrial genomes (Palmer et al., 2000) could explain that the occurrence of mitochondrially encoded male sterility is reserved to plants: their mitochondrial genomes have the space and opportunity to form new coding sequences among which sterility-inducing genes may emerge.

A common feature of CMS-associated genes is their physical association and co-transcription with essential mitochondrial genes. Furthermore, they must be translated from bicistronic mRNAs, since monocistronic mRNAs are not observed. Even when monocistronic mRNAs are observed for the partner gene, such as *atpA* in PET1 CMS (Laver et al., 1991), it is not the case for the CMS-associated gene. Interestingly, the study of rapeseed cybrids carrying Ogura CMS showed that the dissociation of the CMS-associated *orf138* gene from the essential *orfB* gene leads to frequent spontaneous reversion to fertility through loss of the CMS gene (Bellaoui et al., 1998). Therefore, the association and co-transcription of CMS genes with essential mt genes might well reflect not only a 'recruitment' of expression signals, but also express a constraint: if not tightly linked to essential genes, their maintenance is compromised.

Every time the effect of nuclear restorers has been studied, the restoration of fertility results from an impaired expression of the sterility-associated gene. So far, the only possible exception to this rule was the *Rf2* restorer of Texas maize CMS. This gene was cloned and identified as a mitochondrial aldehyde dehydrogenase (Cui, Wise & Schnable, 1996). Recently, it was shown to be required for normal male fertility in maize (Liu et al., 2001). It is therefore not a genuine 'restorer' gene. A number of investigations have been carried out on the effects of restorer genes on the sterility determinant. They appear to be all different, although the vast majority of results suggest an interference with the expression of the CMS mRNA at the post-transcriptional level (see Table 1). The recent identification of the Petunia *Rf* gene (Bentolila, Alfonso & Hanson, 2002) as a PPR-protein encoding gene, reinforces the idea that this large gene family might be involved in the control of organellar gene expression (Small & Peeters, 2000). Two studied restorers do not act at the mRNA level on CMS gene expression: the *Fr* restorer of *Phaseolus*, which dramatically decreases the CMS gene copy number; this reduction hinders the expression of the sterile phenotype (Mackenzie & Chase, 1990); the *Rfo* restorer of Ogura CMS, which leads to the destabilisation of the CMS-associated protein in anthers (Bellaoui et al., 1999).

The polypeptides encoded by CMS-associated genes are in most cases constitutively expressed and are generally associated with mt membranes. Again, the *Phaseolus* CMS is an exception, since the *orf239* gene is constitutively transcribed, but the ORF239 protein is specifically degraded in vegetative tissues and detectable only in reproductive tissues (Sarria et al., 1998), where it seems to accumulate in the microspore cell walls and callose layer (Abad, Mehrtens & Mackenzie, 1995).

The mechanism(s) of CMS is still unclear regardless the CMS system. At this point, two observations seem to be particularly relevant: (1) most CMS-associated genes are constitutively expressed but induce a phenotype which is extremely localised in development; (2) the observed phenotype is not uniform among all types of CMS, although in a majority of cases the first cytological anomalies appear in the tapetal cell layer, followed by abortion of microspores. It must be noticed that some sterile phenotypes can deeply affect flower morphology. In some instances, anthers are totally absent or have some aberrant morphology (petaloid anthers) as in carrot (Kitagawa et al., 1994) and *Plantago* (Van Damme, 1983).

The two main hypotheses that have been proposed to explain CMS mechanism(s) seem mutually exclusive at first glance.

According to the first hypothesis the CMS gene product interacts with and an unknown 'X factor' specifically present in the target tissue (tapetum for instance). The interaction may trigger a deleterious cascade of events and finally lead to the abortion of microspores. This hypothesis was inspired by the huge amount of experimental work performed on the Texas maize CMS (reviewed in Wise et al., 1999a). It is attractive, since it could explain the paradox between the constitutive expression of CMS genes and the developmentally localised phenotype induced. However, no 'X factor' has been identified so far. This hypothesis can be considered as the archetype of 'gain-of-function' model. It is quite difficult to imagine how the association of parts of 'classical' mt genes into chimeric sequences can produce such a precise function. But the CMS-associated coding sequences also include novel sequences of unknown origin. In Texas maize T-*urf13*, this is true of only nine codons. But in other genes, it can be the case of almost the entire coding sequence. On this point, the Petunia CMS-associated S-*pcf* gene is quite interesting: its coding sequence is a fusion of a part of the *coxII* coding sequence and an unknown open reading frame (*urfS*). It encodes a fusion protein, which is post-translationally matured; only the URFS part of the protein is ultimately accumulated (Nivison & Hanson, 1989).

The second hypothesis (Levings, 1993) suggests that CMS gene products somehow interfere with the normal physiology of mitochondria (possibly via the portions of normal mt polypeptides included in their sequences), leading to less efficient respiration and/or ATP production. This would consequently impair pollen production, which is an energy demanding developmental program. Although the model does not really explain why other developmental stages requiring high levels of energy (germination for instance) do not seem to be affected, it is implicitly assumed in most papers on CMS. Interestingly, a recent paper reports a reduced ATP/ADP ratio in flowers of male sterile tobacco (Bergman et al., 2000). It has been suggested that the reduction interferes with cell proliferation and may lead to the abnormal flower morphology of these plants (Farbos et al., 2001).

Table 1. Modes of action of nuclear restorers of fertility

Species; CMS system; restorer gene	Mode of action	Reference
Phaseolus; Fr	Decreasing of *orf239* gene copy number	Mackenzie and Chase (1990)
Phaseolus; Fr2	Post-transcriptional impairment of *orf239* expression	Chase (1994); Abad, Mehrtens and Mackenzie (1995)
Maize; Texas; *Rf1*[1]	Processing of T-*urf13* transcript	Wise et al. (1999b)
Maize; Texas; *Rf8*[1]	Processing of T-*urf13* transcript	Wise et al. (1999b)
Maize; Texas; *Rf**[1]	Processing of T-*urf13* transcript	Wise et al. (1999b)
Maize; CMS-S; *Rf3*	Alteration of transcript pattern of *orf355* and *orf77*	Wen and Chase (1999)
Brassicaceae; Ogura; *Rfo*	Post-translational destabilisation of the ORF138[2] protein	Bellaoui et al. (1999)
Brassicaceae; kosena; *rfk1*, *rfk2*	Translational inhibition of ORF125[2] protein accumulation	Koizuka et al. (1998); Koizuka et al. (2000)
Rapeseed; *polima*; *Rfp*[3]	Processing of *orf224/atp6* co-transcripts	Li et al. (1998); Menassa, L'Homme and Brown (1999)
Rapeseed; *nap*; *Rfn*[3]	*Orf222* transcript processing	Li et al. (1998); Brown (1999)
Sunflower; PET1	Reduction of *orf522* transcript level	Monéger, Smart and Leaver (1994)
Petunia; *Rf*	Reduction of *pcf* transcript level[4]	Hanson et al. (1999)
Sorghum; *Rf3*	*orf107* transcript internal processing	Tang et al. (1996)
Rice; Bo CMS[5]	Alteration of transcription pattern of the *urf-rmc* gene	Kadowaki, Suzuki and Kazama (1990)
Wheat; *timopheevi*	Alteration of transcription pattern of *orf256*[6]	Song and Hedgcoth (1994)

[1] *Rf1*, *Rf8* and *Rf** are not allelic (Wise et al., 1999a,b).
[2] ORF138 and ORF125 are very closely related and Ogura and kosena cytoplasms might be variants of the same cytoplasmic male sterility system (Iwabuchi et al., 1999).
[3] *Rfp* and *Rfn* are allelic (Li et al., 1998).
[4] The effect of the Petunia *Rf* gene may not be limited to reducing transcript levels, but may also affect the PCF protein level directly (Hanson et al., 1999). The Petunia *Rf* gene has been identified by Bentolila, Alfonso and Hanson (2002).
[5] The Bo-CMS phenotype was also suggested to be linked to an incomplete editing of *atp6* transcripts in male sterile plants, and that *Rf-1* gene acts through a better editing of this transcript (Iwabuchi, Koizuka & Shimamoto, 1993).
[6] Altered editing of *atp9* transcript was suggested to be responsible for the sterility phenotype (Begu et al., 1990). This is still controversial (Laser, Oettler & Kück, 1995).

Another recent study on PET1 CMS in sunflower suggests that the underlying mechanism of CMS may interfere with programmed cell death (PDC) of the tapetum (Balk & Leaver, 2001). In their discussion, the authors propose that the premature death of the tapetal cells may be a consequence of their inability to satisfy a high energy demand in a tissue where PDC-associated proteins are prematurely activated because of a dysfunctioning mitochondria ('loss-of-function' model). It is still very difficult to reconcile the 'loss-of-function' model, where CMS plants are less efficient in some basic mitochondrial function with the 'female advantage' which seems necessary for the selection of CMS-inducing cytoplasms in natural populations.

However, there is cytological, molecular and metabolic evidence in favour of the high energy demand during male gametogenesis:

— soon after meiosis, the number of mitochondria increases several fold both in the microspores and in the tapetum (Lee & Warmke, 1979);
— expression levels of nuclear and mitochondrial genes encoding subunits of respiratory enzymes change strikingly during pollen maturation (Monéger et al., 1992; Conley & Hanson, 1994; Smart, Monéger & Leaver, 1994; Lalanne et al., 1998; Wen & Chase, 1999) and additional isoforms of enzymes involved in energy production (the β3 subunit of mitochondrial ATP synthase (De Paepe et al., 1993), pyruvate decarboxylase, alcohol dehydrogenase (Tadege & Kuhlemeier, 1997) and aldehyde dehydrogenase (Op den Camp & Kuhlemeier, 1997) are specifically expressed in pollen.

In summary, the hypothesis according to which pollen abortion results from a deficient mitochondrial metabolism during male gametogenesis fits the 'loss-of-function' model, which considers CMS as an 'ordinary' mt mutation. Two examples of this (maize and *N. sylvestris*) are detailed below.

Respiratory mutants

As outlined above, the reason for which it is the male gametophytic process alone, and not vegetative or female reproductive development, that is affected by chimeric mitochondrial genes is still a matter of debate. None of the naturally occurring cytoplasmic male sterile CMS, either found in natural population or resulting from intra (inter) specific crosses seem to be affected in pollen respiratory behaviour. More generally, as of now, only two plant mitochondrial mutants have been shown to be respiratory deficient.

(i) Two stable near-homoplasmic mtDNA deletion mutants have been obtained by protoplast culture in *Nicotiana sylvestris*. These male sterile mutants, named CMSI and CMSII, are both deleted for the mitochondrial *nad7* gene encoding a respiratory Complex I subunit (Pla et al., 1995) and fully impaired in complex I biogenesis and activity (Gutierres et al., 1997). They are similarly affected in growth and leaf morphology, but are female fertile and can be maintained sexually when outcrossed with wild type *N. sylvestris*. Moreover, male sterility is conditional and, in plants grown under greenhouse conditions, reduced amounts of viable pollen are produced under high illumination. CMS plants are likely to survive thanks to the activity of several non-ATP-producing alternative NAD(P)H dehydrogenases existing in plants and fungi and by-passing complex I (Rasmusson et al., 1998; Moller, 1986). Activities of alternative NAD(P)H dehydrogenases, located either on the internal or the external face of the inner mitochondrial membrane and oxidising respectively matrix and cytosolic NAD(P)H, are markedly higher in CMS than in wild-type (WT) (Sabar, De Paepe & de Kouchkovsky, 2000; Brangeon et al., 2000). The activity of another energetically futile respiratory enzyme specific to plants, the alternative oxidase, by-passing ATP-producing complexes III and IV (Siedow & Umbach, 1995), is also enhanced in CMS (Gutierres et al., 1997). As a result, global leaf respiration is slightly higher in CMS than in WT (Sabar, De Paepe & de Kouchkovsky, 2000; Duranceau, Ghasghaie & Brugnoli, 2001), although it would supposedly be less efficient from an energetic point of view. In contrast, full respiratory activity is not maintained in CMS male reproductive organs anthers and pollen, where it represents about 60% of WT oxygen uptake (Sabar et al., 1998). Such differences between vegetative and reproductive tissues comforts the hypothesis that reduced respiration levels could indeed result in male sterility.

(ii) The NSC2 mutant of maize, carrying a deletion in its mitochondrial genome, which affects Complex I *nad4* gene (Marienfeld & Newton, 1994). As other NCS (non-chromosomal stripe) maize mtDNA deletion mutants carrying a dele-

tion in their mitochondrial genomes (Newton & Coe, 1986), NCS2 plants are usually heteroplasmic, containing a mixture of normal and deleted mitochondrial genomes. They are severely affected in their development, are both male and female sterile, and therefore can only be maintained by somatic propagation. NCS2 mutants have lower rates of internal NADH dehydrogenase activity, probably owing to Complex I missassembly, whereas activity of external NADH dehydrogenase seems unaffected. No changes in the amounts of putative corresponding enzymes could be evidenced (Karpova & Newton, 1999). The NCS5 mutant has a defective cytochrome oxidase gene (Newton et al., 1990), and is therefore likely to be affected in the corresponding activity, but, to the best of our knowledge, no physiological studies have yet been reported.

The study of maize and *N. sylvestris* respiratory mutants leads to address two questions which seem relevant for CMS systems.

Could some alteration in main chain respiratory genes account for at least some of the CMS phenotypes observed in natural populations or inter (intra) specific crosses? Clearly the complete loss of function as observed in *N. sylvestris* mutants cannot be maintained by natural selection (mutants are slow germinating and growing), but less severe defects, such as point mutations (deletions) affecting only the efficiency of a particular respiratory subunit could have a more limited effect on vegetative growth, yet affect male reproductive organs. The case of *B. vulgaris* ssp. *maritima G* mitochondrial variants (Ducos, Touzet & Boutry, 2001) is especially relevant, as abnormal *coxII* and *nad9* genes could indeed affect structure/functioning of the corresponding complexes and therefore respiratory activity. Indeed, in *G* mitochondria, diverting the electron flux from the cytochrome *c* pathway to the over-expressed alternative oxidase may compensate mitochondrial respiration for the impaired cytochrome *c* oxidase activity. Overexpression of alternative oxidase has been associated with lower H2O2 levels (Maxwell, Wang & McIntosh, 1999), and was proposed to protect mitochondrial metabolites and genetic information against oxidative stress during plant life (Wagner & Moore, 1997). Interestingly, cytochrome *c* oxidase-defective mutants of the fungus, *Podospora anserina*, revealed a possible relation between respiration changes and senescence in terms of the prevention of the production of reactive oxygen species (ROSs) by complex III (Dufour et al., 2000).

If the above proposition were true, what could be the selective pressure that maintains plants affected in their main chain respiratory functioning, and thus probably affected in their energetic metabolism? This leads to understand the possible role of non-phosphorylative respiratory enzymes in plant survival and effective reproductive success. Activation of these alternative pathways would prevent the blockage of plant metabolism in case of saturation of main chain electron flow, allowing glycolysis turnover and the Krebs cycle to function. Moreover, it is likely that NAD(P)H dehydrogenases, as already proposed for alternative oxidase (Wagner, 1995; Purvis, 1997), act as detoxification enzymes, controlling the levels of ROS produced by the electron flux through the respiratory chain. High levels of antioxidant enzymes such as superoxide dismutases and catalases present in the different cell compartments are well known to be involved in tolerance to various biotic and abiotic stresses (Allen, 1995). It could then be hypothesised that plants with higher levels of alternative enzymes activities are better equipped to resist to certain kinds of stress, resulting in higher selection values under harmful conditions. Preliminary investigations on *N. sylvestris* CMS plants showing that they have higher levels of certain anti-oxidant activities (Garmier et al., 2002) and activities of certain anti-oxidant enzymes are in agreement with this suggestion. CMS plants also resist better to dehydration than WT plants (De Paepe et al., unpublished results Figure 1) and differ

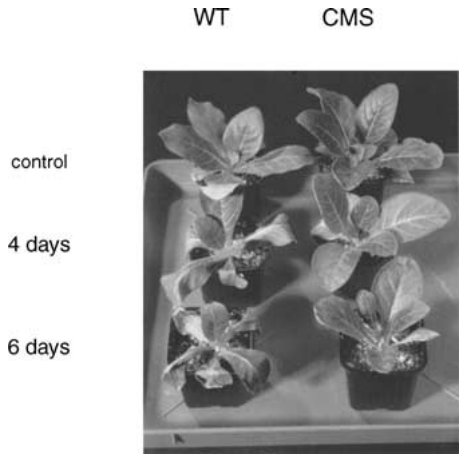

Figure 1. WT and CMS *N. sylvestris* mutants submitted to a dehydration stress in a controlled cabinet (16 h photoperiod, 24°C day/17°C night). Number of days without watering are indicated.

in their reaction to harpin, a bacterial elicitor of the Hyper-Resistance response (Boccara et al., 2001).

The higher resistance to stress of mitochondrial mutants provides us a clue to understand a possible selective force for the maintenance of at least some types of male sterilities. In the future, this resistance-to-stress hypothesis should be tested in natural gynodioecious populations. A candidate species could be *B. vulgaris* ssp. *maritima*, owing to the existence of numerous mitotypes associated with male sterility and polymorphism for respiratory genes. More effort should also be made to determine whether certain chimeric sterility genes are associated with pollen-specific physiological defects. It is likely that, during the course of evolution, different systems have been tested and acquired by mitochondria to ensure the success of their genome, in (or not) competition with that of their symbiotic host.

Conclusion and perspectives

From the survey of our present knowledge of natural CMS, alloplasmic CMS, CMS-associated mt genes and their restorers, and mt mutants, we can draw some relevant conclusions and raise a few questions.

It seems clear that mitochondria are involved whenever there is a cytoplasmic effect on sex determinism in plants. Although they might support the same type of evolutionary forces, chloroplasts have never been implicated. This could be an indication that mitochondria play an active role in male gametogenesis. Is this role strictly limited to the necessary energy provision of this exacting developmental program, or will we be able to unravel a specific role of mitochondria in male gametogenesis? In this regard, the recent involvement of programmed cell death in sunflower CMS may prove particularly relevant (Balk & Leaver, 2001).

Understanding the underlying mechanism of CMS seems crucial for the comprehension of the selective forces that maintain it in natural populations. So far, two possible mechanisms that are apparently mutually exclusive have been proposed, opposing the 'loss-of-function' mt mutants to the 'gain-of-function' mt chimeric new genes. Maybe we should reconsider our views and try not to oppose both hypotheses. Can a model be constructed where a single genetic determinant could impair some physiological pathway in male tissues while opening up a new way for the plant to increase its female reproductive success, at least in some environmental conditions? Maybe it would be easier to identify this increase of female reproductive success in natural populations if we have some idea of what type of pathway is modified in female mitochondria?

The theoretical models have suggested that restorers should not be maintained in the absence of their corresponding male-sterility inducing cytoplasm (unless there is a population structure effect). Some recent works on *Lobelia* (Dudle, Mutikainen & Delph, 2001) and *Raphanus* (Yamagishi & Terachi, 1997) suggest that this may not necessarily be the case. Obviously, we need to undertake more work on the evolutionary impact of nuclear genes in order to better understand the evolutionary constraints on such genes. Once more, the mechanism(s) of restoration could provide clues: if the restorer studied is an allele of a 'modifier of mitochondrial transcripts' (*Mmt*) as it is the case for *Brassica* (Brown, 1999), maybe no 'cost' will be linked with its presence, even in the absence of the sterility-inducing cytoplasm. This might not be the case for other mechanism(s) of restoration. The recent identification of a PPR-protein as a restorer in the Petunia CMS (Bentolila, Alfonso & Hanson, 2002) will undoubtedly open a new route for understandings of male sterility evolution and might reveal a general implication of this peculiar protein family in the gene recruitment for male fertility restoration. The PPR-protein family seems to have been extensively enlarged in plants and its impressive amplification has been related to a possible role in the control of organellar gene expression (Small & Peeters, 2000). Obviously the identification of other restorer genes (whatever they encode PPR-proteins or not) will allow new advances in our understanding of selective forces in action during evolution of these systems.

For some time, population studies on natural CMS and molecular studies on CMS used in crop breeding have been performed in parallel by different teams, with different scientific and technical backgrounds, and with very limited exchanges. Now may be the time to open up the communication doors between communities, for the mutual benefit of both. The way has been paved for two species, *Beta* (Ducos, Touzet & Boutry, 2001) and *Raphanus* (Yamagishi & Terachi, 1996, 1997, 2001), showing that we have now the means to address questions which are relevant for both mechanisms and evolution of CMS. Answering such questions will undoubtedly take us several steps further towards the understanding of genetic conflicts between mitochondria and nucleus in higher plants.

Acknowledgements

The authors are grateful to Richard Berthomé, Joël Cuguen, and Hakim Mireau for the critical reading of the manuscript. They gratefully acknowledge the help from Licia Touzet during its writing.

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