# Tuning a ménage à trois: Co-evolution and co-adaptation of nuclear and organellar genomes in plants

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**Plastids and mitochondria arose through endosymbiotic acquisition of formerly free-living bacteria. During more than a billion years of subsequent concerted evolution, the three genomes of plant cells have undergone dramatic structural changes to optimize the expression of the compartmentalized genetic material and to fine-tune the communication between the nucleus and the organelles. The chimeric composition of many multiprotein complexes in plastids and mitochondria (one part of the subunits being nuclear encoded and another one being encoded in the organellar genome) provides a paradigm for co-evolution at the cellular level. In this paper, we discuss the co-evolution of nuclear and organellar genomes in the context of environmental adaptation in species and populations. We highlight emerging genetic model systems and new experimental approaches that are particularly suitable to elucidate the molecular basis of co-adaptation processes and describe how nuclear-cytoplasmic co-evolution can cause genetic incompatibilities that contribute to the establishment of hybridization barriers, ultimately leading to the formation of new species.**

**Keywords:**
chloroplast capture; co-evolution; cytoplasmic incompatibility; cytoplasmic male sterility; hybridization barrier; plastome-genome incompatibility; speciation

## Introduction

By combining the capabilities of two entirely different organisms, endosymbioses can result in evolutionary quantum leaps. The plant cell is the result of two such quantum leaps: the creation of the mitochondrion from an endosymbiotically acquired α-proteobacterium and, somewhat later, the creation of the chloroplast (plastid) from an engulfed cyanobacterium. While the former established the eukaryotic lineage, the latter event, which occurred more than a billion years ago, equipped the cell with the unique capability to harness solar power by performing photosynthesis. The endosymbiotic uptake of the two formerly free-living bacterial species was followed by massive rearrangements in both the genome of the host cell (the nuclear genome) and the genomes of the endosymbionts (the mitochondrial genome or chondriome, and the plastid genome or plastome). The most dramatic process contributing to these rearrangements involved the large-scale physical translocation of genetic information from the organellar (mitochondrial and plastid) genomes into the nucleus. Thousands of prokaryotic genes disappeared from the organellar genomes and were integrated into the nuclear genome [1]. These intracellular (endosymbiotic) gene transfer processes have recently become amenable to mechanistic investigation [2–4]. Using experimental evolution approaches, the translocation of organellar DNA to the nuclear genome can be observed in real time and the fate of the transferred gene in the nucleus can be followed on its way to either functionalization or evolutionary deterioration [5–7]. As expected from the sheer scale of the contribution of the genomes of the endosymbionts

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**Abbreviations:**
**CI,** cytoplasmic incompatibility; **CMS,** cytoplasmic male sterility; **DMI,** Dobzhansky-Muller incompatibilitiy; **ORF,** open reading frame; **PGI,** plastome-genome incompatibility; **PPR,** pentatricopeptide repeat; ***Rf***, restorer of fertility.



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to the nuclear genome of the host cell, a large portion of the nuclear genome of present-day plant cells is devoted to controlling the two DNA-containing organelles. This is mainly done by re-routing many of the proteins encoded by the transferred genes to the plastid and/or mitochondrial compartment. In addition to many biosynthetic enzymes and structural components of the gene expression machinery, the photosynthetic apparatus and the respiratory chain, these imported proteins also include most of the regulatory factors that determine the expression levels of organellar genes in response to ever-changing environmental conditions. In addition to this nuclear control of organellar function (also referred to as anterograde signaling), signaling pathways emanating from the organelles and influencing the expression of the nuclear genome have evolved (so-called retrograde signaling pathways; [8]). The enormous complexity of anterograde and retrograde signaling pathways suggests an extraordinarily tight co-ordination of nuclear and organellar functions.

To distinguish between the set of genes in the nuclear genome and the genes in the organellar genomes, the terms genotype (referring to the genetic information in the nucleus) and plasmotype (referring to the sum of the information contained in the plastid and mitochondrial genomes) can be used. Genotype and plasmotype evolve by different mechanisms and at different pace. While nuclear genes and their alleles are subject to continuous reshuffling by sexual recombination, organellar genes are largely excluded from recombination. This functional asexuality of organelles is due to their usually uniparental (typically maternal) mode of inheritance, and, in the case of the plastids, is additionally secured by the lack of organelle fusion even in those cases, where plastids are regularly transmitted biparentally [9–11]. Asexual reproduction is expected to result in gradual genome deterioration due to the accumulation of deleterious mutations, a process known as Muller's ratchet. However, strangely, mutation rates in the plastid and plant mitochondrial genomes are significantly lower than in the nuclear genome [12]. How plastids and mitochondria escape Muller's ratchet and stay fit without sex is not yet fully clear, but the high copy numbers of the plastome and the chondriome and the ability of organellar genomes to undergo copy correction by gene conversion [13, 14] may contribute to keeping mutation rates low and, in this way, slowing down the ratchet.

Although the nuclear and organellar genomes are physically separated in different compartments and show different modes and speeds of evolution, the tight functional and regulatory interactions between the genotype and the plasmotype offer limited leeway for independent evolution. For example, nearly all proteins encoded in organellar genomes are subunits of large multiprotein complexes, such as the 70S ribosome, the photosystems in the thylakoid membrane of the chloroplast, or the complexes of the respiratory chain in the inner mitochondrial membrane. All of these protein complexes are of dual genetic origin in that one part of their subunits is encoded in the organellar genome and the other part in the nuclear genome. Thus, organellar genes and nuclear genes for organellar proteins co-evolve and it is conceivable that, in many cases, appearance of a mutation in an organellar gene requires compensatory mutation(s) in nuclear gene(s) and vice versa. Consequently, if adaptation to a new environment calls for genetic changes in a process that is partially encoded in an organellar genome (e.g. the requirement to optimize the efficiency of photosynthetic electron transport under stressful conditions), this adaptation is likely to occur genetically as co-adaptation of genotype and plasmotype.

The tight genetic interactions between the nucleus and the organelles and the co-evolution of genotype and plasmotype provide ample opportunities for genetic incompatibilities under the Dobzhansky-Muller model, as illustrated in Fig. 1. Although initially developed to explain genetic incompatibilities between nuclear alleles of closely related species, the model also applies to incompatibilities between genotype and plasmotype ([15]; Fig. 1). In its simplest form, the Dobzhansky-Muller model posits that changes in two genetic loci are required to cause a fitness decline in the progeny of individuals from two allopatric populations that originally stem from a common ancestral population (Fig. 1). In the most extreme case, the fitness decline entails the inability to reproduce (hybrid sterility) or even the death of the offspring (hybrid lethality; [16, 17]). Incompatibilities of the Dobzhansky-Muller type (also called Dobzhansky-Muller incompatibilities, DMI) are considered a major driving force in incipient speciation, where two populations (or subspecies) interbreed only rarely or with limited success [18].

Here, we discuss the contributions of the two DNA-containing organelles of plant cells to plant adaptation and to the establishment of genetic barriers between species and populations. We describe how new combinations of genotypes and plasmotypes arise in nature and can be created experimentally, and how their study can further our understanding of co-evolution and co-adaptation processes and the selective forces that drive them.

## Organelles can be exchanged between species and ecotypes by sexual or asexual mechanisms

The selective value of the plasmotype and the effects of natural selection on a pool of available plasmotypes are best studied by comparatively testing different organellar genotypes in a constant nuclear background. Introduction of a new plasmotype into a given nuclear genotype can occur naturally, but also can be achieved experimentally using in vitro techniques or substitution crosses.

## Organelle capture occurs naturally and can be induced by grafting experiments

The natural acquisition of a new organellar genotype is also referred to as organelle capture [19–21]. Organelle capture is often suggested as a possible reason for strong incongruences between phylogenetic trees constructed with nuclear marker sequences and those derived from plastid or mitochondrial sequences. If the position of species on the chloroplast or mitochondrial tree is much closer than their position on the nuclear tree, organelle capture provides a plausible

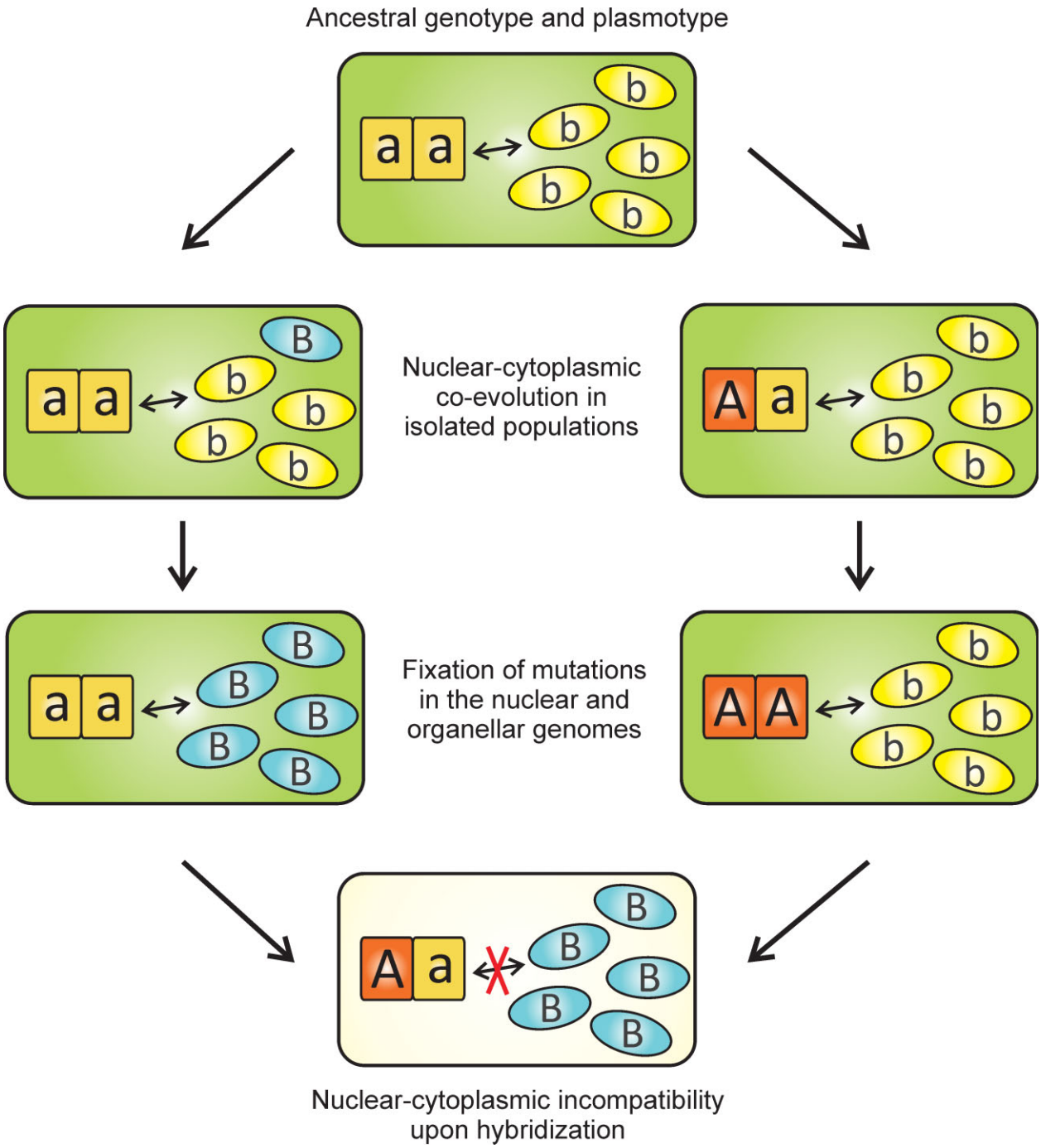


**Figure 1.** The Dobzhansky-Muller model of hybrid incompatibility adapted for nuclear-cytoplasmic interactions. In an ancestral population, the nuclear (aa) and cytoplasmic genomes (b) are co-adapted and interact functionally (compatible interaction; arrow). A population split results in reproductive isolation, which can be followed by the accumulation of mutations and the fixation of new cytoplasmic (B) and nuclear genotypes (AA) in the two subpopulations. Subsequent breakdown of the population borders leads to de novo combination of the independently evolved nuclear genotypes and plasmotypes, which now can result in incompatibility due to negative nuclear-cytoplasmic epistasis, since allele A has not co-evolved with cytoplasm B. The model shown here reflects the dominant case (F1 incompatibility), in which the newly evolved nuclear allele is dominant (in that already the heterozygous nucleus Aa is incompatible with the B cytoplasm). If the newly evolved nuclear allele is recessive, incompatibility can be induced in later generations by hybrid breakdown (recessive incompatibility). Modified after [15].

explanation. The mechanism(s) of organelle capture are not clear. Most proposed models invoke differential lineage sorting of ancestral polymorphisms in the plasmotype and/or introgression of the new cytoplasm by interspecific hybrid formation (e.g. through accidental pollination) followed by recurrent back-crossing with the pollen-donating species [20, 22]. While introgression-based models certainly can explain some cases of organelle capture, sexual transfer mechanisms are

unlikely to account for all captures. This is because molecular traces of the introgression event (i.e. DNA sequences from the species that donated the new cytoplasm) are often not found in the nuclear genome of the recipient [21]. The recent finding that mitochondrial DNA can be subject to horizontal gene transfer [23–25] and the discovery that chloroplast genomes can be transferred horizontally between grafted plants [26–28] have revealed asexual mechanisms that potentially could be involved in organelle capture. Especially the transfer of entire chloroplast genomes between different species across graft junctions precisely mimics the outcome of chloroplast capture events. It is important to note that grafting is not just a technique used by humans, but also occurs naturally (especially, but not exclusively, among woody plants; [29]). Interestingly, many of the reported chloroplast capture events involve woody species (e.g. [19, 20]). It, therefore, seems possible that at least some organelle capture events do not involve sexual processes and, instead, may be the result of horizontal gene transfer [27]. This then would be the most complete conceivable form of horizontal gene transfer: the transfer of an entire genome [29].

A most remarkable feature of chloroplast capture is that plastid genotypes (haplotypes) are often much better correlated to geographic locations than to taxonomic relationships [19]. It, therefore, seems reasonable to assume that the captured cytoplasm provides a selective advantage under specific environmental conditions [22]. In the case of chloroplast capture, this advantage could conceivably lie in superior photosynthetic performance, because most genes encoded in the plastid genome are directly or indirectly related to photosynthesis [30]. Divergence in photosynthetic traits is common within species, indicating that these traits are under selection in natural populations [31]. However, the possible driving forces underlying organelle capture have not been addressed experimentally and, therefore, currently remain speculative.

## In vitro technologies facilitate the experimental transfer of organelles between species

Combinations of nuclear genomes with new plasmotype can also be produced via cell biological manipulations in vitro. In plants, interspecific hybrids can be readily generated by fusion of protoplasts from two different species [32]. Fusion of untreated protoplasts results in so-called symmetric hybrids, which combine both the nuclear genomes and the cytoplasmic genomes of the two species. Partial elimination of the nuclear genome of one of the fusion partners (e.g. by X-ray irradiation or $\gamma$-ray irradiation of the protoplasts) produces so-called asymmetric hybrids, while complete elimination of the nuclear genome of one of the fusion partners results in so-called cybrids (or cytoplasmic hybrids). Following segregation of the two plasmotypes during cell division and plant regeneration, cybrid plants can harbor new combinations of nuclear and cytoplasmic genomes (i.e. the nuclear genome from the non-irradiated species and the plastid and/or mitochondrial genomes from the irradiated species). The interactions between the nucleus and the new cytoplasm can be either compatible (in that no visible phenotype is observed) or lead to various degrees of nuclear-cytoplasmic incompatibility also simply referred to as cytoplasmic incompatibility or CI ([33, 34] and see below).

In summary, natural (vertical or horizontal) organelle transfer as well as experimental exchange of plasmotypes generate new combinations of nuclear genomes and organellar genomes and, in this way, provide a rich platform for future investigations into the mechanisms underlying co-evolution and co-adaptation acting on the three genomes present in plant cells.

## Organellar genotypes vary in natural populations and this variation can cause nuclear-cytoplasmic incompatibilities

While natural genetic variation and its impact on fitness in different environments has been extensively studied in the nuclear genomes of plants (reviewed, e.g., in [35]), relatively little attention has been paid to natural variation in plant plasmotypes. However, already at the beginning of the last century, several geneticists recognized that functional variation of plasmotypes between and within natural populations exists (e.g. [36, 37]). This early work also triggered extensive speculations about a possible role of organellar genome variation in selection (e.g. [38]). Most of these initial studies focused on compatibility/incompatibility relations between the nucleus and the organelles and were performed in two classical models of plant genetics: the genus *Oenothera* (evening primroses; Fig. 2) and the genus *Epilobium* (willowherbs). Incompatibilities were revealed by so-called substitution crosses, in which the cytoplasm from one species or ecotype was combined with the nucleus of a different species or ecotype. In *Epilobium*, substitution crosses were shown to frequently result in various degrees of cytoplasmic male sterility (CMS), when strains of different geographic origin were introgressed. In addition, cases of growth abnormalities, often modifiable by abiotic factors, or seedling lethality were identified. For example, *Epilobium* strains collected in the area around Jena, Germany, were found to have similar (though not identical) cytoplasms. Substitution of cytoplasms between these strains led to phenotypic alterations, such as mild morphological changes, mottled leaves, or sterility. In contrast, much more pronounced incompatibilities, including severe vegetative disturbances, were seen when these cytoplasms were introduced into strains from other parts of Germany, northern Europe, or Asia [37, 38].

## Uncovering nuclear-cytoplasmic incompatibilities requires multiple methods

Detection of CI is challenging, and hence, the overall presence of CI may be underestimated. Since organelles are inherited maternally in most vascular plant species [9–11], the occurrence of reciprocal differences in hybrid (in)compatibility in the F1 generation may be indicative of the involvement of a cytoplasmic genetic component. However, the absence of reciprocal differences does not rule out the involvement of the cytoplasm in a hybrid incompatibility per se, because the plasmotypes of both parents could be incompatible with the

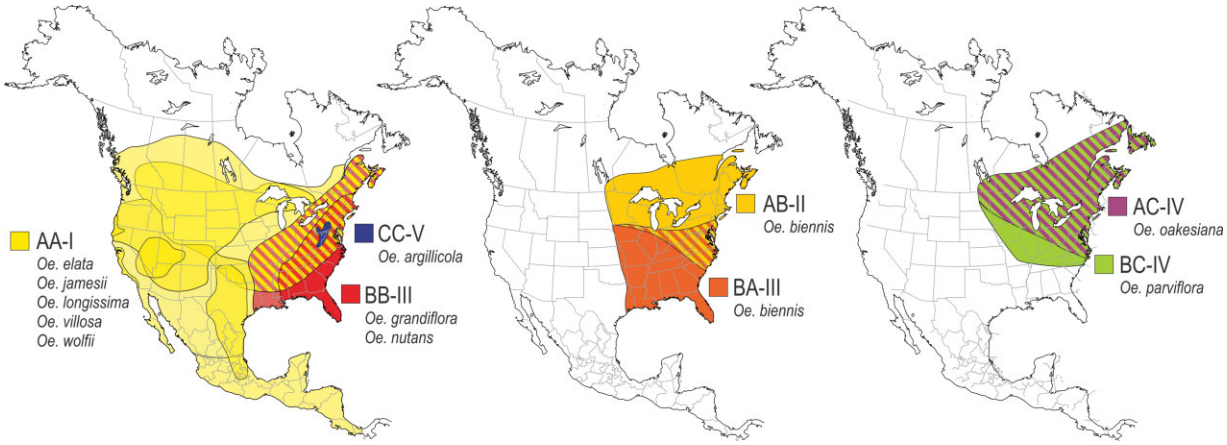


**Figure 2.** Geographic distribution, genotypes and plastome types of the eleven native North America *Oenothera* species (in the subsection *Oenothera*). The maps summarize data from [46] and [45] and exclude naturalized populations. The homozygous species are shown in the left map, the heterozygous species in the other two maps. Yellow and red gradients indicate occurrence of distinct species or subspecies. Note that the distributions of all genotypes overlap. Plastome-genome combinations are heterogeneous within the species *Oe. biennis* (middle map). *Oe. biennis* is a diploid structural heterozygous species and displays sex linkage of its haploid nuclear genomes A and B. Due to the genetic phenomenon of permanent translocation heterozygosity, the haploid genomes are inherited as entire units. AB-II corresponds to a *biennis* subgroup that habors A as maternal (egg cell-derived) and B as paternal (pollen-contributed) haploid genome, whereas the situation in BA-III is reversed.

hybrid nuclear background (cf. [39]). Furthermore, even if a reciprocal difference is observed, other maternal effects, such as genomic imprinting [40], must be ruled out to ascertain a causal role of disturbed nuclear-cytoplasmic interactions (see below). To exclude these alternative explanations, typically reciprocal F2 hybrid populations (having similar nuclear backgrounds, but different cytoplasms) and/or backcross populations are analyzed (e.g. [41, 42]). CI can come in different forms of genetic manifestation. It can be expressed already in the hybrid nuclear background of the F1 generation (Fig. 1), it can be recessive (manifesting itself in the F2 or in higher generations, also referred to as hybrid breakdown), or can be additive (i.e. a dominant F1 incompatibility is enforced in higher generations by hybrid breakdown; for details see [15, 43]).

A further challenge lies in the separation of chloroplast and mitochondrial effects. Since in most plants, the transmission of both organellar genomes is restricted to the egg cell (maternal inheritance), sexual crosses do not allow the clear distinction between plastid and mitochondrial causes of CI. Consequently, the only well-characterized examples of plastid-mediated hybrid incompatibility (plastome-genome incompatibility (PGI)) in crossable species come from plants exhibiting biparental plastid transmission ([15]; Figs. 3 and 4). Here, if one of the plastid types involved is compatible and the other one is incompatible, so-called hybrid variegation is observed (Fig. 4F). The occurrence of distinct variegation patterns (periclinal and/or sectorial chimeras) that are typical of somatic chloroplast segregation in leaves (“sorting-out”), allows a relatively clear assignment of the incompatibility to the chloroplast genome [11, 15, 44]. Theoretically, hybrid variegation could also be conferred by regular biparental transmission of the mitochondria. However, no such case has been reported to date.

## Plastome-genome incompatibilities occur frequently in natural populations

PGI is widespread in diverse genera, both among species colonizing the same habitat and between populations of the same species (for references, see [15]). The genetics of compatible and incompatible interactions between the nucleus and the plastid was worked out in detail for the evening primrose system. In the genus *Oenothera* (and especially in its best-studied North American subsection *Oenothera*), three basic nuclear genomes (A, B, and C) were identified, which can occur in the homozygous state (AA, BB, CC) or in a stable heterozygous state (AB, BC, AC; Figs. 2 and 3). The nuclear genotypes can be associated with five genetically distinguishable plastome types (numbered I to V). Systematic studies of the North American *Oenothera* populations revealed that an important aspect of species identity in *Oenothera* is the combination of a particular nuclear genome with a particular plastome type. The various species are distributed over the whole North American continent and colonize defined areas, at the borders of which hybrid zones are present (Fig. 2). Incompatible genome-plastome combinations in interspecific hybrids (which readily form in evening primroses and are usually fertile) have been proposed to contribute to maintaining the hybrid zones and preventing breakdown of species barriers [15, 45, 46].

From the altogether 30 possible genome-plastome combinations in *Oenothera*, only 12 are compatible (green circles in Fig. 3). The remaining ones display varying degrees of PGI and are not normally found in nature [47, 48]. Phenotypically, PGI often manifests itself as defects in leaf pigmentation. These can be relatively mild and appear only periodically (so-called *virescent* phenotypes), or can be more severe and give rise to yellow-green (*lutescent*), yellow or even completely white leaves (Figs. 3 and 4). Moreover, the development of floral organs or embryos and the fertility of pollen or egg cells (CMS

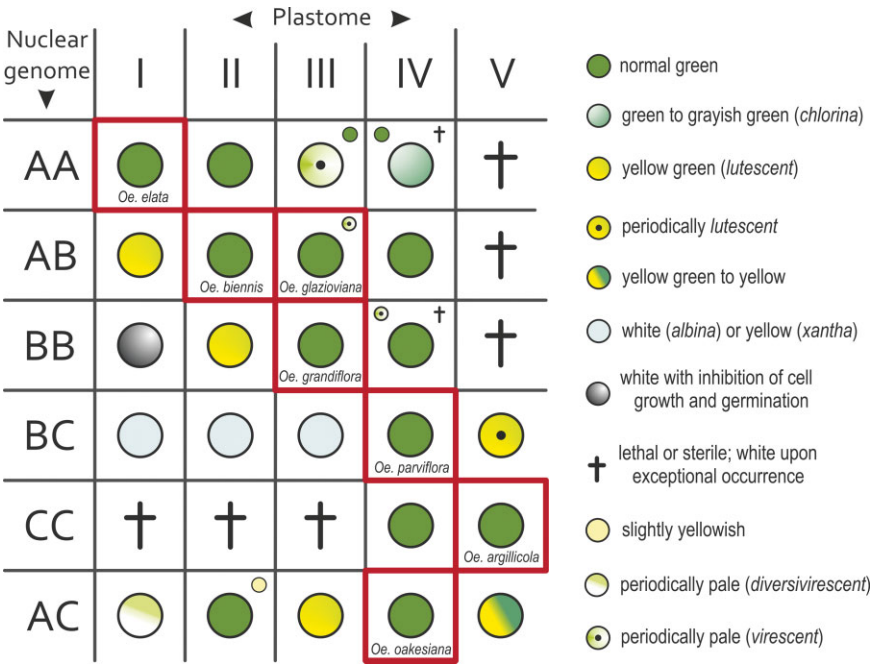


**Figure 3.** Plastome genome compatibility and incompatibility relations in the evening primrose subsection *Oenothera* (redrawn and modified from [47, 48]). The three basic nuclear genotypes A, B, and C can occur in the homozygous state or in stable heterozygous states. Existence of five genetically distinguishable plastome types (I–V) can potentially result in 30 different combinations of nuclear genotypes and plastome types. Combinations boxed in red occur naturally in defined species (Fig. 2). Representative species are given in the respective squares. Most combinations of a given nuclear genotype with an alien plastome type show PGI to varying degrees. Small symbols indicate phenotypic variation noted for some subgenotypes.

or cytoplasmatic female sterility) can be affected by PGI [15]. Phenotypically similar PGI phenotypes can occur in very different combinations of nuclear and plastid genomes (e.g. combinations AB-I, BB-II, and AC-III in *Oenothera* all show a *lutescent* phenotype; Fig. 3). Interestingly, all PGI phenotypes described for evening primroses also occur in other taxa (for references, see [15]). For example, white and *virescent* PGI phenotypes are known from *Pelargonium* hybrids (Fig. 4B). Unfortunately, the molecular determinants of PGI are still largely unknown and, at present, it remains unclear whether macroscopically similar phenotypes are caused by similar disturbances in plastid function.

## The molecular causes of PGI can be manifold

Molecular data on PGI are sparse. Sequence comparison of the five *Oenothera* plastome types (Fig. 3) revealed only relatively minor differences in coding regions of the plastome [49]. This is in line with the fact that most plastome-encoded genes encode components of the translational apparatus and the photosynthetic machinery, both of which have evolved billions of years ago, and are highly conserved throughout the plant kingdom. With the possible exception of *rbcL* (the gene for the large subunit of Rubisco, the key enzyme in photosynthetic carbon fixation; Box 1), their major structural components are not expected to be under strong positive selection in microevolution. In contrast, the mechanisms involved in fine-tuning of photosynthesis upon environmental adaption are probably under strong positive selection. Hence, the molecular causes of PGI may predominantly lie in regulatory pathways. Nucleus-encoded proteins that regulate photosynthesis [50], or factors controlling plastid transcription, mRNA maturation, translation or protein turnover [15, 51] may be good candidates. Consistent with this idea, the lack of the ability to perform mRNA editing at a specific site was implicated in an experimentally generated incompatibility between the *Nicotiana tabacum* (cigarette tobacco) plastome and the *Atropa belladonna* (deadly nightshade) nuclear genome. While cybrids harboring the *Nicotiana* genome and the *Atropa* plastome are green and grow autotrophically, the reciprocal cybrids combining the *Atropa* genome with the *Nicotiana* plastome are white, photosynthetically incompetent and grow only on sucrose-containing medium. Molecular analysis of this incompatibility revealed that the pale phenotype is due to the loss of C-to-U RNA editing at a single site in the plastid *atpA* mRNA (encoding the ATPase α-subunit; [52]) suggesting that the *Atropa* nuclear genome does not encode the site-specific trans-acting factor required for the recognition of this particular plastid RNA editing site. This case of PGI represents a particularly intriguing example of the co-evolution between the plastid and the nuclear genome, in that the *Atropa atpA* gene does not contain this particular editing site and, therefore, its nuclear genome also lacks the gene for the corresponding specificity factor, presumably a member of the pentatricopeptide repeat (PPR) protein family [51, 53].

## Cytoplasmic male sterility is often due to chondriome-genome incompatibility

CMS is the by far best-investigated example of chondriome-genome incompatibility. Although CMS can also be caused by PGI [15], in most cases, it results from a disturbed interaction between the nuclear and the mitochondrial genomes [54]. Examples for CMS have abundantly been documented in natural populations of gynodioecious plants (characterized by coexistence of male-sterile individuals with hermaphroditic individuals). The underlying CMS cytoplasms usually display a wide geographic distribution and are found in multiple

## Box 1

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) – a special enzyme in many respects. Rubisco is the most abundant protein on Earth. It can comprise up to more than 50% of the total soluble protein in green leaves. Rubisco consists of two subunits, the nucleus-encoded small subunit RbcS and the plastid-encoded catalytic large subunit RbcL. In seed plants, Rubisco is a hexadecameric protein complex comprised of eight large and eight small subunits. Rubisco is the key enzyme in the photosynthetic dark reactions (Calvin-Benson cycle) and catalyzes $CO_2$ fixation. It mediates transfer of $CO_2$ to the acceptor molecule ribulose-1,5-bisphosphate resulting in two molecules 3-phosphoglycerate (which are subsequently used for carbohydrate synthesis). $CO_2$ fixation by Rubisco is rather inefficient and, in a competing reaction, the enzyme also catalyzes transfer of $O_2$ to ribulose-1,5-bisphosphate. This side reaction (referred to as photorespiration) produces only one molecule of 3-phosphoglycerate and, in addition, one molecule of 2-phosphoglycolate. The latter cannot be utilized in the Calvin-Benson cycle and its production incurs a net reduction of the carbon fixation rate. Rubisco is regulated by a dedicated (nucleus-encoded) enzyme called Rubisco activase [85–87, 89, 108, 109].

From an evolutionary perspective, the inefficiency of Rubisco and its dual function as carboxylase and oxygenase are extremely puzzling. Recent studies suggest that the poor substrate specificity of Rubisco is due to a trade-off between carboxylation velocity and $CO_2$ affinity [108]. The Rubisco enzymes of different species are probably optimized for different (intracellular) $CO_2/O_2$ concentrations and thermal environments [109]. These factors, in turn, are linked to water availability, because gas exchange is unavoidably accompanied by transpirational water loss (in that both are controlled by stomatal conductance; [86]). Because of its variability, the plastid *rbcL* gene is widely used for phylogenetic reconstructions, although the enzymatic properties of Rubisco do not only correlate with phylogenetic relatedness, but also significantly with environmental factors [85, 89]. It is therefore unsurprising that the plastid gene for the catalytic subunit of Rubisco is under positive selection in a wide range of terrestrial plant species. Interestingly, only 20 codons are responsible for more than 70% of all cases of positive selection observed in *rbcL*. Some of the affected amino acid residues are located close to the active site. Others are quite close to each other in the three-dimensional structure of the enzyme and are involved in subunit interactions or in interaction with Rubisco activase. Mutant analyses have shown that the interactions between the subunits contribute to thermal stability, catalytic efficiency and $CO_2/O_2$ specificity of the enzyme [89].

populations. However, in the otherwise hermaphroditic genus *Mimulus* (monkey flower), a locally restricted CMS locus was described, possibly suggesting a role in adaptation [55].

Due to its great importance in hybrid seed production, CMS has been intensely studied at the molecular level and several CMS loci have been identified, predominantly from crop species. CMS can manifest itself very differently and defects can range from complete failure to develop male floral organs to arrest of pollen development at different stages [54]. CMS alleles typically arise from rearrangements in the mitochondrial DNA involving genes encoding subunits of the ATP synthase (complex V) or, less frequently, the NADH dehydrogenase (complex I). Often sequences of unknown (possibly extramitochondrial) origin are additionally involved in the chondriome rearrangements. The rearrangements frequently produce new chimeric open reading frames (ORFs), whose expression can lead to the production of short toxic polypeptides. However, there are also a few examples known, where CMS is induced by loss-of-function mutations in genes or operons for components of the mitochondrial respiratory chain [18, 54]. The corresponding restorer of fertility (*Rf*) loci mostly encode members of the large PPR protein family, which act as regulators of mitochondrial (and plastid) gene expression [51]. An interesting exception is an *Rf* locus in *Zea mays* (maize) that was identified as a putative aldehyde dehydrogenase possibly involved in detoxification of byproducts of (mitochondrial?) metabolism [56].

The above examples illustrate that the mechanisms leading to CI in plastids and mitochondria can be very different. Plastid DNA rearrangements that could lead to new expressed chimeric ORFs have not been observed. On the other hand, clear cases of mitochondrial CI that, similar to PGI, could be readily explained by disturbed interactions of nuclear factors with functional mitochondrial gene products have not yet been identified in plants (but are known from animal and fungal systems [43, 57–59]). Thus, it seems possible that CMS and *Rf* loci in plants do not represent typical cases of nuclear-mitochondrial co-evolution and functional adaptation, but rather control sex determination and optimize reproduction (see below).

## The plasmotype contributes to environmental adaptation

In general, plastid and mitochondrial genes and genomes are under strong purifying selection (negative selection). This is evident also from the conserved gene content of both genomes [30, 60]. However, in recent years, indications for positive selection have been obtained for an increasing number of organellar genes and genomes [61–66]. Positive selection is usually evidenced by a high ratio of non-synonymous to synonymous substitutions (also referred to as Ka/Ks ratio or dN/dS) and/or elevated amino acid substitution frequencies. Amino acid substitution rates in different genes and lineages can be quite variable and even in the highly conservative genome of seed plant plastids, some genes or genomic regions seem to evolve at higher speeds than the rest of the genome [49, 67, 68].

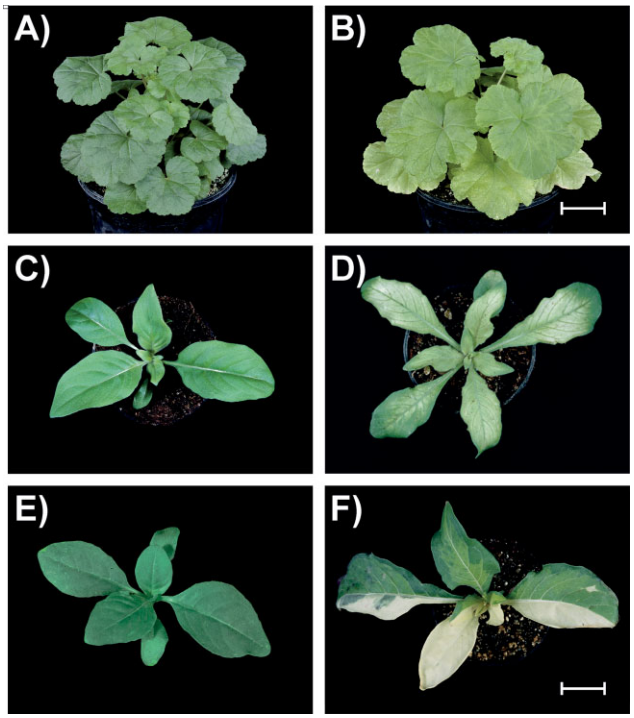


**Figure 4.** PGI in *Pelargonium* and *Oenothera*, two genera displaying biparental chloroplast inheritance. **A:** Hybrid from a cross between *Pelargonium zonale* hort. var. Trautlieb and *Pelargonium zonale* var. Roseum harboring the plastids from Roseum. This plastome is compatible with the hybrid nuclear background. **B:** Hybrid from the same cross as in (A) but harboring the plastids from Trautlieb [110]. This plastome is mildly incompatible with the hybrid nuclear background, as evidenced by the light green color of the leaves (*virescent* phenotype). **C:** *Oenothera elata* carrying the genome-plastome combination AA-I (cf. Figs. 2 and 3). **D:** *Oe. elata* combined with plastome III from *Oe. glazioviana*. The hybrid with the genome-plastome constitution AA-III is incompatible (showing the *virescent* type of PGI; Fig. 3). **E:** *Oe. grandiflora* carrying the genome-plastome combination BB-III, in which genome and plastome are fully compatible (Figs. 2 and 3). **F:** *Oe. grandiflora* harboring the incompatible plastome type I from *Oe. elata*. Note that here the incompatibility is complete in that the white tissue is photosynthetically incompetent. Plants with a homogeneous population of plastids with plastome I therefore cannot grow autotrophically. The individual shown here is heteroplasmic and contains plastome III in its green sectors and plastome I in its white sectors. The plant survives in soil, because the green sectors and cell layers nourish the white tissue (BB-III/I hybrid). Scale bars: 5 cm (for panels A and B), and 2 cm (for panels C-F), respectively.

## The plasmotype influences multiple traits

The plasmotype can confer phenotypic variation, e.g. in leaf shape, plant architecture or flower morphology, but it can also contribute to fitness differences by influencing seed yield, germination efficiency, and plant vigor [15, 38, 39, 42, 69]. The systematic molecular analysis of the geographic distribution of plasmotypes in relation to phenotypic or physiological traits has begun only very recently [70]. The few published studies suggest that plasmotype distribution in natural populations may be the result of selection. The so far most comprehensive study was performed in the model plant *Arabidopsis thaliana* [42]. 65 plastid haplotypes and 11 groups of mitochondrial haploptyes were identified in an analysis of 95 *Arabidopsis* accessions that had been collected from widely different geographic locations. In germination assays of 27 pairs of reciprocal F2 families, a significant effect of the genotype of the cytoplasm on the efficiency of seed germination could be demonstrated [42]. In the genus *Schiedea* (Caryophyllaceae), fixation of plastome markers during island radiation was observed, whereas significant gene flow was detected for nuclear alleles [71]. Unfortunately, while sequence divergence in plastid and mitochondrial genomes has been extensively used for the construction of phylogenetic trees, the exploitation of plant organelles in phylogeography is still in its infancy [72]. Also, the functional significance (i.e. fitness effects) of the allelic diversity underlying the distribution patterns of different plasmotypes and the specific environmental conditions correlating with them remain elusive.

## Evidence for an adaptive value of the plasmotype is accumulating

Cytoplasmic contributions to plant adaptation in natural environments have been suggested mainly based on ecological studies that reported genotype-environment interactions of local cytoplasms. In these studies, plant survival, germination rate or seed production were shown to vary between plasmotypes upon reciprocal transplanting of individuals from different habitats [73]. In addition, traits for ecological differentiation, such as flower and seed size or flowering time were found to be influenced. However, in many of these studies (analyzing intraspecific or interspecific hybrids), maternal effects other than a contribution of the plasmotype (e.g. maternal predetermination or imprinting [40]) could not be ruled out with certainty [41, 70, 73–79]. In some cases, a potential adaptive value of the cytoplasm, such as altered drought stress tolerance, water use efficiency or photosynthetic performance, was observable [41, 80, 81], suggesting possible driving forces for ecological adaptation of cytoplasms.

For example, plasmotype-dependent drought tolerance was detected in the genus *Helianthus* (sunflower, [41]), and the recent isolation of a drought and temperature-tolerant sunflower plastome mutant [82] provided further evidence for the plastid genome influencing this trait. This is in line with photosynthesis and respiration being tightly connected to water balance as well as with the involvement of the chloroplast and mitochondrial compartments in the signaling cascades regulating drought tolerance [83, 84]. Moreover, Rubisco, the key enzyme in photosynthetic carbon fixation, is known to be affected by drought stress and shows signs of evolutionary adaptation to water availability [85–87]. Its large subunit encoded by the plastid *rbcL* gene was repeatedly associated with positive selection ([15, 65, 70, 88, 89]; Box 1).

Evidence for a role of the cytoplasm in adaptation to water availability and in habitat differentiation has also been obtained in *Potamogeton*. The natural hybrid species *Potamogeton anguillanus* (*P. malaianus* x *P. perfoliatus*,

Potamogetonaceae) is an aquatic plant with facultative terrestrial organs. Hybrids with *P. malaianus* as the mother (M-hybrids) form more terrestrial shoots and exhibit higher tolerance to drought stress than the hybrids with *P. perfoliatus* as the mother (P-hybrids). This difference, coinciding with the maternally inherited plastid genome, is likely of adaptive value, because M-hybrids dominate in shallow waters and inshore areas, but are nearly absent from deepwater and offshore areas (where the P-hybrids prevail; [90]). Again, the *rbcL* gene seems to be under positive selection [88] indicating that, in addition to the morphological adaption, also the efficiency of carbon fixation could contribute to the observed reciprocal differences in drought tolerance between the hybrids. Although the involvement of a maternal effect other than the genotype of the cytoplasm still needs to be ruled out, *Potamogeton* provides an intriguing example of ecological differentiation (and, perhaps, incipient speciation) that is conferred by the crossing direction in interspecific hybrid formation.

In general, differences in photosynthetic performance between cytoplasms [31] can account for fitness differences by affecting plant vigor and/or reproductive success (seed yield). Besides drought stress, other selection pressures acting on the cytoplasm are the susceptibility to diseases and pests [38, 39, 72, 91] and cold tolerance. In *Cucumis sativus* (cucumber), chilling tolerance co-segregates maternally with the plastome type. Here, plastid and mitochondrial effects can be cleanly separated, because the mitochondria are paternally inherited in cucumber [92]. Sequence comparison of plastomes from chilling-sensitive and chilling-tolerant varieties uncovered three polymorphic sites correlating with the difference in cold stress tolerance: a single basepair deletion in an intergenic region and two SNPs in coding regions (in the *atpB* gene encoding a subunit of the chloroplast ATP synthase, and in *ycf1*, an ORF of unknown function). However, the functional relevance of these sites in determining chilling tolerance still needs to be examined [93].

It is important to note that the potential of plastid and mitochondrial genomes to contribute to plant adaption is influenced by both the gene content of the nuclear genome and that of the organelle genomes [70]. For example, cold tolerance was associated with the plastid genome in *Cucumis sativa* and in *Brassica napus* cybrids [92, 94], but the plastid genome does not appear to play a role in chilling sensitivity of *Solanum lycopesicum* (tomato) or *Oenothera* species [95]. It therefore seems reasonable to assume that the architecture of the genetic network determining cold tolerance differs between taxa, which in turn may result in different degrees of involvement of the plasmotype.

## Co-evolution and co-adaptation potentially contribute to speciation

Environment-dependent fitness differences conferred by the plasmotype can be strong enough to preserve a hybrid zone (by the hybrid offspring being less fit than the members of the two parent species). Genotype-environment interactions can build hybridization barriers probably even before pre-zygotic or post-zygotic isolation mechanisms (e.g. classic DMI alleles conferring hybrid sterility or inviability) arise [77, 96, 97]. Hence, the cytoplasm can confer species separation via plasmotype-environment interactions [77] and/or via CIs of the Dobzhansky-Muller type ([98]; Fig. 1).

Interestingly, in *Helianthus* and *Chamaecrista fasciculata* (Fabaceae), two taxa in which locally adapted cytoplasms were described [41, 73], also CMS occurs [39, 99, 100]. Since the maternally transmitted organelles benefit from a female advantage in male sterile plants (e.g. due to lower investment in pollen production), this trait can become fixed in populations. At the same time, counter-selection for nuclear *Rf* loci sets in [101, 102]. However, since CMS alleles can readily spread in a population, their possible contribution to reproductive isolation remains somewhat unclear. Male fertile hybrid offspring will either not carry the CMS cytoplasm or harbor both the CMS cytoplasm and the nuclear *Rf* allele [18, 103]. The possible contribution of CMS to reproductive isolation, therefore, should depend on the fitness costs of the *Rf* allele. These costs, in turn, may depend on the environment [103]. It was also proposed that expression of CMS determinants in vegetative tissues could trigger cellular stress responses, providing a potential selective force that could act on CMS alleles in natural populations [101]. Thus, adaptive forces may have played a role in the evolution of some, but not all, CMS loci [104]. Similar arguments can be made for other organelle-mediated speciation barriers, including PGI [15].

Strong DMI loci, such as PGI and to some extent CMS loci, may or may not differ from loci involved in cytoplasmic adaptation in their physical location, their evolutionary history and the selection pressures that created them. It is important to note that neither the evolution of plasmotypes nor their co-evolution with the nuclear genomes are solely driven by adaptive selection. In addition to genome conflicts, genetic drift and other selection forces potentially can also create DMI loci [104, 105]. On the other hand, since cytoplasmic incompatibilities are usually caused by disturbed interactions between cytoplasmic and nuclear alleles that did not co-evolve, adaptive forces shaping organelle-nuclear co-evolution are likely to create loci for reproductive isolation (Fig. 1). From the genetic point of view, adaptive incompatibility loci and those evolved because of genome conflicts or other selection forces are difficult to separate. First, maternal inheritance of organelles and lack of genetic recombination create a strong linkage between all mitochondrial and all plastid genes in the majority of plant species [9–11]. Second, the adaptive value of a DMI locus may not become obvious from elucidating its molecular basis. Criteria for distinguishing between the different possibilities how a cytoplasmic DMI locus could have evolved are not easy to formulate, but a thorough physiological and molecular analysis of incompatibility loci in natural populations and environments certainly will be essential. Interesting examples for linkage of CMS with putative adaptive traits, such as cold tolerance or disease resistance, have been reported for several crop species [91, 106]. At least in some cases, these traits are conferred by different loci or even different cytoplasmic genomes, only one of which is probably adaptive. In *Brassica napus* cybrids, a particular *Raphanus sativus* (radish)

cytoplasm confers both CMS and chilling sensitivity. While the CMS locus is encoded in the chondriome, the locus for chilling sensitivity turned out to reside in the plastome [94]. In contrast, susceptibility to a fungal pathogen and CMS are encoded by the same mitochondrial locus in the Texas cytoplasm of maize [107].

## Conclusions and outlook

Local adaptation of cytoplasms may be much more common than currently believed. Although in recent years, a number of striking examples have been described [15, 41, 65, 70], most of them are the result of serendipitous discovery and thus likely represent just the tip of the iceberg. The phenomenon clearly deserves much more systematic studies, which – with the rapid advance in genomics research – become increasingly feasible. At present, even very fundamental questions remain open. For example, it is still largely unclear, whether loci for cytoplasmic adaptation and incompatibility are distinct or are largely identical or must be thought of as co-occurring along a continuum. Also, in many cases, the only genetic evidence for cytoplasmic adaptation effects that has been obtained is their non-Mendelian (usually maternal) inheritance. Often this does not allow to distinguish between plastid (plastome-encoded) and mitochondrial (chondriome-encoded) contributions to cytoplasmic adaptation. Therefore, future research should be directed toward identifying loci for both CI and local cytoplasmic adaptation, and localizing them on the genomes of the two DNA-containing cell organelles. Suitable study systems include species that are interfertile, display biparental inheritance of organelles (a useful feature also to separate plastid from mitochondrial effects) and allow the easy substitution of cytoplasms (such as evening primroses; Figs. 2–4; [15]). Likewise, the increasing number of cases of organelle capture that have arisen naturally or can be produced experimentally [27, 42] represent a treasure trove that waits to be tapped. Clearly, all this is not just a task for geneticists. It necessitates the combination of genetic and phylogeographic studies with rigorous physiological investigations to pinpoint clear-cut selective advantages associated with local cytoplasmic adaptation under defined environmental conditions. Likewise, the identification of incompatibility loci will have to be followed by thorough physiological and ecological investigations as well as population biology studies to elucidate the selective forces producing these loci and to gain a better understanding of the role that cytoplasmic genes play in species formation. The time is ripe for scholars in these various fields to join forces and develop new interdisciplinary approaches to address these challenges.

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