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Merging theory and mechanism in studies of gynodioecy

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In gynodioecious species, females and hermaphrodites coexist and the genetics of sex determination is usually nuclear cytoplasmic. Maintaining nuclear-cytoplasmic gynodioecy requires polymorphism for the feminizing genes (contained in the mitochondria) and the genes that restore male fertility (contained in the nucleus). This complex polymorphism depends, in part, on there being negative pleiotropic effects (i.e. costs) of the nuclear restorer alleles. Here, we combine information from theoretical studies and studies on the molecular action of restorer alleles in crops to interpret the probable costs of such alleles, and suggest how various aspects of the theoretical models could be tested. In doing so, we highlight how crops can be used to address evolutionary questions about the maintenance of nuclear-cytoplasmic gynodioecy.

Introduction

The maintenance of polymorphism has long intrigued evolutionary biologists. Gynodioecy, a widespread gender polymorphism that has been well studied in plants, refers to species containing two genetically determined morphs, females and hermaphrodites [1–6]. The genetic control of this gender dimorphism is often nuclear cytoplasmic [7], involving two sets of genes. One set is in the cytoplasm and causes male sterility, turning plants into females [8]; the other set is in the nucleus and restores male fertility ('nuclear restorers'), turning plants back into hermaphrodites [9,10]. Nuclear-cytoplasmic gynodioecy is therefore a mix of two sexual morphs whose sex is determined by genes in two genomes, both of which must be polymorphic for nuclear-cytoplasmic gynodioecy to be maintained [11].

The maintenance of this polymorphism is often the focus of studies of nonagricultural plant species, from both theoretical and empirical perspectives. Theory suggests that compensation (the seed-fertility advantage of females) and cost (the negative pleiotropic effects of nuclear restorers; see Glossary) control the frequency of females and the evolution of gynodioecy [12–15]. Consequently, field studies often attempt to quantify compensation and/or cost (e.g. [2,4]). By contrast, studies using agricultural species focus on identifying the molecular action of the genetic components in crops in which latent male sterility has been uncovered in order to produce outcrossed or hybrid seed [9,10]. These studies describe the practical dynamics of facultative gynodioecy in important crops and rarely include any theoretical construct based on the evolutionary dynamics of gynodioecy. Many of these studies have been motivated by a desire to understand interactions between nuclear and mitochondrial genes [16].

Here, we review theoretical studies of gynodioecy as well as the molecular-action literature on restorers in crops, and categorize restorer actions within cells as a way of evaluating when and why restorers might exhibit costs. We hope to bring an evolutionary perspective to crop researchers about how the sex-determination system is maintained and to bring a mechanistic understanding of how the different genetic components work to theoreticians and researchers interested in studying the maintenance of nuclear-cytoplasmic gynodioecy.

Glossary

Chimeric gene: gene formed by the novel fusion of fragments of other genes.
Compensation: the seed-fertility advantage of females relative to hermaphrodites.
Constitutive cost: cost in which restorers always have negative pleiotropic effects regardless of whether they restore male fertility (i.e. cost is independent of the CMS background).
Cost: negative pleiotropic fitness effects of restorers.
Cytoplasmic male sterility (CMS): maternally inherited traits that cause plants that would otherwise be hermaphroditic to not make pollen and become female.
Cytotypes: cytoplasms containing different mitochondrial haplotypes.
Dominant cost: when individuals with a single restorer allele have lowered seed and/or pollen production relative to those without the restorer allele.
Dominant restoration: when only a single restorer allele is necessary to restore male fertility.
Epistatic restoration: when restorer alleles at more than one locus are needed to cause complete restoration.
Expressed cost: cost that is a consequence of the restorer being with a cytotype that it can restore; that is, the act of restoration carries a cost.
Pollen cost: cost affecting pollen production and/or viability.
Polycistronic transcripts: co-transcription of multiple genes, resulting in a single strand of mRNA.
Recessive cost: when only individuals homozygous for the restorer allele have lowered seed and/or pollen production.
Recessive restoration: when individuals must be homozygous for the restorer allele for male fertility to be restored.
Restorer: nuclear alleles that restore male fertility (pollen production) in individuals carrying CMS genes.
Seed and pollen cost: a cost affecting seed and pollen production or viability, either via action in all reproductive tissues or effects in somatic tissues that affect the survivorship or overall vigor of the individual.
Seed cost: a cost that affects seed production and/or quality.
Silent cost: cost only expressed when the restorers do not affect sex determination (they are 'silent') because they are matched with a CMS that they are unable to restore.

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The evolutionary dynamics of CMS and restorer genes

In nuclear-cytoplasmic gynodioecious species, cytoplasmic male sterility (CMS) genes, which prevent plants from producing pollen (i.e. turn them into females), are usually found in the mitochondrial genome [8,9,16]. CMS genes are widespread among angiosperm families and are likely to arise often as a consequence of the fluid nature of the plant mitochondrial genome (Box 1). Given that mitochondrial genes are usually only inherited maternally [17], a female need only make slightly more or better seeds than a hermaphrodite to invade a population [17], thus compensation must be non-zero.

When a CMS gene confers compensation, the proportion of the population that carries the CMS gene and is female will increase in each generation via natural selection [18]. Any nuclear mutation that counteracts the effect of CMS (i.e. restores male fertility) should have an advantage when seed production is pollen limited (e.g. when female frequencies are extremely high) as it can convert female offspring into hermaphrodites that can pollinate the remaining females, further spreading the mutant nuclear restorer gene. This nuclear restorer increases in frequency by increasing the total number of gametes produced by restored individuals and by its association with the rare gamete type, pollen [19].

However, restorers do not always spread to fixation. If females make substantially more and/or better seeds than hermaphrodites (compensation is greater than twofold), females will easily be maintained at frequencies up to 50% [18]. By contrast, a cost of restoration is required to explain the maintenance of females when compensation is low or female frequencies are >50% at equilibrium (Table 1). Such negative pleiotropic effects or costs favor hermaphrodites with fewer restorers than average. How restorers are counted and which ones impart a cost, vary from model to model (Table 1).

The cost of restoration has been modeled with different assumptions about whether the restorer allele is dominant, whether the cost itself is dominant, the mode of cost (silent, expressed or constitutive), and whether cost affects seed and/or pollen production (Table 1). Whether the cost of restoration is dominant (occurs when only one copy of the restorer allele is present) or recessive (occurs only in individuals that are homozygous for the restorer allele) has a relatively large impact on female frequency, as well as on the maintenance of nuclear-cytoplasmic gynodioecy *per se* [15]. If the cost is dominant, then polymorphism at the restorer locus is maintained by rare advantage; by contrast, if the cost is recessive, polymorphism is maintained by overdominance [15] (Figure 1). The cost of a restorer can be dominant or recessive independent of whether the action of the allele (i.e. whether or not pollen is produced) is dominant. The highest frequencies of females are possible with dominant costs, but recessive costs enable females to be maintained when compensation and costs are extremely small [15].

Different modes of cost also produce different predictions. Nuclear-cytoplasmic gynodioecy is stable over a larger parameter space when the costs of restorers are constitutive (i.e. occur regardless of the CMS background) relative to those with only expressed or silent costs (i.e. occur when the restorer does or does

Table 1. Models of sex-ratio evolution for nuclear–cytoplasmic gynodioecy

Dominance of restoration	Dominance of cost	Mode of cost	Pollen and/or seed cost	Results	Refs
Dominant	Dominant	Silent	Pollen	Stable[a]	[14]
			Seed	Stable[a]	[14]
		Expressed	Pollen	Unstable	[15]
			Seed	Stable	[50]
				Unstable	[15]
		Constitutive	Pollen	Stable[b]	[7]
				Stable[a]	[15]
			Seed	Stable	[12]
				Stable[c]	[13]
				Stable	[50]
				Stable[a]	[15]
			Seed and pollen	Stable[c]	[13]
				Stable[a]	[15]
	Recessive	Silent	Pollen	Stable	[14]
			Seed	Stable	[14]
		Expressed	Pollen	Stable[c]	[15]
			Seed	Unstable	[15]
		Constitutive	Pollen	Stable[c]	[15]
			Seed	Stable[c]	[15]
Recessive	Dominant	Expressed	Seed	Stable	[50]
		Constitutive	Seed	Stable	[50]

[a]Female frequencies tend to a single value or cycle at equilibrium.
[b]Females are maintained only at the level of the metapopulation.
[c]Female frequencies tend to a single value at equilibrium.

Box 1. CMS and nuclear restorer genes

CMS

CMS is caused by chimeric genes, formed from segments of other mitochondrial genes as well as from segments of unknown origin; together, these segments form open reading frames(ORFs) that are transcribed and translated to produce a novel protein [8]. Moreover, they are commonly co-transcribed with other mitochondrial genes [9]. This co-transcription is one factor that could cause negative pleiotropic effects of nuclear restorer alleles, although this remains untested. There are instances in which CMS has been traced to 'loss-of-function' mutations; however, most evidence is consistent with an active role for the new allele and its products, including several cases in which the new protein appears to act as a toxin that interferes with mitochondrial respiration [9,47]. CMS genes are thought to arise as a consequence of intragenomic recombination [8].

Researchers are currently determining which proteins CMS genes encode and what their possible functions within the cell might be [16]. The answer is typically species specific; in addition, not only are several different processes involved, but in some cases CMS also affects the gametophyte and, in others, the sporophyte (see main text).

Nuclear restorer genes

Restorers are thought to arise from the many nuclear genes that control mitochondrial gene expression. Recent evidence in petunia, radish and rice suggests that restorer genes have been recruited from the pentatricopeptide (35 amino-acid) repeat gene family (PPR) gene family, which is involved in organelle gene expression (reviewed in [49]). Restorers appear to work primarily through preventing translation of CMS genes (Box 2). This is achieved by destabilizing the transcript produced by the CMS gene before translation or, if the transcript is dicistronic (i.e. includes the transcript of the CMS gene as well as that of an essential gene), it cuts the CMS-gene transcript from that of the essential gene [52].

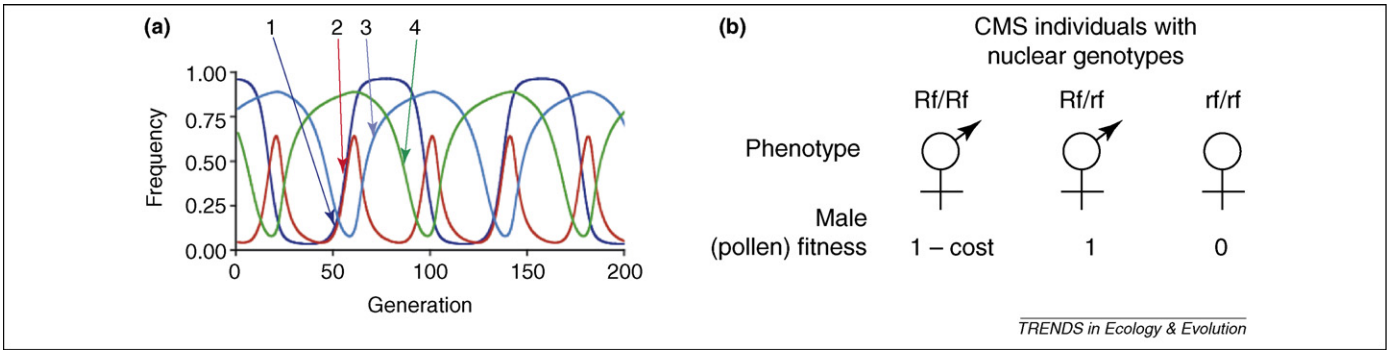

Figure 1. Dominant and recessive costs. When restoration is dominant, whether the restoration cost is dominant or recessive leads to different selection patterns on restorers [15]. In both cases, females are maintained in the population only if compensation and cost values are sufficiently large. **(a)** Dominant costs cause negative frequency-dependent selection (rare advantage) and cycling of the different CMS genes and their restorers. We consider a system with two CMS types, 1 and 2, with dominant restorers, R1 and R2, respectively. Arrow 1 (dark blue): first, CMS1 (dark-blue line) and its restorer, R1 (light-blue line), are both rare; therefore, the few CMS1 individuals are female. Arrow 2 (red): next, because of compensation, CMS1 females increase in frequency, thereby increasing the overall frequency of females (red line). Arrow 3 (light blue): R1 is then selected for given that CMS1 is common, turning most CMS1 individuals into hermaphrodites. Arrow 4 (green): fourth, R2 (green line) is selected against (if the cost is silent or constitutive in this example) because it carries a dominant cost in CMS1 hermaphrodites. At this point, CMS2 and R2 are both rare in the population, causing the cycle to restart. **(b)** Recessive costs cause overdominance of the restorer loci. If we consider a single CMS type and its restorer *Rf*, individuals heterozygous at the restorer locus have the highest male (pollen) fitness: heterozygous individuals are hermaphrodites because restoration is dominant; however, they do not express a cost because that is recessive.

not affect the phenotypic expression of CMS, respectively) [15]. However, the impact of when the cost of restoration occurs on the maintenance of nuclear-cytoplasmic gynodioecy is less than that of the dominance of the cost of restoration.

Characterizing the probable costs of restoration in agricultural species

Box 2 highlights ways in which nuclear restorers of crop species interact with mitochondria and CMS genes, from the actual removal of the sub-molecule containing the CMS gene to interactions with the transcripts produced by the CMS gene to post-translational destabilization of the protein encoded by the CMS gene. Here, we highlight research on three crops, brassica *Brassica napus*, maize *Zea mays* and bean *Phaseolus vulgaris*, which are well studied and cover the range of characteristics of nuclear restorer genes that we used to predict aspects of the cost of restoration. Table 2 summarizes this information and our predictions for these and other crops.

Brassica napus

In terms of CMS, *B. napus* contains an ancestral male-fertile cytoplasm (termed *cam*) and two male-sterile cytoplasms (termed *nap* and *pol*) [20]. The two CMS genes are related to each other, and both contain fragments of the essential gene *atp8*, suggesting that they are chimeric. It is still unknown which of these two CMS genes was the progenitor of the other. The two CMS genes differ in their placement along the mtDNA, and are co-transcribed with different essential mitochondrial genes.

There are two restorers, *Rfp* and *Rfn*, as well as a recessive non-restoring form, *rf*, which occur at the same, possibly novel, locus (i.e. are allelic) [21]. Each restorer restores only one of the CMS types. Both restorers enhance mtDNA transcript processing in anthers; specifically, they mediate cleavage of the CMS-associated open reading frame (ORF) transcripts, which reduces or prevents translation of these transcripts into proteins (thereby countering the action of the CMS gene). However, the two restorers differ in other ways. *Rfp* has no known pleiotropic effects and it appears to only cleave transcripts produced by *pol* [22]. Hence, *Rfp* should carry a cost only when in combination with *cam* or *nap* (a silent cost), and even then, the cost is likely to be relatively small because the only effect would be to produce a protein with no function. By contrast, *Rfn* has pleiotropic effects [20]. It cleaves the ORF transcript produced by *nap*, as well as at least two additional mtDNA transcripts: *nad4*, an essential mitochondrial gene and *ccmFn*, which is involved with cytochrome *c* biogenesis ([23], G. Brown pers. commun.). If these pleiotropic effects occur regardless of the cytoplasm type (and there is evidence that they occur in both *nap* and *pol* cytoplasms [23]), then the cost will be constitutive. This cost might explain why *Rfn* is rare, except when the *nap* cytoplasm is common [21]. The tissue specificity of both restorers (their expression is enhanced in anthers) should lead to a reduction in either pollen production or pollen viability.

Altering which restorers are present on a *cam* cytoplasm would enable our conclusions about the magnitude of the costs to be tested. We predict the following hierarchy for pollen production and/or viability based on the hypothesis that a restorer that interferes with transcription of essential genes (*Rfn*) will carry a higher cost than one with no known pleiotropic effects (*Rfp*): *rf/rf* > *Rfp/Rfp* > *Rfn/Rfn*.

Zea mays

Zea mays contains two male-fertile cytotypes as well as at least three different CMS cytotypes [24,25]. The most studied are CMS-T (sporophytic) and CMS-S (gametophytic). In CMS-S, only pollen grains carrying the restorer allele are viable.

Male sterility in CMS-T is caused by the expression of a chimeric gene, *T-urf13*. Restoration of fertility to CMS-T requires the combined action of the dominant alleles of two nuclear restorer loci, *rf1* and *rf2* (i.e. epistatic restoration) [24]. *Rf1* is rare among maize lines, whereas *Rf2* is present in almost every maize line, even though most of them have never been exposed to the T-cytoplasm.

Box 2. Restorer effects on mitochondrial gene expression

Restorers prevent the accumulation of CMS-associated proteins through several different processes. Figure Ia represents the open reading frame (ORF) associated with CMS and Figure Ib represents other genes in the mitochondrial genome not associated with CMS. There are two steps involved in producing a protein from a gene, the first of which is transcription, which results in the production of strands of mRNA. In crops, there are a total of five known molecular actions of restorers on mRNA that prevent the CMS-associated protein from being made, four known from CMS-associated ORFs (Figure Ia; each crop and specific restorer are listed in the parentheses following each action) and three from non-CMS-associated genes in the mitochondria (Figure Ib):

(i) cleavage, when the restorer cuts the mRNA in one or more places;
(ii) alteration of size, when the restorer reduces the length of the mRNA;
(iii) destabilization, when the restorer causes the mRNA to degrade prior to translation;
(iv) suppression, when the restorer 'suppresses' mRNA translation (the exact action is not described in Figure I); and
(v) RNA editing, when the restorer edits the base pairs within the mRNA (shown In Figure Ib as C → U).

Other restorers act after the second step in protein production, translation of the mRNA into a protein. Specifically, the *Rfo* restorer of radish acts post-translationally, degrading the protein encoded by the CMS-associated ORF. In addition to these examples (see also Table 2, main text), the *Fr* restorer of the common bean causes permanent restoration by eliminating the portion of the mitochondrial genome that contains the CMS-associated ORF (shown in Figure Ia).

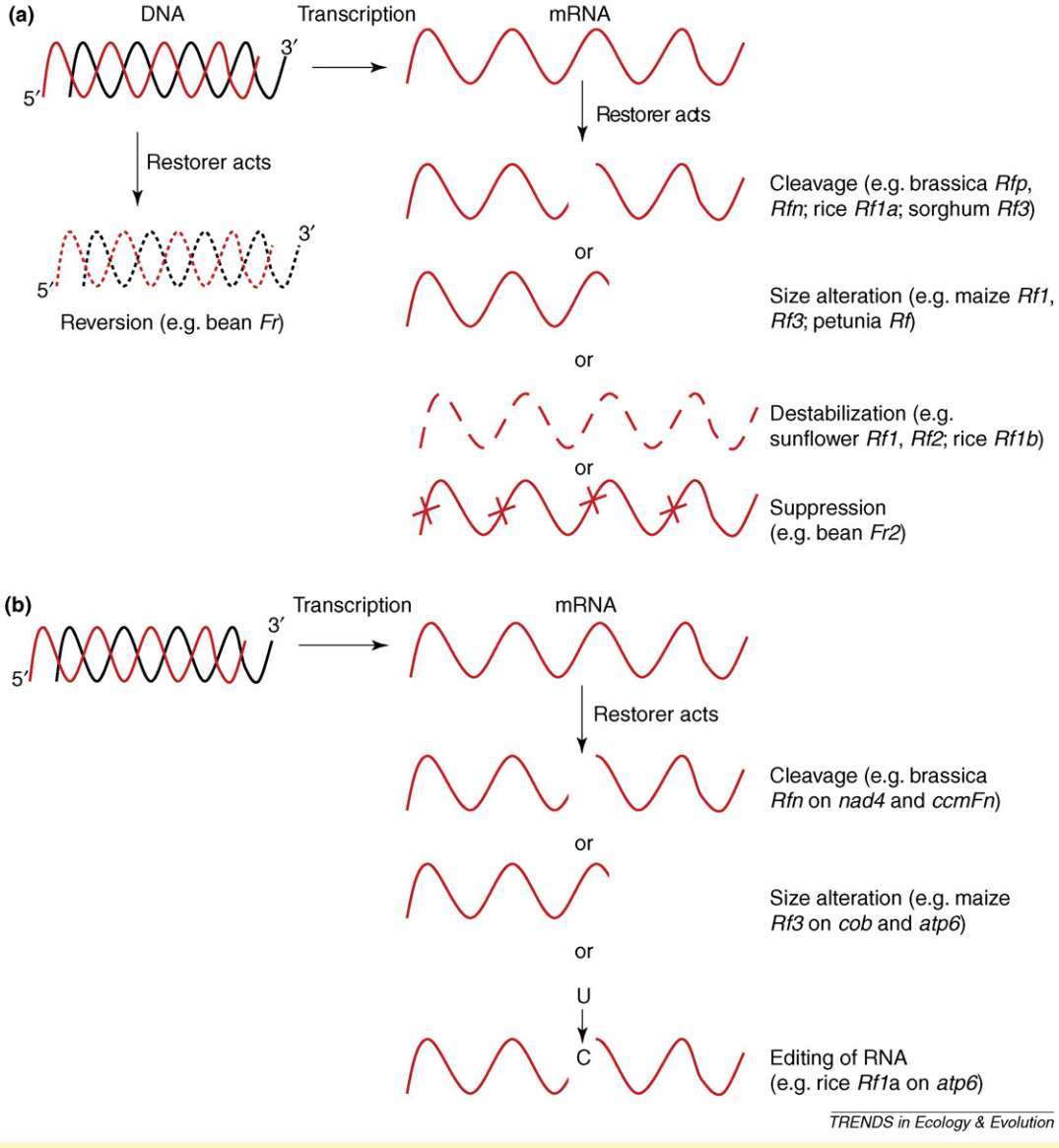

Figure I.

The accumulation of the CMS-T associated protein, URF13, which is toxic, is reduced by ~80% in plants carrying *Rf1*, presumably as a consequence of the ability of this allele to alter the accumulation and nature of *T-urf13* transcripts [26,27]. The action of *Rf1* is believed to occur throughout the plant as its effect on URF13 accumulation occurs in all tissues currently analyzed [26]. *Rf8* and *Rf** are additional partial restorer loci, which,

Table 2. Restorer alleles of CMS-associated genes of crops, their predicted mode of cost, and whether the cost would lead to a reduction in either seed and/or pollen quantity or quality

Species	CMS gene (cytotype)	Restorer	Restorer dominance	Molecular action of restorer	Predicted mode of cost[c]	Predicted effects based on tissue expression[d]	Refs
Brassica *Brassica napus*	*orf224* (*pol*)	*Rfp*[a]	Dominant	Mediates cleavage of *orf224*/*atp6* transcripts	Silent	Pollen	[20–23]
	orf222 (*nap*)	*Rfn*[a]	Dominant	Mediates cleavage of *orf222*, *nad4* and *ccmFn* transcripts	Constitutive	Pollen	
Maize *Zea mays*	*urf13* (CMS-T)	*Rf1*	Dominant	Alters accumulation and size of *urf13* transcripts	Silent	Seed and pollen	[24–35]
	orf355/orf77 (CMS-S)	*Rf3*	Dominant	Alters accumulation and size of *orf355*/*orf77*, *cob* and *atp6* transcripts	Constitutive	Seed and pollen	
Bean *Phaseolus vulgaris*	*orf239* (*pvs*)	Fr_2	Dominant	Suppresses *orf239* transcripts	Silent	Pollen	[36–46]
Sunflower *Helianthus annuus*	*orf522* (PET1)	*Rf1*[b]	Dominant	Destabilizes *atpA*/*orf522* transcripts via polyadenylation	Silent	Pollen	[53–56]
		Rf2[b]	Dominant	Destabilizes *atpA*/*orf522* transcripts via polyadenylation	Silent	Pollen	
Petunia *Petunia* x *hybrida*	*pcf*	*Rf*	Dominant	Alters accumulation and size of *pcf*/*nad3*/*rps12* transcripts	Silent	Seed and pollen	[57–61]
Radish *Raphanus sativus*	*orf138*	*Rfo*	Dominant	Destabilizes the ORF138 protein	Silent	Pollen	[51,62,63]
Rice *Oryza sativa*	*orf79* (CMS-BT)	*Rf-1a*[b]	Dominant	Mediates cleavage *of B-atp6*/*orf79* transcripts and RNA editing of *atp6* transcripts	Constitutive	Seed and pollen	[47,52,64–66]
		Rf-1b[b]	Dominant	Destabilizes *B-atp6*/*orf79* transcripts	Silent	Seed and pollen	
Sorghum *Sorghum bicolor*	*orf107* (IS1112C)	*Rf3*[b]	Dominant	Mediates cleavage of *orf107* transcripts possibly via RNA editing	Insufficient data	Seed and pollen	[67–70]
		Rf4[b]	Dominant	Unknown	Insufficient data	Insufficient data	

[a]Restorers are allelic (i.e. occur at the same locus).
[b]Restorers are epistatic.
[c]The mode of cost is considered constitutive when the restorer alters expression of necessary mitochondrial genes that are not co-transcribed with the CMS gene and is considered silent when the restorer has no known pleiotropic effects other than producing an unnecessary protein when silent in sex expression.
[d]The effects of cost are assumed to be limited to pollen grain production or viability when the restorer is only expressed in anther tissue and to act on both seeds and pollen when expression of the restorer is not limited to anther tissue.

although not allelic to *Rf1*, can partially supplement it by acting on *T-urf13* transcript accumulation [28]. As the effect of *Rf1* is specific to the CMS-related transcripts, we assume that the cost would be expressed in plants that are not CMS-T (silent cost). Seed and pollen production might both be affected given the expression of *Rf1* in multiple tissue types.

In contrast to the other restorers of CMS-T, *Rf2* does not affect the novel polypeptide accumulation. *rf2* (now called *rf2a*) encodes an aldehyde dehydrogenase (ALDH), which could reduce the amount of toxic aldehyde present as a result of residual *URF13* expression [29–31]. Nevertheless, this allele is also necessary for male fertility in plants with normal cytoplasm [30]. Hence, plants homozygous for the non-restorer allele, a loss-of-function mutant, exhibit an intermediate phenotype containing both male-fertile and male-sterile florets on the same inflorescence [30]. The question remains as to whether *rf2a* is a genuine restorer locus or a locus involved in regulating mitochondrial gene expression and, hence, is necessary regardless of the cytoplasm. If *rf2a* were a restorer locus, selection on the restorer allele should be driven by the presence of CMS-T. Under this hypothesis, the encoded mitochondrial ALDH would exhibit specific features not found in other species; data on this gene in more plant species are needed to evaluate whether this is the case. However, given that the allele has a selective advantage regardless of the cytoplasmic background with which it is interacting, it is not surprising that it has spread in maize. We do not include it in Table 2 because we cannot characterize it as a restorer in the same sense as others that are listed.

For CMS-S, several independent restorer loci have been found in both maize and its ancestor teosinte, highlighting the fact that both the CMS gene and its restorers evolved prior to domestication of the species. Restorer alleles appear to be frequent in plants with fertile and S cytoplasms [32]. The most studied and frequent restorer allele is *Rf3*, which affects the transcript pattern of the novel chimeric CMS gene. In addition, *Rf3* has a pleiotropic effect on the processing of *cob* and *atp6* transcripts, two essential mitochondrial genes, regardless of the cytoplasm. This effect is dominant and is observed in both gametophytic and sporophytic tissues [33]. We predict that the cost should be constitutive and should potentially affect seed and pollen production. The question remains as to whether the high frequency of *Rf3* is the signature of a low cost or the recruitment of a gene that controls mitochondrial functioning. Nevertheless, the gametophytic effect of restoration (only pollen containing *Rf3* are viable) has probably favored its spread in the species.

In addition to the naturally occurring restorer alleles in the CMS-S system, another class of alleles has been described that occurs spontaneously in CMS-S plants. They are gametophytic and recessive in their restoration

effect, but are homozygous-lethal to developing seeds (reviewed in [34]). Pollen containing these alleles is viable, because maturing pollen depends more on fermentation pathways than on respiration, unlike maturing seeds. One of these loci has been shown to disrupt the accumulation of one of the subunits of the ATPase complex, an essential component of the mitochondrial respiratory chain [35]. This marginal example would not lead to gynodioecy in natural populations, but would cause reversion to hermaphroditism, in which all individuals were heterozygous for the recessive, gametophytic, lethal allele. It is therefore moot to discuss the cost of these restorers in an analogous way to that of other naturally occurring restorers.

Phaseolus vulgaris

CMS in *P. vulgaris* is caused by the mitochondrial sequence *pvs*, which occurs on a subgenomic molecule (containing only a portion of the mitochondrial genome) that appears to be maintained by autonomous replication [36]. It contains two ORFs, at least one (*orf*239) of which encodes a protein involved in male sterility [37,38]. Accumulation of ORF239 occurs only in reproductive tissues, because of its rapid degradation in other tissues [39,40].

Although *pvs-orf*239 occurs in all studied accessions of *Phaseolus*, suggesting that it arose prior to the divergence of common bean, it only induces male sterility when present at high copy number [41]. Thus, the stoichiometry (concentration) of the subgenomic molecule containing *pvs* affects male sterility. This subgenomic molecule can be spontaneously lost, resulting in male fertility [36]. Male fertility also can be restored when the molecule is eliminated or reduced to substoichiometric levels by a nuclear 'reversion' allele, *Fr* [36,42,43]. This reversion results in permanent, nonsegregating male fertility; *Fr* is therefore fundamentally different from the other restorers that we are characterizing and we do not list it in Table 2.

A second, and more typical nuclear restorer, Fr_2, is dominant [44] and works by suppressing the expression of the *pvs-orf*239 sequence post-transcriptionally: the *pvs-orf*239 region is transcribed, but ORF239 is not present in anthers of Fr_2-restored lines [39,45]. Two additional restorers, $Fr_{PI207228}$ and Fr_{XR235}, are 'genetically indistinguishable' to Fr_2 and are therefore either allelic or tightly linked [46]. Although all three restorers occur in lines of common bean of Mesoamerican origin, little else is known about them, making it difficult to characterize their costs fully. Based on current knowledge, the cost is predicted to be silent, presumably small, and likely to affect only pollen production or pollen viability.

Merging data with theory

Our review of the literature on molecular action of restorers shows that they usually act by silencing CMS-associated messenger RNAs (mRNAs) [47]. Moreover, in some cases, restorers have been shown to alter the expression of other mitochondrial genes that are unassociated with the CMS gene. We have predicted what the costs of restorers might be based on such distinctions, as well as on the tissue(s) in which they are expressed (Table 2).

Dominance of cost

Dominance of cost is an important feature of models dealing with the maintenance of nuclear-cytoplasmic gynodioecy (Figure 1). Unfortunately, not enough is known about most of the crop restorers to characterize their costs fully in this respect. The nucleus is known to control mitochondrial gene expression, and genes involved in this control are called 'housekeeping' genes. Restorers are thought to be recruited from these genes [48,49]. If such a restorer locus were heterozygous, it could restore pollen production and perform housekeeping duties simultaneously [5,15]. By contrast, if the locus were homozygous, it would restore pollen production but lack the ability to perform its housekeeping duties, thus exhibiting a recessive cost. Comparisons of the fitness of homozygously restored individuals with those that are heterozygous would be useful to determine whether costs are recessive. A suitable candidate would be *Rf3* in maize, which is dominant, expressed everywhere, and is pleiotropic in its action [33]. If *Rf3* is an allele of a gene that is also a housekeeping gene, we predict that the cost will be recessive.

Mode of cost

In models, silent and constitutive costs can both maintain nuclear-cytoplasmic gynodioecy (Table 1). Most of the crop species reviewed here appear to have nuclear restorers that are likely to only express their cost when silent (i.e. in combination with a cytoplasm that they do not restore; Table 2). Moreover, this cost might sometimes be small, such as when the gene is expressed only in one or a few tissues and in which the only cost would be the production of a protein that had nothing to act on because it targets specific transcripts that are not present. A good example of this is *Rfp* in *Brassica*, which does nothing other than cleave the transcript produced by *pol* and only in anthers. Interestingly, *Brassica* also contains an example in which the cost is likely to be constitutive, *Rfn*. Other examples are *Rf3* in maize and *Rf1a* in rice (Box 2). In all three cases, the restorers alter transcripts of essential mitochondrial genes even when in combination with male-fertile cytoplasm or CMS genes that they do not restore.

We have characterized restorers as fitting two of the cost categories (silent and constitutive) from models, but what about expressed costs? In this case, both models and data agree that this scenario is probably less prevalent. None of the crop species reviewed had a restorer that appeared to fit this category. Moreover, models of expressed cost have only allowed for stable nuclear-cytoplasmic gynodioecy under restricted conditions, either allelic restoration [50] or overdominant selection [15]. However, more work is needed before expressed costs can be considered irrelevant. For example, if cleavage of polycistronic transcripts alters protein accumulation of necessary enzymes, then this could lead to an expressed cost.

Seed and/or pollen cost

Models that include a pollen cost predict higher frequencies of females and dynamic equilibria for relatively low costs compared with seed-cost models

(Table 1). In a conclusive test for a cost of restoration in a natural gynodioecious species, *Lobelia siphilitica,* a pollen-viability cost (but not a seed cost) of restoration was found [4]. One of the more striking conclusions from our crop review is that the cost of restoration was predicted to affect either only pollen or both seeds and pollen (Table 2). The dependence of pollen production on high mitochondrial functioning [51] makes the connection between the restoration of pollen production by restorers and the expression of cost of restoration on pollen production seem mechanistically consistent.

Conclusions

Just like the CMS genes that they restore, the various nuclear restorer alleles reviewed here differ markedly from each other, with the exception that all are likely to lower pollen production and/or viability as a component of their cost relative to wild-type alleles. Some nuclear restorers appear to occur at novel loci, whereas others are likely to have been recruited from a locus that was already involved in the regulation of mitochondrial gene expression. Furthermore, they differ, even within species, in their molecular action, with some targeting only the mRNA transcripts of the CMS genes that they counteract and others also affecting transcripts of essential mitochondrial genes. These differences suggest that silent and constitutive costs are both likely for nuclear restorers.

Given the complexity of the genetic mechanisms involved in nuclear-cytoplasmic gynodioecy, we suggest that the CMS systems found in crop species and their wild relatives are convenient systems for testing the predictions of current theoretical models, and, in particular, for estimating the cost of restorer loci in model systems. Indeed, crop species provide unique material with which to assess the effect of a restorer allele in a fixed genetic background, something unavailable in most wild species.

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