

Male sterility in plants: occurrence, determinism, significance and use

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Abstract – Most of higher plant species are hermaphroditic and male-sterility is often considered as an accident of development. In fact among the multiple possible causes of male-sterility, the most frequently met in nature is cytoplasmic male-sterility (cms) which is a maternally inherited trait playing an active role in the evolution of gynodioecious species. Recent molecular studies have shown that this trait is determined by additional genes created in plant mitochondrial genomes due to their high recombinogenic activity. The physiological mechanisms by which the products of these genes interfere with the formation of male gametophytes are still the subject of intense research. © 2001 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

cytoplasmic male-sterility / mitochondria / gynodioecy / F1 hybrids

Résumé – Stérilité mâle chez les plantes : présence, déterminisme, signification et utilisation. La plupart des espèces de plantes supérieures sont hermaphrodites et la stérilité mâle est souvent considérée comme une anomalie du développement. En fait, parmi les multiples causes possibles de stérilité mâle, la plus fréquemment rencontrée dans la nature est la stérilité mâle cytoplasmique (smc) qui est un caractère à hérédité maternelle jouant un rôle actif dans l'évolution des espèces. Les études moléculaires récentes ont montré que ce caractère est déterminé par des gènes supplémentaires créés au sein du génome mitochondrial grâce à sa très forte activité recombinogène. Les mécanismes physiologiques par lesquels ces gènes interfèrent avec le développement des gamétophytes mâles sont encore l'objet d'une recherche intense. © 2001 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

stérilité mâle cytoplasmique / mitochondrie / gynodioecie / hybrides F1

. Version abrégée

Parmi les multiples causes génétiques possibles de l'avortement du pollen, le gamétophyte porteur des gamètes mâles, la stérilité mâle à hérédité cytoplasmique (ou smc), qui est déterminée par des gènes mitochondriaux, apparaît comme l'explication la plus fréquente (pour ne pas dire exclusive) de la gynodioecie. Ce dimorphisme sexuel, où coexistent, dans des populations de la même espèce, des plantes hermaph-

rodites et des plantes femelles, est le système de reproduction le plus fréquent, après l'hermaphrodisme, chez les plantes supérieures.

Le mode d'hérédité de cette stérilité mâle explique son maintien et éventuellement son expansion dans l'espèce : en effet, à condition que les plantes hermaphrodites assurent la pollinisation, le caractère est transmis à toute la descendance des plantes femelles puisque les mitochondries sont transmises uniquement

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par le gamète femelle. Une prolificité légèrement supérieure des plantes femelles suffit à augmenter la fréquence du cytoplasme inducteur de stérilité mâle dans la population.

Chez plusieurs espèces comme le maïs, le tournesol, le colza, le haricot, le pétunia, les déterminants moléculaires de ces stérilités ont été étudiés en détail. Il s'agit dans tous les cas de gènes mitochondriaux « supplémentaires » dont l'origine réside dans des recombinaisons intragénomiques conduisant à la création fortuite de phases ouvertes de lecture qui utilisent des signaux d'expression de gènes pré-existants. Ces gènes apparaissent comme des gènes parasites du génome mitochondrial et codent pour des protéines à localisation membranaire. Ils sont exprimés de manière constitutive, mais leur seul effet notoire est l'avortement des spores mâles au cours de leur formation ou pendant le déroulement de la gamétogenèse. La genèse de ces gènes est rendue possible par l'organisation particulière du génome mitochondrial des plantes : une grande taille (variable, plusieurs centaines de kb) qui s'explique en partie par des transferts de matériel génétique depuis le noyau et les chloroplastes, une répartition en molécules sub-génomiques dont certaines peuvent être sélectivement amplifiées et une grande plasticité dans

l'organisation (l'ordre des gènes) résultat d'une forte activité recombino-gène.

Des gènes nucléaires de restauration de la fertilité pollinique se trouvent généralement présents dans les populations gynodioïques et peuvent être fixés dans ces populations qui redeviennent par conséquent hermaphrodites. Ils agissent à différents niveaux de l'expression des gènes de stérilité (pré- ou post-traductionnel), mais leur identification reste à faire et devrait apporter des données intéressantes sur le contrôle de l'expression des gènes mitochondriaux.

La production de variétés hybrides bénéficie de ces systèmes naturels de castration qui permettent de contrôler la pollinisation entre individus des deux lignées parentales et de récolter les semences sur les lignées femelles. La production d'hybrides de maïs qui a permis une progression extraordinaire des rendements dans tous les pays a pu en bénéficier pendant une trentaine d'années. C'est sur de tels systèmes que reposent aujourd'hui la production de variétés hybrides chez le riz, le tournesol, la betterave à sucre, le sorgho, le colza, et plusieurs légumes. Depuis quelques années, des recherches visent à élaborer des systèmes de substitution dérivés du génie génétique.

1. Introduction

Gynodioecy is the co-existence of hermaphroditic and female (male sterile) individuals in populations of the same plant species. This term was introduced by Darwin in 1877 [1] who was the first to suppose that the loss of the male function could play an important role in plant adaptation and evolution. He considered this dimorphism as a step towards the separation of sexual types in different individuals (dioecy) which represents the achievement of an obligate cross-fertilizing mating type.

Male sterility may have multiple causes. It can result from adverse growth conditions, from diseases, or from mutations. Naturally occurring genetically male sterile plants in hermaphrodite species generally maintain fully normal female functions. The phenotypic manifestations of male sterility are very diverse from the complete absence of male organs, the failure to develop normal sporogenous tissues (no meiosis), the abortion of pollen at any step of its development, the absence of stamens dehiscence or the inability of mature pollen to germinate on compatible stigma.

Although there exist multiple causes for pollen abortion, cytoplasmic male sterility, which depends on mitochondrial genes, is the determinant of gynodioecy most frequently met in natural populations. The molecular data accumulated over the last two decades by molecular biologists on sterility-inducing mitochondrial genes and their expression permit a better understanding of the main-

tainance and the role of this sexual dimorphism in plant species in relation to theoretical models established by population geneticists.

2. The maintenance of gynodioecy in natural populations

Gynodioecy has been described for as many as 7.5 % of European angiosperm species and is the second most frequent reproductive system, after hermaphroditism which represents 72 % of them [2].

In theory, a nuclear (Mendelian) recessive mutation leading to male sterility (*ms*) can be maintained in a natural population if the resulting female plants (homozygous *ms/ms*) produce at least twice the number of viable seeds produced by a hermaphroditic plant [3]. This is the consequence of the disymmetry created by the mutation. As shown in *figure 1A* a female plant transmits the *ms* gene to the next generation exclusively through the female side and only if it is pollinated. On the contrary the corresponding *Mf* allele in normal hermaphrodites is transmitted through both male and female gametes. A female superiority, necessary for the maintenance of the *ms* gene, can result from phenomena like the hybrid vigor resulting from cross pollination, or different developmental behaviour of females, allowing them to colonise a wider environment. However, to our knowledge, a nuclear mutation leading to male sterility and associated with high female superiority

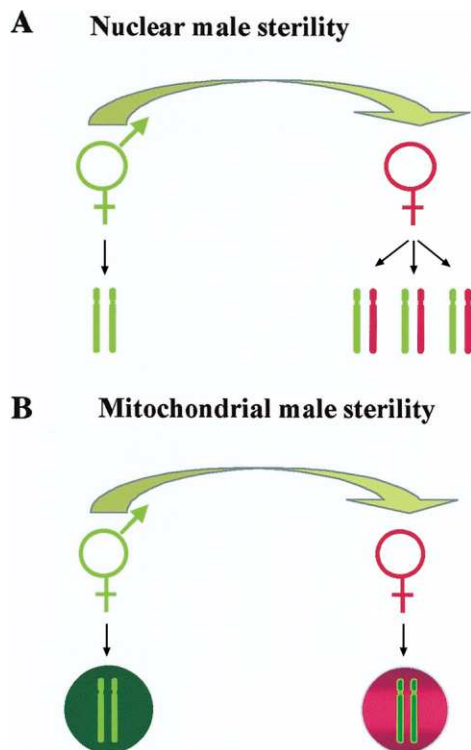


Figure 1. Schematic representation of conditions for maintenance of the male sterility determinant in populations. **A.** in the case of a Mendelian recessive gene (symbolised by a red chromosome) the female must produce more seeds than the hermaphrodite in order to compensate the transmission of the male fertile gene (green chromosome) to the next generation by the hermaphroditic parent. **B.** in the case of maternal inheritance (mitochondria) the male sterility determinant (symbolised by a red cytoplasm) and the male fertility determinant (green cytoplasm) are equally transmitted to the next generation.

allowing its maintenance in natural populations has not been reported so far.

Early examples of gynodioecy studied by Darwin [1] and Correns [4] were proved to be controlled by cytoplasmic factors. After a long period of mystery, it is now generally admitted that the male sterility determinant in these cases is borne by the mitochondrial genome which is transmitted only through the cytoplasm of the female gamete (the oosphere). Due to this mode of inheritance, the theoretical scheme for maintenance of the male sterile determinant is different. The female plants need as previously the presence of pollinators, but as shown in *figure 1B* all the progeny of female plants carries the male sterility inducing cytoplasm. As soon as female plants produce more seeds than hermaphrodites, the male-sterility-inducing cytoplasm will be more successful than the 'normal' cytoplasm. It also may be maintained by genetic drift. In any case, the spread of the 'male sterile' cytoplasm is only limited by the necessity for the presence of pollinators. From these considerations one can predict that cytoplasmic male sterilities would be more easily maintained in natural populations than Mendelian male sterilities. This will be illustrated below.

Unlike cytoplasmic genes, nuclear genes which are associated with a sterility inducing cytoplasm are severely handicapped since they are not transmitted by the pollen. Consequently, any nuclear gene able to restore the pollen production (male fertility) to plants carrying a sterility-inducing cytoplasm will be selected. This situation should lead to the rapid spreading and finally the fixation of restorers in a given population/species, a number of generations after the appearance of a novel cms. After this fixation, the population is back to sexual monomorphism : hermaphroditism.

However, gynodioecy is still observed, indicating that the studied populations have not reached equilibrium and/or other selective forces are involved in the phenomenon [5, 6].

3. Genes responsible for male sterility

Nuclear male sterilities are generally recessive mutations which affect a huge number of functions. In maize for example, several hundred loci are involved. These mutations may affect for example proteins involved in male meiosis [7], the metabolism of plant hormones [8] the biosynthesis of complex lipid molecules [9] or the synthesis of secondary metabolites [10].

Cytoplasmic male-sterility (cms) is usually defined as "maternally inherited deficiency in producing viable pollen" and the mitochondria are responsible for this trait. This definition leads us to call cms any mitochondrial mutation which impairs the proper functioning of mitochondria, leading to male sterility. For example, in *Nicotiana sylvestris*, mitochondrial mutants were obtained from in vitro culture [11]. They exhibit male sterility but also many other abnormal characters throughout all their development. Such mutated mitochondrial genomes would obviously never have been selected for in natural conditions. Therefore, a better definition for cms would be: "maternally inherited male sterility resulting from a specific (mitochondrial) gene whose expression impairs the production of viable pollen without otherwise affecting the plant".

The present knowledge about cms genes results from studies in several cultivated species, where this trait is used for F1 hybrid production (see below).

We have particularly studied the case of the Ogura [12] type of cytoplasmic male sterility first discovered in Japanese wild radish. The male sterility inducing gene (*orf138*) was determined [13–15] by studying the recombined mitochondrial DNA of 'cybrids' (cytoplasmic hybrids) obtained through protoplast fusion [16] between fertile *Brassica* (*napus* or *oleracea*) and male-sterile Brassicas (Ogura bearing cytoplasm) derived from interspecific crosses [17]. The '*orf138* locus' (*figure 2*) comprises two open reading frames coding respectively 138- and 158-amino-acid polypeptides which are co-transcribed. The latter, also called *orfB*, corresponds to the subunit 8 of the ATPase complex [18], and the *orf138* gene has some similarities

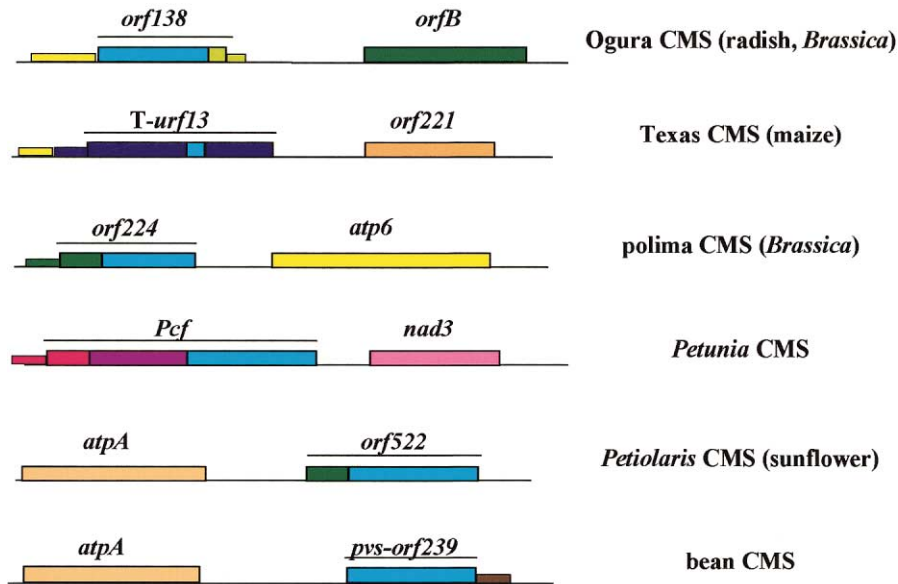


Figure 2. Some examples of cytoplasmic male sterility genes identified in different plant species. [After 14, 19, 20–23, 26, 29]. The open reading frames are indicated as tall rectangles, non coding regions as lines or small rectangles. The male sterility inducing genes are represented with the adjacent normal and indispensable mitochondrial gene with which they are co-transcribed. The cyan color identifies regions for which no obvious homology with known genes have been detected in databases. Other colors refer to similarities with known mitochondrial genes.

with different radish mitochondrial DNA sequences indicating that it could result from intragenomic recombination. The particular structure of this locus determines the stability of the *orf138* gene. For example, we observed some cases of dissociation of the Ogura cms gene (*orf138*) from its co-transcribed partner (*orfB*) after mitochondrial recombination following protoplast fusion experiments. In such plants, the male sterility was unstable due to a frequent loss of the cms-inducing gene [19]. An instability of the cms is also observed when a second copy of the *orfB* gene allows the loss of the copy associated with the *orf138* gene [14, 19].

Very similar situations were described in other cms systems and in other species as shown in figure 2. In the case of maize [20], the *T-urf13* gene responsible for the Texas type of cms is a chimaeric sequence composed of a part of the *atp6* gene, the 3'-flanking region of *rrn26* (ribosomal RNA) gene, an unidentified sequence and a part of the coding sequence of *rrn26*. The resulting protein, URF13 is localised in the internal membrane of the mitochondria and is responsible of the male sterility and, a unique case in cms plants, leads also to a strong susceptibility to two pathogenic fungi, *Bipolaris maydis*, T strain, and *Phylosticta maydis*. In the case of 'polima' cms in *Brassica napus*, the *orf224* gene is associated with the *atp6* gene [21]. In the case of *Petunia hybrida* [22] the *S-pcf* locus is composed of the 5'-end of the *atp9* subunit, parts of two exons of *coxII* and a sequence of unknown origin. This *S-pcf* gene is cotranscribed with the *nad3* and *rps12* genes.

In the case of sunflower [23, 24] (*Helianthus annuus*), the cms PET1, produced after interspecific hybridization with *H. petiolaris* by Leclercq [25], is due to the expression of *orf522*, which has similarities with the *orfB* gene at its 5'-end. This gene is associated with the *atpA* gene as in the case of *Phaseolus vulgaris*, where the *orf239* gene is responsible for the 'cms-Sprite' [26].

Cms-inducing genes are not only associated and cotranscribed with essential mitochondrial genes, but also translated from the resulting bicistronic messenger RNAs (mRNAs), since monocistronic mRNAs are not observed. Even when monocistronic mRNAs are observed for the partner gene, such as *atpA* in sunflower PET1 cms [23], it is not the case for the cms associated gene. The above mentioned monocistronic mRNAs for indispensable genes are very likely produced by maturation of the co-transcripts. Such maturation leads to the degradation of the cms part of the co-transcript. As we can find cms inducing sequences in the 5'-half or the 3'-half of these co-transcripts, we can expect the stabilization/degradation of mRNA in plant mitochondria to involve both 5'- and 3'-signals.

The physiological function of the polypeptides encoded by these genes remains unknown. They are constitutively synthesized in all tissues of the plant. They are generally found in the membranous fraction and could interfere, but only in some circumstances, with one or other (or several) enzymatic complexes, or create abnormal physical properties of the mitochondrial inner membrane.

4. The plasticity of the plant mitochondrial genome

A great deal of sequence and mapping data converge to the conclusion that the plant mitochondrial genome is very stable in sequence but highly variable in organisation [27].

Compared to algae or animal mitochondrial genomes it is characterized by a larger size. This larger size seems to result from expansion instead of the conservation of an ancestral size since at the same time the number of genes transferred from the mitochondria to the nucleus is higher in plants than in algae (which have smaller genomes), and viral, plastid and nuclear sequences have been integrated into the mitochondrial genomes [28]. In the case of *Arabidopsis* for which the mitochondrial genome composed of 367 kb has been fully sequenced, 58 'true' genes (of known or predictable function) have been detected, in addition 156 reading frames of at least 100 codons of unknown function have been identified. Although none of these potential ORFs are conserved in other species and are thus unlikely to be functional, these potential coding sequences could constitute a reservoir for male sterility genes. When they are stable, cms are very often controlled by ORFs that are associated and cotranscribed with another mitochondrial gene, indispensable for proper mitochondrial functioning as in the case for example of *Brassica*, *Petunia* and sunflower. The cms-inducing genes known so far likely result from genetic recombinations putting together unrelated mitochondrial sequences and building up from this genetic shuffling open reading frames surrounded by proper expression signals. Although the expression signals of the normal associated genes are thus used for expression of cms-inducing genes, such an association is probably more necessary for the maintenance of the cms-inducing genes than for their expression. The cms genes therefore appear at the level of the mitochondrial DNA as parasitic sequences which use for their expression signals pre-existing mitochondrial genes absolutely necessary for the cell survival.

The large majority of nuclear restorers whose effect on cms genes have been described so far act on the maturation of the bicistronic mRNA associated with cms. They seem to increase the production of monocistronic mRNAs for the indispensable partner gene. It is the case for the sunflower PET1 cms [23], for the *B. napus* polima *Rfp1* restorer [29], for the maize Texas cms restorers *Rf1*, *Rf8* and *Rf** [30], for example.

The permanent restorer *Fr* of bean cms is the only known restorer gene acting at the level of mitochondrial DNA [31]. This *Fr* gene acts by eliminating a 25-kb molecule bearing the cms determinant, the *atpA-orf239* region, the *atpA* function being maintained because there is a second copy of this gene not associated with *orf239* [26]. We may infer from this observation that the nuclear factors which are involved in mitochondrial genome maintenance are probably under strict selective constraints. This could explain the paradoxical observations that mito-

chondrial genomes appear extremely stable in structure when observed at the level of restriction gel electrophoresis patterns but that many substoichiometric recombinant forms can be observed using PCR amplification.

The study of nuclear restorers will undoubtedly provide much information about mRNA maturation and stabilization in plant mitochondria. The relative abundance of nuclear restorers acting at this level of cms gene expression probably indicates a flexibility of the mechanisms involved, either due to redundancy of factors or a large tolerance for variation due to relaxed selective constraints.

5. The possible role of cms and restorer genes in evolution

A common feature among the gynodioecious species studied by population geneticists, such as *Thymus vulgaris* [32], *Plantago lanceolata* [6] or *Beta vulgaris* [33] is the mitochondrial polymorphism in natural populations. It is likely that mitochondrial recombination and mechanisms involved in equilibrium shifts between major and substoichiometric molecules are involved in the production of mitochondrial polymorphism. Similar situations are scarce in cultivated plants which usually possess a single cytoplasm or only a limited number of cytoplasms. A molecular analysis of the mitochondrial polymorphism in natural populations should provide clues for understanding how this polymorphism is generated.

The association of the nuclear genome of a plant species with an alien cytoplasm generally from a distinct species (alloplasm) has long been known to induce cms [34]. In alloplasmic situations, the observed phenotype is often complex, resulting from the association of both chloroplastic and mitochondrial genomes with an alien nuclear counterpart. Some of the cms induced by alloplasmic situations could also be a revival of ancient cms in the cytoplasm donor species which have been silenced by fixation of nuclear restorers in this species. A new nuclear background, which has never selected restorer genes for this cms, allows the cytoplasm to induce sterility again. One can expect to detect the cms inducing gene in such alloplasmic situations by looking for an ORF, present but silent in the cytoplasm donor species and expressed (and probably co-transcribed with an indispensable mitochondrial gene) in the species where cms is expressed. This is what is observed in the case of the alloplasmic *Brassica napus* and *B. juncea* plants carrying *B. tournefortii* cytoplasm. In the *B. tournefortii* mitochondrial genome, a chimaeric ORF (*orf263*) is present 3' to the *atp6* gene but not expressed. In alloplasmic lines of *B. napus* or *B. juncea* carrying the *B. tournefortii* cytoplasm, *orf263* is cotranscribed with *atp6* and the bicistronic mRNA is accumulated [35]. Although the responsibility of *orf263* in the male sterile phenotype of alloplasmic plants remains to be proven, the expression behavior of this mitochondrial gene in different nuclear backgrounds conforms to what is expected from a 'silent' cms gene reactivated in an alloplasmic context.

One of the most intriguing features of cms, still open to hypotheses, is the precise localisation in development of the phenotype induced by a mitochondrial gene which, in all cases except the bean *pvs* gene [36], is constitutively expressed. This observation is not so surprising when one considers that these genes have recruited expression signals from other mitochondrial genes and are therefore submitted to the same controls. One can expect that cms genes which have been maintained for a long time (and probably selected for) in natural populations, such as the *Ogura* cms present in different *Rhaphanus* species [37, 38] or the bean cms determinant present in different *Phaseolus* species [39, 40] will have an effect strictly limited to pollen production. Hence, the limited phenotype induced by these genes must result from a specific 'activity' in male organs. It is likely that the possible means of impairing male gametogenesis via a mitochondrial defect without affecting the female fitness of the plant are limited. This remark might appear to be in contradiction with the observed absence of homology between cms gene sequences. However, it is noteworthy that at least one hydrophobic domain and a possible or shown association with the mitochondrial membrane are shared between the encoded proteins [41]. Some stretches of amino acids are sometimes shared by these otherwise unrelated proteins [42, 43], but an interpretation of this latter observation in terms of function of the encoded proteins is still premature.

6. Male sterility and hybrid production

The concept of hybrid vigor emerged gradually [44] since the first observations in the eighteenth century by J.G. Koelreuter of interspecific crosses in *Nicotiana*, *Dianthus*, *Verbascum*, *Mirabilis*, *Datura*, confirmed by Darwin [45] in vegetables, and W.J. Beal in maize in 1880 [46]. Subsequently this effect was exploited in plant breeding [47] when the tools to produce the necessary amount of seeds became available in hermaphrodite species: the first male sterility system was developed in onion in 1943 [48] and others were developed in a wide range of species such as sugar beet, (maize), sorghum, sunflower, rice, rapeseed, carrot [49]. Cytoplasmic male sterilities are far more convenient for the production of hybrid seeds than Mendelian ones because of their mode of maintenance and restoration of fertility. Furthermore, cms which have been successfully selected in natural populations are less prone to be associated with characters which are not convenient for seed production. They are even likely to be associated with a better seed production than hermaphrodites. So, our definition of cms, previously suggested, is not only in accordance with the situation observed in natural populations but also with the demands of plant breeders, who need high performance seed-producing female parents for their hybrids.

The production of hybrid varieties of maize (from the thirties in the US) and of rice (since 1976 in China) represents the most significant and successful breeding efforts of the twentieth century. A 6-fold increase was observed between 1930 and 1990 for US corn yield after the introduction of hybrid breeding, compared to uniform performances for selected open pollinated populations during the previous 60 yr [50]. In China, the hybrid rice production increased from $2.1 \cdot 10^6$ ha in 1977 to $15.3 \cdot 10^6$ ha in 1997, with an advantage of 20–30 % compared to inbred varieties, increasing the rice production of this country by more than $310 \cdot 10^6$ t during this period [51]. In both cases the development of male sterile lines and fertility restorer lines was essential in order to allow the seed production of fertile F1 hybrids from the intercrossing of parental lines and to prevent self pollination of the seed-bearing plants. However in maize, the cms-T system was practically abandoned after 1970 due to the susceptibility of cms-T corn to 'southern leaf blight' fungi (*B. maydis*) pointed out above and hybrids are now produced by manual or mechanical emasculation. Breeders of other important species such as sugar beet, sunflower, rapeseed, sorghum have taken advantage of cytoplasmic male sterile systems. Nevertheless, since these systems are different and cannot be transposed from one species to another, efforts based on genetic engineering are being made in several laboratories to generate new methods for the production of hybrid seeds in a wider range of species [52].

7. Conclusion

The high recombinogenic activity of the plant mitochondrial genome produces new coding sequences which are potential cytoplasmic male sterility genes. These genes which have been proven in several instances to precede domestication, are maintained in wild plant populations and can be fixed in these populations if nuclear restorer genes are also present. One can imagine several cycles of this type of evolution involving different cms-restorer partners while subspecies are diverging and can predict that this would lead to male sterile plants when nuclei and cytoplasm of different hermaphroditic species are combined by crosses. This phenomenon, representing an interspecific barrier, is in fact commonly observed by plant geneticists, and is exploited for hybrid seed production.

The development of the male gametophyte is an aspect of plant development around which converge multiple scientific questions such as the evolution of sexual dimorphism in natural populations or the privileged physiological relationship between mitochondria and the male function of plants. This explains the growing interest of scientists for identifying and studying male sterilities.

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