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REVIEW ARTICLE

# From chloroplasts to "cryptic" plastids: evolution of plastid genomes in parasitic plants

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**Abstract** To date, more than 130 plastid genomes (plastomes) have been completely sequenced. Of those, 12 are strongly reduced plastid genomes from heterotrophic plants or plant-related species that exhibit a parasitic lifestyle. Half of these species are land plants while the other half consists of unicellular species that have evolved from photosynthetic algae. Due to their specialized lifestyle, parasitic lineages experienced a loss of evolutionary pressure on the plastid genome and, in particular, on the photosynthesis-related genes. This made them tolerant for the accumulation of detrimental mutations and deletions in plastid genes. That parasitic plants are naturally occurring plastome mutants makes them a rich source of information concerning plastome evolution and the mechanisms that are involved. This review reports on the progress made in recent years with parasitic plant plastomes and attempts to summarize what we can learn from analysing the genomes of functionally reduced, or cryptic, plastids. Particularly, the loss of genes for a plastid-encoded RNA polymerase as well as an intron maturase and the retention of the gene for the large subunit of the Calvin cycle enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in selected species will be discussed.

**Keywords** Cryptic plastids · MatK intron maturase · Parasitic plants · Plastid genome evolution · Plastid polymerases · Rubisco

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**Abbreviations**

| | |
|---|---|
| bp | Basepairs |
| NEP | Nuclear-endoded RNA polymerase |
| PEP | Plastid encoded RNA polymerase |
| Rubisco | Ribulose-1,5-bisphosphate carboxylase/oxygenase |

## Introduction

Photosynthesis is one of the hallmarks of plant life and is the most fundamental biochemical process on earth. In eukaryotic cells, the acquisition of the potential to convert solar energy into chemical energy is rooted within the endosymbiotic uptake of a photoautotrophic cyanobacterium some 1.5 billion years ago. This initial endosymbiosis seems to have been the trigger to the evolution of all plastid-containing organisms that exist today and is generally referred to as primary endosymbiosis (see recent review by Gould et al. 2008). All land plants possess plastids that evolved from such a primary endosymbiosis whereas a large number of unicellular marine algae and some heterotrophic pathogens like the apicomplexans contain secondary plastids that evolved when eukaryotes in possession of a plastid became subsequent victims of endosymbiotic uptake and reduction (Gould et al. 2008).

Although the aspect of photosynthesis has certainly attracted the most attention, it is well known that plastids also fulfil other essential metabolic roles such as the synthesis of fatty acids, tetrapyrroles and aromatic substances (Neuhaus and Emes 2000). These anabolic pathways have in common that they are dependent on an import of precursors from the cytosol (Fischer and Weber 2002) and are most evident in plastids from heterotrophic plant tissues such as amyloplasts and leucoplasts or in plastids from

species that have resorted to a heterotrophic or even parasitic lifestyle.

A remnant of the original endosymbiont is the plastid genome whose expression is driven by the plastids' own gene expression apparatus. Compared to the genomes of free-living cyanobacteria this genome is characterized by vast losses of genes that have been transferred to the nucleus or have been eliminated altogether. Only about five percent of the proteins that contribute to the plastids metabolic activity are still encoded inside this organelle (Abdallah et al. 2000). Despite this enormous reduction in its coding capacity, the plastome still codes for many key components of the photosynthetic complexes. It is, therefore, an integrative and indispensible part of the plant cell.

Plastid chromosomes are present in high copy numbers per cell. This as well as their relatively small size has made them much more amenable to sequencing than the plant nuclear genomes. The first complete plastid genome sequences, namely that of the bryophyte *Marchantia polymorpha* and of *Nicotiana tabacum*, were reported as early as 1986 (Ohyama et al. 1986; Shinozaki et al. 1986). Since then, more than 130 complete plastid genomes have been deposited in public databases and are available for analysis. A comprehensive comparative study of numerous plastomes of photosynthetic land plants performed by Jansen et al. (2007) showed that genome structure, coding capacity and intron content are remarkably conserved across photosynthetic species and that a constant subset of genes has not been transferred to the nucleus but has remained on the plastid genome instead. The high degree of conservation of coding capacities in plastomes of different plant taxa has been discussed extensively and many attempts to explain the relative uniformity are focussed on the influence of photosynthesis on the constraints of plastome coding capacities (Race et al. 1999; Zerges 2002; Allen 2003). Meanwhile, a few of the more recent discussions take into account that there could be more than one 'raison d'etre' for a conserved plastid genome (Bungard 2004; Barbrook et al. 2006).

The strongest deviations of the standard gene repertoire of 113 coding sequences and 18 introns in land plants have been reported in species that have given up photosynthetic carbon fixation in favour of a parasitic or mycoheterotrophic lifestyle. Such species are in essence naturally occurring photosynthesis mutants and therefore provide a means to dissect the consequences of evolutionary pressure exerted by photosynthesis versus evolutionary forces provided by one or more of the other plastid metabolic functions. Non-photosynthetic flowering species that have lost the unique autotrophic properties of plants in favour of a parasitic lifestyle have evolved in at least 11 independent angiosperm lineages and account for approximately 1% of all angiosperm species (Barkman et al. 2007). These species have developed an extremely specialized set of morphological, biochemical and molecular traits. Morphological changes can include, for instance, the loss of leaves and the development of characteristic feeding organs, the haustoria, with which the parasites can extract water and inorganic as well as organic nutrients from the parasitized host. Parasites can attach to the roots of their host plants (root parasites) or live epiphytically on branches or stems of their hosts (shoot parasites) where they can inflict serious damage and even represent a problem for agriculture. Typical shoot parasites are members of the dodder family with over 150 species in the only genus Cuscuta (Fig. 1a–c). A family of root parasites, on the other hand, is the Orobanchaceae or broomrapes by common name (Fig. 1d, e).

Whereas species like the mistletoes, which remove only water and inorganic nutrients from their hosts but generate their own organic carbon compounds through photosynthesis, are classified as hemiparasites, the above mentioned dodders and broomrapes are holoparasites that obtain both inorganic and organic nutrients from their hosts and have often lost the ability to perform photosynthetic carbon fixation. The border between these groups is, however, not clear-cut and allows for a large range of intermediate forms. A number of such intermediate forms are found in the genus Cuscuta, where some species are still able to perform photosynthesis, albeit not beyond the compensation point.

As the holoparasites adapted to their parasitic lifestyle, a relaxation of the stringent demands normally associated with photosynthesis, led to significant changes in the chloroplast genome, and ultimately resulted in reduced organelles that are referred to as "cryptic". The first known cryptic plastid genome of a non-photosynthetic plant was that of the Orobanchaceae *Epifagus virginiana* (Beechdrops) (Wolfe et al. 1992). With a size of only 70 kbp, the plastid genome of *E. virginiana* is the smallest plastid genome of any land plant known. Its coding capacity is reduced to 42 genes of which all but four genes are involved in the plastids' gene expression machinery (Wolfe et al. 1992).

For a long time, this plastid genome alongside that of the non-photosynthetic unicellular alga *Euglena longa* (also previously referred to as *Astasia longa,* Gockel and Hachtel 2000) and those of the parasitic apicomplexan species *Toxoplasma gondii* and *Plasmodium falciparum* were the only ones that shaped our knowledge on the coding capacity and regulatory mechanisms of reduced plastid genomes (see Table 1). Lack of ready access to sufficient quantities of fresh plant tissues for plastid or plastome isolation as well as a lack of methods tailored specifically to isolate these organelles or their DNA from non-photosynthetic holoparasites are among the chief reasons for the slow progress in this promising field of plant genomics.

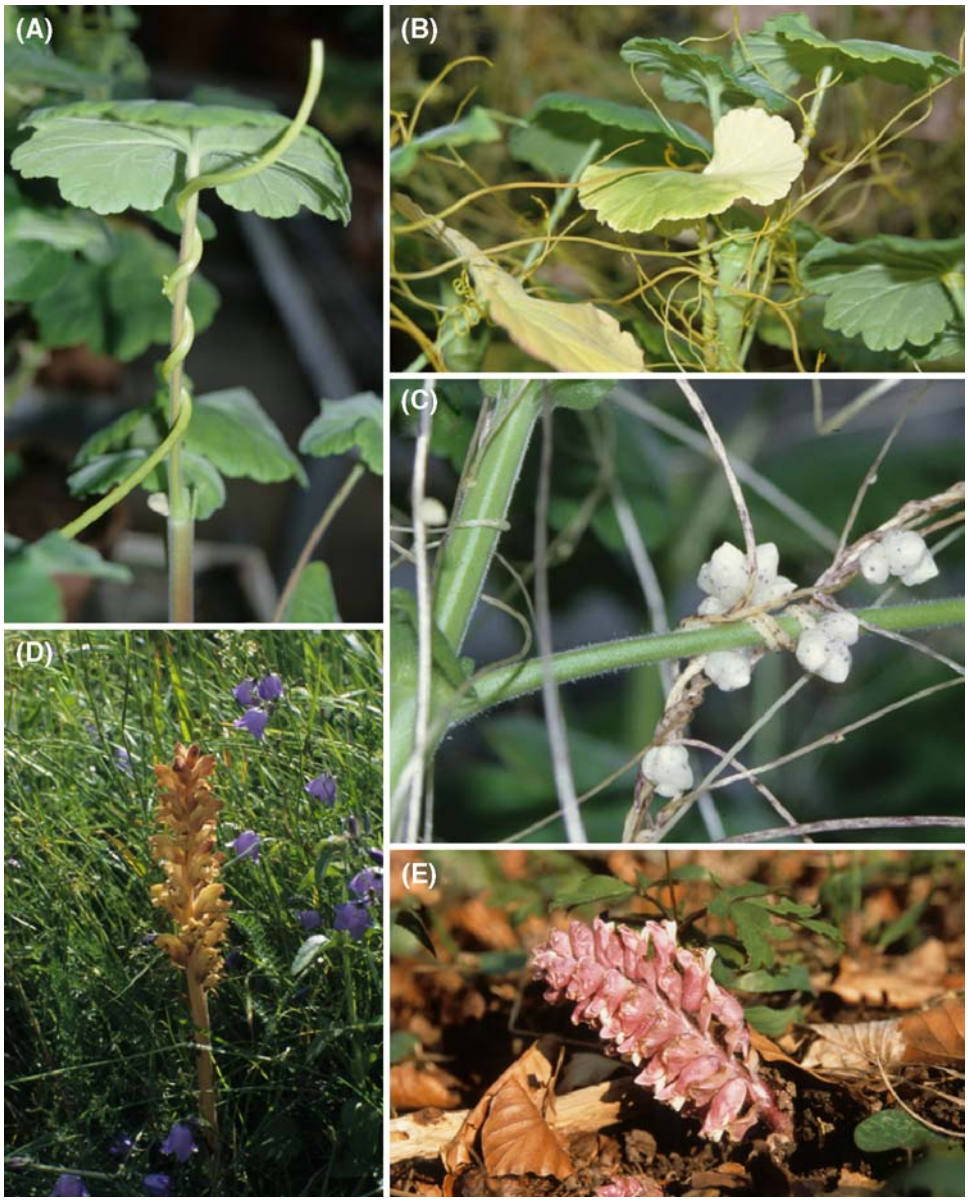


**Fig. 1** Habitus of parasitic land plants. *Cuscuta reflexa* (**a**), *Cuscuta campestris* (**b**) and *Cuscuta odorata* (**c**) on Pelargonium host plants. These three Cuscuta species represent the large spectrum of pigment composition and photosynthetic capacity that exists in this genus. *Orobanche spec* (**d**) and *Lathrea squamaria* (**e**) are achlorophyllous representatives of the group of root parasites

Recent publication of the complete plastome sequences of four species of the genus Cuscuta (Funk et al. 2007, McNeal et al. 2007b) and of *Aneura mirabilis*, the only parasitic liverwort known to date (Wicket et al. 2008) (see Table 1, Fig. 2), has changed this situation and has enabled us for the fist time to test and re-evaluate previous hypotheses on the mechanistics of plastid genome evolution. In part, this progress can certainly be attributed to the development of techniques tailored to facilitate the sequencing of plastid genomes (Jansen et al. 2005; McNeal et al. 2006).

The wealth of new sequence information is further enhanced by the reports on partial or complete plastome sequences of some non-photosynthetic unicellular algae (Knauf and Hachtel 2002; Tartar and Boucias 2004; Borza et al. 2005; deKoning and Keeling 2006). Finally, the recent identification of a photosynthetic alveolate that is a close relative of apicomplexan parasites and therefore links them to their algal ancestors (Moore et al. 2008) holds the promise for further insights into the ancient evolutionary events.

## Plastome reduction in parasitic angiosperms

The average photosynthetic land plant possesses a plastome of a size between 120 and 160 kbp in size. These plastomes typically contain the genes for subunits of the photosynthetic electron chain complexes, but also for many ribosomal proteins, transfer RNAs and ribosomal RNAs. Further typical genes include the *rpoA*, *rpoB*, *rpoC1* and *rpoC2* genes for the subunits of a plastid-encoded RNA polymerase (PEP) as well as the *accD* gene for the acetyl-CoA carboxylase, the *clpP* gene for a Clp protease subunit,

**Table 1** Complete plastome sequences of parasites in chronological order of annotation

| Species | Accession no. | Size (nt) | Number of genes | Number of pseudogenes | References |
|---|---|---|---|---|---|
| *Epifagus virginiana* | NC_001568 | 70,028 | 71 | 15 | Wolfe et al. (1992) |
| *Plasmodium falciparum* | ***DQ642846*** | ***34,682*** | ***66*** | **–** | ***Wilson et al.*** (1996) ***and Birren et al. (2006, direct sub-mission)*** |
| *Toxoplasma gondii* | ***NC_001799*** | ***34,996*** | ***63*** | **–** | ***Kissinger et al. (1997, direct sub-mission)*** |
| *Euglena longa* | *NC_002652* | *73,345* | *84* | *1* | *Gockel and Hachtel* (2000) |
| *Eimeria tenella* | ***NC_004823*** | ***34,750*** | ***65*** | ***7*** | ***Cai et al.*** (2003) |
| *Theileria parva* | ***NC_007758*** | ***39,579*** | ***70*** | **–** | ***Gardner et al.*** (2005) |
| *Helicosporidium sp.* | *NC_008100* | *37,454* | *54* | *0* | *deKoning and Keeling* (2006) |
| *Cuscuta gronovii* | NC_009765 | 86,744 | 98 | 5 | Funk et al. (2007) |
| *Cuscuta reflexa* | NC_009766 | 121,521 | 113 | 4 | Funk et al. (2007) |
| *Cuscuta obtusiflora* | NC_009949 | 85,286 | 98 | 1 | McNeal et al. (2007b) |
| *Cuscuta exaltata* | NC_009963 | 125,373 | 117 | 7 | McNeal et al. (2007b) |
| *Aneura mirabilis* | **NC_010359** | **108,007** | **128** | **21** | **Wicket et al.** (2008) |

Details for angiosperm species are printed in *normal*, for Apicomplexa in *bold italic*, for algae in *italic* and for the bryophyte *Aneura mirabilis* in *bold*

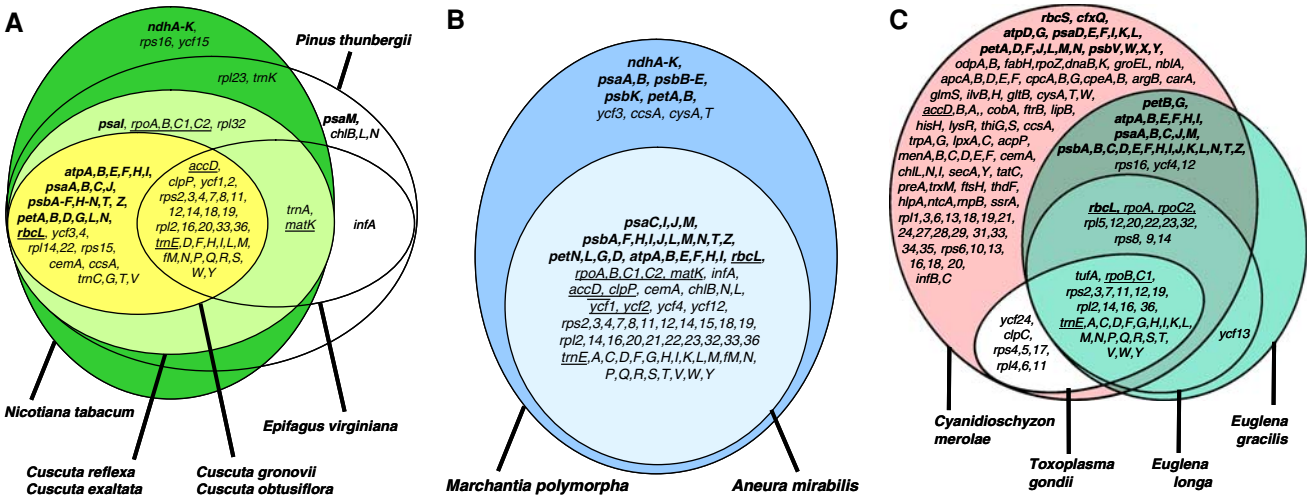


**Fig. 2** **a** Venn diagram comparing the protein and tRNA coding gene content of plastid genomes of six angiosperm and one gymnosperm species. Coding capacities were inferred from data deposited in the GenBank database. Multiple synonymous tRNAs coding for the same amino acid were not considered. All genes appearing within one circle are encoded by the plastome of the respective species as indicated at the periphery. Photosynthesis-related genes are printed in *bold*, key genes discussed in the text are *underlined*. **b** Venn diagram comparing the protein and tRNA coding gene content of two bryophyte plastid genomes. *Marchantia polymorpha* as reference genome is depicted in darker color than the parasitic *Aneura mirabilis*. Coding capacities were inferred from data deposited in the GenBank database. Photosynthesis-related genes are printed in *bold*, key genes discussed in the text are *underlined*. **c** Venn diagram comparing the protein and tRNA coding gene content of the plastomes of three unicellular algae and the apicomplexan *Toxoplasma gondii*. The photosynthetic red alga *Cyanidioschyzon merolae* and the eugleoid alga *Euglena gracilis* are used as reference genomes to the heterotrophic *E. longa* and *T. gondii*. Coding capacities were inferred from data deposited in the GenBank database. Not all of the 30 *ycf* genes identified in *C. merolae* are listed. Photosynthesis-related genes are printed in *bold*, key genes discussed in the text are *underlined*

the gene for an intron maturase, *matK*, and a number of conserved open reading frames of unknown function (*ycf* genes). Surprisingly, not only the genes but also the amount and position of intron sequences that occurr in a number of genes were conserved (Jansen et al. 2007).

In the plastome of the holoparasitic *E. virginiana* which is characterized by an extreme reduction to a size that is about half of that of average photosynthetic land plants (Wolfe et al. 1992) all genes associated with the bioenergetic processes of photosynthesis and photorespiration were lost or degraded to pseudogenes (Fig. 2a). In addition, *E. virginiana* has also lost functional genes for the plastid-encoded subunits of the PEP, several ribosomal protein genes and a substantial number of tRNA genes (Morden et al. 1991; Lohan and Wolfe 1998). In total, the *E. virginiana* plastome codes for 17 tRNAs, 21 protein coding genes

and the ribosomal RNA operon (Lohan and Wolfe 1998). Among the protein-coding genes is the *infA* gene that codes for a translation initiation factor and that has been lost, for example, in all Solanaceae (see Fig. 2a). Despite its low gene content, the plastome size of *E. virginiana* is larger than that of some other cryptic plastid genomes, for example that of parasitic algae (Table 1). This is largely due to the retention of many partially conserved albeit dysfunctional pseudogenes as well as large areas of non-coding sequence between the genes.

Unlike *E. virginiana*, different species of the shoot parasitic genus Cuscuta, which are all classified as holoparasites, exhibit a gradient of photosynthetic capacities ranging from almost fully photosynthetic green species via intermediate species with a significantly compromised photosynthetic apparatus to completely achlorophyllous, non-photosynthetic species (van der Kooij et al. 2000, 2005). Such large physiological range within closely related species is without precedence in other examined parasitic lineages and thus offers a unique opportunity to obtain information on the biochemical and evolutionary mechanisms that are involved in shaping plastid structure and function. Consequently, a number of investigations have been focussed on the plastid genomes in these lineages (Krause et al. 2003; Berg et al. 2003, 2004; Revill et al. 2005; Stefanovic and Olmstead 2005; Funk et al. 2007; McNeal et al. 2007a, b). Total plastome sequences of Cuscuta species are, to date, available for four species that possess some residual photosynthetic activity. Two species, *Cuscuta reflexa* and *Cuscuta exaltata*, possess chlorophylls and, at least in the case of *C. reflexa*, photosynthetic carbon fixation was documented (Hibberd et al. 1998; van der Kooij et al. 2000). However, the rate at which this carbon fixation occurs does not exceed the compensation point. The photosynthetic activity of *Cuscuta gronovii* (van der Kooij et al. 2000) is even lower than in *C. reflexa* and is mostly limited to the growing shoot tips. No physiological data have so far been published on *Cuscuta obtusiflora*. The plastome sequences of these four species together with that of *E. virginiana* reveal that both their physical size and their coding capacity decrease in parallel with the photosynthetic activity (Fig. 2). The frequency, with which base substitutions, insertions and deletions (indels) occur, therefore seems to correlate with the removal of the selective constraints normally exerted by photosynthesis. Within *C. obtusiflora*, accelerated non-synonymous and synonymous nucleotide substitution rates were observed in comparison to photosynthetic plants. Especially, the non-synonymous rates for ribosomal protein genes and the ATP synthase subunit genes were significantly elevated (McNeal et al. 2007b). When comparing these rates to Epifagus, however, the authors could find no clear trend of selective constraints, since some genes appear to be more conserved in Epifagus, others in Cuscuta.

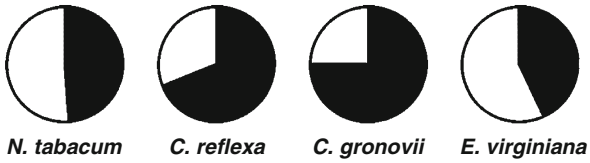


**Fig. 3** Relative ratios of coding (*black*) to non-coding (*white*) plastome sequences in one non-parasitic (tobacco) and three parasitic angiosperms. The ratios are based on the numbers published by Funk et al. (2007)

Unlike Epifagus, Cuscuta species exhibit also a strong tendency towards the reduction of non-coding intergenic regions and intron sequences (Fig. 3). Non-coding sequences account for 51% of the tobacco plastome and 57% of the *E. virginiana* plastome. In contrast, the non-coding regions amount to only 31% in *C. reflexa* and 25% in *C. gronovii*, respectively (Funk et al. 2007 and Fig. 3). Similarly, McNeal et al. (2007b) have calculated that in *C. obtusiflora* all of the 63 non-coding, intergenic regions between homologous, functional genes are decreased by 49% when compared to tobacco. *C. exaltata* on the other hand exhibits only a 16% decrease over the same area relative to tobacco. This condensation in the non-coding regions that commonly contain regulatory sequence motifs coincides with a simplification of plastid gene expression in *C. gronovii* and in other species with largely impaired photosynthetic capacity (Krause et al. 2003; Berg et al. 2004). Overall, as pointed out by McNeal et al. (2007b), the plastomes of Cuscuta species appear to be much more "streamlined" than that of *E. virginiana*.

## Switch from PEP- to NEP-based transcription of photosynthesis genes

The analysis of Cuscuta plastome sequences has uncovered two surprising findings that have implications far beyond a simple loss of a gene and deserve a closer examination: (1) the deletion of the genes for the plastid-encoded RNA polymerase (PEP) together with a switch from PEP- to NEP-based transcription of photosynthesis-related genes and the ribosomal RNA operon and (2) the concommitant loss of a plastid-encoded splicing factor and its target, the group IIa introns from plastid genes.

As none of the Cuscuta species, whose plastome sequences are known, is completely achlorophyllous they have retained a full set of photosynthesis genes (with the exception of *psaI*, which is missing in *C. gronovii* and *C. obtusiflora*) (Fig. 2). A fascinating surprise came with the discovery that *rpo*-genes coding for the PEP are absent from the plastome of both *C. gronovii* and *C. obtusiflora*. Its role appears to have been fully taken over by a nuclear-encoded polymerase (NEP) that is imported into the plastids (Krause et al. 2003; Berg et al. 2004). This loss is

intriguing in that a NEP-driven expression of photosynthesis-related genes is unknown from any photosynthetic plant. Artificial deletion of the *rpo* genes leads to a loss of photosynthetic capacity (deSantis-Maciossek et al. 1999) despite the presence of low but detectable amounts of the photosynthesis-related gene transcripts (Krause et al. 2000; Legen et al. 2002). These transcripts arise from an unspecific transcriptional activity of the NEP and are seemingly unable to function as templates for protein biosynthesis probably because they are lacking the normal 5′ and 3′ ends and could therefore be subject to incorrect processing (Legen et al. 2002). In higher plants the intertwined roles of PEP and NEP (Demarsy et al. 2006; Courtois et al. 2007) aggravate an analysis of the regulation of each of the two polymerases separately. The mutagenesis of PEP subunits from photosynthetic plants does not mimick the situation that can evolve under natural selection. Hence, the two species, *C. gronovii* and *C. obtusiflora* offer a unique possibility to study at least the transcriptional regulation in plastids that are exclusively dependent on one polymerase, namely the NEP. It remains to be seen if any insight from this special situation can be transferred to higher plants.

It is noteworthy in this context that the plastid *trnE* gene coding for the glutamyl-tRNA was reported to play a role in the regulation of the transcriptional activity of the NEP (Hanaoka et al. 2005). Despite the loss of many other transfer RNA genes, *trnE* is retained in all plastomes analyzed to date. Alltogether, *trnE* fulfills a triple role in the plastids—that as transfer RNA, a role in heme biosynthesis and a role in transcriptional regulation (reviewed in Barbrook et al. 2006). The retention of this particular tRNA gene strongly supports a key role of the phage-type nuclear-encoded RNA polymerase for plastid biogenesis in parasitic plants. Why the PEP was replaced by the NEP in these organisms is a matter that is still subject to debate. The fact that it was possible for *C. gronovii* and *C. obtusiflora* to loose the *rpo* genes before loosing photosynthesis-related genes might in this case point at a directed, active change of the regulation of plastid gene expression rather than a passive event due to lack of selective pressure due to photosynthesis. While it is possible that a sophisticated regulation by PEP was simply no longer needed in plastids with limited photosynthetic capacity, a switch to NEP-based transcription with all its concomitant adjustments seems to be more of an investment for the plant than maintaining a dispensable PEP activity.

## Parallel losses of group IIa introns and of a highly conserved intron maturase MatK

The fully sequenced plastomes of *C. gronovii* and *C. obtusiflora* provide the very first accounts of plastid genomes from higher plants where the *matK* gene, which is usually located within the intron of the transfer RNA *trnK-UUU* and putatively codes for a plastid intron maturase, is completely absent. *MatK* was found in more than 100 land plants (including angiosperms, gymnosperms, ferns, liverworts and hornworts) and in charopycean algae but not in the plastid genomes of other algae (Hausner et al. 2006) or of the apicomplexan lineage (Wilson et al. 1996, Cai et al. 2003). Empirical evidence from ribosome-deficient plastid mutants of monocotyledonous plants (Hübschmann et al. 1996; Vogel et al. 1997) suggests that the MatK maturase governs the splicing of the *trnK* intron, in which it is contained, as well as the splicing of the other group IIA introns of the plastome. Up until recently, *matK* was regarded as one of the most persistent genes of the plastome, being retained in both photosynthetic and nonphotosynthetic land plants, alike. For this reason, *matK* has also been a popular choice for plant systematic studies.

The absence of *matK* from the plastome of *C. gronovii* and *C. obtusiflora* forces us to re-evaluate the role of the MatK protein and to take a closer look at the introns that are the presumable target sequences of this maturase.

From the eight group IIA introns that are regarded as putatively dependent on MatK maturase activity, only one, namely intron 2 of the *clpP* gene was retained in *C. gronovii* (Funk et al. 2007) as well as in *C. obtusiflora* (McNeal et al. 2007b). The four group II intron-containing transfer RNA genes—including *trnK-UUU*, were lost altogether, or are dysfunctional pseudogenes. The remaining three introns that are normally contained within the *rps12*, *atpF* and *rpl2* genes were precisely lost without impairing their corresponding host genes (Funk et al. 2007). The same precise loss has also occured with both introns of the *ycf3* gene. These losses seem to be significant in the context of *matK* gene deletion, as *E. virginiana* has retained two of these introns (those of *rpl2* and *rps21*) while the two *ycf3* introns and the *atpF* intron of Epifagus were lost together with their genes (Ems et al. 1995). The plastome sequence data from *C. gronovii* and *C. obtusiflora* therefore provide a direct, phylogenetic correlation between *matK* and certain introns of the group II-type.

*MatK* was identified as a free standing gene in *Epifagus* as well as in the *Cuscuta* species belonging to the subgenus Monogyna (*C. reflexa*, Funk et al. 2007 and *C. exaltata*, McNeal et al. 2007b) and therefore differs from the *matK* genes of most other plant species where it is typically intron-contained. That the free standing *matK* genes nevertheless are functional can be deduced from the conservation of the protein's putative RNA-binding domain. This approximately 100 amino acid long motif, referred to as domain X, consists of a core consensus sequence of 45 amino acids (Hausner et al. 2006). A sequence comparison shows that this region is highly preserved in photosynthetic and non-photosynthetic plants, independent of whether

*matK* is intron-encoded or free-standing (Fig. 4). The only other known free-standing plastid *matK* gene is that of the fern *Adiantum capillus-veneris* (Wolf et al. 2003). However, in this case the domain X shows a significantly lower degree of conservation to both, angiosperms and to the fern *Psilotum nudum* (Fig. 4) despite the fact that group II introns seem to exist in the Adiantum plastome. A final conclusion on the significance of domain X conservation, the position of the gene within the genome and the functionality of the protein can therefore only be drawn when more experimental data are available.

Although the possibility that the *matK* gene could have been transferred to the nuclear genome of *C. gronovii* and *C. obtusiflora* is not excluded yet, the precise losses of several of its putative target sequences without the impairment of the rest of the gene provide strong hints that MatK activity has been relinquished in these species. However, whether the loss of *matK* was pivotal to a predisposition to loose all group IIa introns but one, or if it was the other way round, is still a matter for speculation.

## Alternative functions of Rubisco in parasitic plants?

Another riddle of many plastomes of parasitic plants is the retention of the *rbcL* gene independent of whether photosynthetic capacity is still prevailing or not. Open reading frames for *rbcL* were detected in many lineages of holoparasitic Scrophulariaceae. *Lathrea clandestina*, a parasite of alder (*Alnus glutinosa*), was reported to possess a functional and expressed *rbcL* gene (Lusson et al. 1998) despite the fact that this plant lacks chlorophyll. Similar situations have been described for other holoparasites (Thalouarn et al. 1994; Wolfe and dePamphilis 1997; Wolfe and dePamphilis 1998; Delavault and Thalouarn 2002). The presence or absence of other photosynthesis genes cannot be ruled out since complete plastome sequences for none of these species are yet available. However, the lack of any photosynthetic pigments provides strong empirical evidence that a loss of genes for the photosystems has occured there. If this assumption is correct, the selection pressure to maintain the gene for the large subunit of Rubisco must be sought outside of its function for photosynthesis (Bungard 2004). The solution to this puzzle might indeed be close at hand, since our understanding of Rubisco's role in the plastids has recently received a new angle when Schwender et al. (2004) presented evidence for an alternative function for Rubisco in lipid biosynthesis. According to their data, Rubisco is active under low light conditions in developing seeds of oilseed rape (*Brassica napus*) and is involved in a conversion of hexose to pyruvate that bypasses glycolysis. This novel Rubisco-dependent metabolic conversion improves the carbon efficiency during the formation of acetyl-CoA and lipids (Schwender et al. 2004). Lipids are present in the phloem sap only in very limited amounts and their composition is quite different from that of other plant tissue (Madey et al. 2002). It is, therefore, conceivable that parasitic land plants might have to rely on their own metabolic apparatus to provide their cells with the necessary lipids. A role of Rubisco in lipid biosynthesis is particularly intriguing as one of the genes that seem to be present in all parasite plastomes is the *accD* gene. *AccD* encodes a subunit of the acetyl-CoA carboxylase that plays a fundamental role in the biosynthesis of fatty acids. On the other hand, there are caveats in a scenario where Rubisco was retained to facilitate lipid biosynthesis from the resources available to parasitic plants. One of them is that the new pathway requires cofactors that would normally be provided by the photosystems (Schwender et al. 2004).

However, this alternative function does still not explain why the large subunit of Rubisco has not been replaced by a nucleus-encoded duplicate of the protein as it in principle can also be functionally encoded in the nucleus (Kanevski and Maliga 1994). An independent additional function for Rubisco, that would also explain the retention of the gene inside the plastids, was suggested by Yosef et al. (2004) who showed that the N-terminus of the protein possesses RNA binding activity. A model was later proposed where the RNA binding domain of the nascent Rubisco chain becomes exposed under oxidising conditions whereupon it binds to RNA in its vicinity, particularly its own transcript

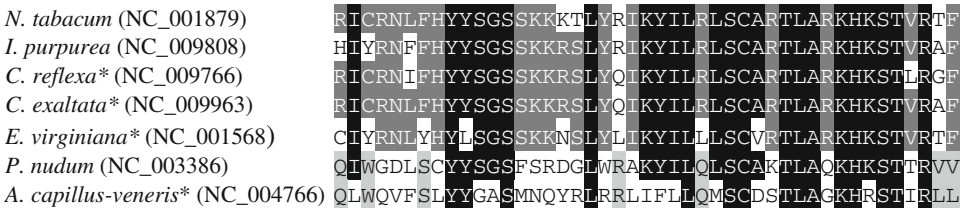


**Fig. 4** Alignment of the core consensus sequence of the domain X region of MatK from two non-parasitic angiosperms, three parasitic angiosperms and two non-parasitic ferns. *MatK* is contained within the *trnK* intron in *Nicotiana tabacum*, *Ipomoea purpurea* and the fern *Psilotum nudum* whereas it is a free-standing gene in the plastome of the fern *Adiantum capillus-veneris* and the three angiosperm parasites *E. virginiana*, *C. reflexa* and *C. exaltata*. Amino acids that are shared between angiosperm and and one or both fern lineages are shaded in *black*. Amino acids that are conserved only among the angiosperms are shaded with *dark grey* whereas aminoacids conserved between the two ferns are depicted with *dark letters* on *light grey* background. Plastomes in which matK is free-standing are marked with an *asterisk*

(Cohen et al. 2005). This would provide a negative feedback regulation of the translational activity under conditions of oxidative stress in the plastids. Whether or not this potential for RNA binding activity is utilized also in non-green tissue has not been investigated yet.

## The plastome of a parasitic liverwort *Aneura mirabilis*

*A. mirabilis* is to date the only known seedless land plant that has resorted to a completely non-photosynthetic life style. Its recently published complete plastome sequence (Wickett et al. 2008) allows for comparisons with the above discussed parasitic seed-bearing plants. At 108 kbp, its plastome size lies between that of the parasite group *C. reflexa/C. exaltata* and the more reduced group consisting of *C. gronovii/C. obtusiflora*. The plastome of the only sequenced photosynthetic relative, *M. polymorpha,* is in contrast 121 kbp (Ohyama et al. 1986). Apart from the loss of all *ndh* genes and a significant number of genes for photosystem subunits, the *A. mirabilis* plastome is relatively unaltered. The set of retained genes includes the *matK* gene and the *rpo* genes (Fig. 2b). This contrasts, for example, with the situation in *C. gronovii* where photosynthesis genes were retained but features of plastid gene expression like *matK*, *rpoA, rpoB, rpoC1* and *C2* were sacrificed (Fig. 2a). It still remains to be seen, though, if the plastid RNA polymerase encoded by the *rpo* genes of *A. mirabilis* is transcriptionally active and, if so, what genes are transcribed by it. Because this plant has a completely non-photosynthetic lifecycle, (Wickett et al. 2008), the *rpo* genes would, in theory, be dispensable. However, as long as we don't know why the *rpo* genes were lost in some parasitic angiosperms, the reason for their retention in *A. mirabilis* cannot be resolved.

With *trnE, accD, ycf1 and ycf2*, *A. mirabilis* has retained the entire set of key genes around which a justification for the retention of cryptic plastomes could be based according to Bungard (2004) and Barbrook et al. (2006). Moreover, *A. mirabilis* has also retained a seemingly functional *rbcL* gene (Fig. 2b). In the absence of any apparent photosynthetic carbohydrate production, the retention of *rbcL* provides yet another strong hint towards a separate function of the large subunit of Rubisco.

## Achlorophyllous unicellular algae

Although often less conspicuous, species of colourless unicellular algae with reduced, or cryptic, plastids have been also described among the trebouxiophytes (Knauf and Hachtel 2002; deKoning and Keeling 2004; Tartar and Boucias 2004; Borza et al. 2005), euglenoids (Siemeister and Hachtel 1990) and dinoflagellates (Sanchez-Puerta et al. 2007; Matsuzaki et al. 2008).

The plastome of *Euglena longa* (alias *Astasia longa*) exhibits one peculiarity in that it does not possess the typical tetrapartite structure known from most other plastomes. Instead, the ribosomal RNA operon is present in three successive copies (Gockel and Hachtel 2000), a distinct feature that is also found in other Euglenoids. As in *A. mirabilis* and *E. virginiana*, all photosynthesis-related genes are absent except for *rbcL* (Fig. 2c), which is functional and transcribed. On the other hand, all four *rpo* genes were identified on the plastid DNA (Gockel and Hachtel 2000; Sheveleva et al. 2002). In the case of *rpoB*, *rpoC1* and *rpoC2*, even transcripts were detected, indicating that these genes are functional and expressed (Gockel and Hachtel 2000). The *accD* and *matK* genes, in contrast, are absent from the photosynthetic *E. gracilis* as well as from the non-photosynthetic *E. longa* (Fig. 2c) whereas *accD* at least is present in the reduced plastome of the unicellular parasite *Helicosporidium spec*. (not shown).

A feature which the *E. longa* plastome shares with plastid genomes of other parasitic algae is that they have considerably less non-coding DNA compared to their free-living photosynthetic relatives (deKoning and Keeling 2006). This coincides with similar observations in the plastome sequences of some species of the flowering plant genus Cuscuta (see Fig. 3 and above text). In Helicosporidium, the structure of the plastome was streamlined to an even greater extent with no redundancy in the set of retained genes at all. As a result, deKoning and Keeling (2006) postulate the existence of common forces that shape plastid genomes even after the relaxation of photosynthetic selection pressure has led to the loss of photosynthesis in these organelles.

## Apicomplexan parasites

Surprisingly, cryptic plastids were found in a number of unicellular parasites with algal ancestors that were not previously suspected to be related to plants. The most spectacular case was the discovery of a remnant plastid genome in a cryptic plastid—the apicoplast—in apicomplexan parasites like the malaria parasite *P. falciparum* (Williamson et al. 1994; Wilson et al. 1996). The completely sequenced plastomes of two related apicomplexan parasites, *T. gondii* (Kissinger et al. direct database submission 1997) and *Eimeria tenella* (Cai et al. 2003), possess plastid genomes of just below 35 kbp in size (Table 1) and therefore are ranked among the smallest plastome sequences known to date. As with holoparasitic plants, these plastomes have a very compact tetrapartite organization (i.e., two single copy regions are separated by two inverted repeats) and are

characterized by the retention of a suite of genes involved in plastid gene expression (Fig. 2c). Genes like *accD*, *rbcL* and *matK* are absent from these parasites' plastomes (Fig. 2c). As discussed earlier, *matK* along with all group II introns is also missing in red algae (Hagopian et al. 2004) and was therefore probably not present in the ancestor of apicomplexan lineages. In contrast to land plants and other parasitic algae where plastomes are found to be closely related to the plastomes of their photosynthetic relatives, it can be noticed that apicomplexan plastid genomes are quite different from those of their presumed algal ancestors. This probably reflects the longer time period of independent evolution that these parasites have had in contrast to their plant and algae counterparts. In this context, the recent discovery of a photosynthetic alveolate that is closely related to apicomplexan parasites is of extreme importance since it provides a link to their algal ancestors (Moore et al. 2008) and will allow taking a closer look at the differences.

Matsuzaki et al. (2008) recently described a cryptic plastid in an algal group that is sister to the apicomplexans, the perkinsids. This report shows that the species with cryptic plastids identified so far probably represent just the tip of the iceberg and that much more is to be expected along these lines in the future.

## Perspectives

Unexpected findings in the analysis of cryptic plastome sequences, such as the retention of the *rbcL* gene in otherwise non-photosynthetic plastids or the loss of the *matK* and *rpo* genes from two Cuscuta species, indicate that the deletion or maintenance of specific features follow no general rules and demonstrate the complexity of the selection processes governing holoparasite evolution. The available evidence points to the fact that the regulation of gene expression from cryptic plastomes is decisively different from that of normal chloroplasts. In chloroplasts, redox control of the PEP plays a major role in ensuring fast and individual responses of each chloroplast to changing conditions. In colourless plastids of parasitic plants, where the NEP is prevailing, such regulatory mechanisms are no longer available. Whether control at the level of transcription still plays the same important role as in chloroplasts or whether the decisive regulatory steps are at posttranscriptional levels in non-green plastids will have to be investigated in the future. In view of the complex regulatory connections between plastid and nuclear genomes that have come to light in the recent past (Woodson and Chory 2008), an investigation of nuclear-encoded plastid proteins in parasitic plants is long overdue. A recently launched parasitic plant genome project funded by the US National Science Foundation with the goal to sequence the entire genetic information of three species of the Orobanchaceae family, *Triphysaria versicolor*, *Striga hermonthica* and *Orobanche ramosa*, promises at long last to shed some light into this current black hole in our knowledge on holoparasite genomics.

As to the constantly asked question why plastomes where retained in non-photosynthetic organisms after all, the answer is perhaps quite simply that despite its dominating role, photosynthesis is still only one of many plastid metabolic pathways that is restricted to only one specialized plastid type, the chloroplast. While photosynthesis certainly has a documented ability to influence the evolutionary constraints on plastid genomes, it is in my opinion not surprising that it is NOT the only shaping factor that acts on the genetic information in the plastids and ensures the retention of a functional plastome. It has been predicted that the sequencing of the genetic information from additional holoparasite species will reveal highly reduced plastomes in which *trnE* is the only gene that is always present (Barbrook et al. 2006). So far, all newly sequenced plastome sequences fulfil this prediction. Whether the decisive "molecular sculptor" in cryptic plastids is heme synthesis (*trnE* gene) or lipid biosynthesis (*accD* gene and perhaps *rbcL* gene) or rather one of the other pathways that have been discussed in the past (Bungard 2004), needs to be, and most certainly will be, revealed in the future.

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## References

Abdallah F, Salamini F, Leister D (2000) A prediction of the size and evolutionary origin of the proteome of chloroplasts of Arabidopsis. Trends Plant Sci 5:141–142

Allen JF (2003) The function of genomes in bioenergetic organelles. Philos Trans R Soc Lond B 358:19–38

Barbrook AC, Howe CJ, Purton S (2006) Why are plastid genomes retained in non-photosynthetic organisms? Trends Plant Sci 11:101–108

Barkman TJ, McNeal JR, Lim S-H, Coat G, Croom H, Young N, dePamphilis CW (2007) Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. BMC Evol Biol 7:248

Berg S, Krupinska K, Krause K (2003) Plastids of three Cuscuta species differing in plastid coding capacity have a common parasite-specific RNA composition. Planta 218:135–142

Berg S, Krause K, Krupinska K (2004) The *rbcL* genes of two Cuscuta species, *C. gronovii* and *C. subinclusa*, are transcribed by the nuclear-encoded plastid RNA polymerase (NEP). Planta 219:541–546

Borza T, Popescu CE, Lee RW (2005) Multiple metabolic roles for the non-photosynthetic plastid of the green alga *Prototheca wickerhamii*. Eukaryot Cell 4:253–261

Bungard RA (2004) Photosynthetic evolution in parasitic plants: insight from the chloroplast genome. Bioessays 26:235–247

Cai X, Fuller AL, McDougald LR, Zhu G (2003) Apicoplast genome of the coccidian *Eimeria tenella*. Gene 321:39–46

Cohen I, Knopf JA, Irihimovitch V, Shapira M (2005) A proposed mechanism for the inhibitory effects of oxidative stress on Rubisco assembly and its subunit expression. Plant Physiol 137:738–746

Courtois F, Merendino L, Demarsy E, Mache R, Lerbs-Mache S (2007) Phage-type RNA polymerase RPOTmp transcribes the rrn operon from the PC promoter at early developmental stages of Arabidopsis. Plant Physiol 145:712–721

deKoning AP, Keeling PJ (2004) Nucleus-encoded genes for plastid-targeted proteins in *Helicosporidium*: functional diversity of a cryptic plastid in a parasitic alga. Eukaryot Cell 3:1198–1205

deKoning AP, Keeling PJ (2006) The complete plastid genome sequence of the parasitic green alga *Helicosporidium* sp. is highly reduced and structured. BMC Biol 4:12

Delavault P, Thalouarn P (2002) The obligate root parasite *Orobanche cumara* exhibits several *rbcL* sequences. Gene 297:85–92

Demarsy E, Courtois F, Azevedo J, Buhot L, Lerbs-Mache S (2006) Building up of the plastid transcriptional machinery during germination and early plant development. Plant Physiol 142:993–1003

deSantis-Maciossek G, Kofer W, Bock A, Schoch S, Maier RM, Wanner G, Rüdiger W, Koop HU, Herrmann RG (1999) Targeted disruption of the plastid RNA polymerase genes *rpo*A, B and C1: molecular biology, biochemistry and ultrastructure. Plant J 18:477–489

Ems SC, Morden CW, Dixon CK, Wolfe KH, dePamphilis CW, Palmer JD (1995) Transcription, splicing and editing of plastid RNAs in the nonphotosynthetic plant *Epifagus virginiana*. Plant Mol Biol 29:721–733

Fischer K, Weber A (2002) Transport of carbon in non-green plastids. Trends Plant Sci 7:345–351

Funk HT, Berg S, Krupinska K, Maier UG, Krause K (2007) Complete DNA sequences of the plastid genomes of two parasitic flowering plant species, *Cuscuta reflexa* and *Cuscuta gronovii*. BMC Plant Biol 7:45

Gardner MJ, Bishop R, Shah T, deVilliers EP, Carlton JM, Hall N, Ren Q, Paulsen IT, Pain A, Berriman M et al (2005) Genome sequence of *Theileria parva*, a bovine pathogen that transforms lymphocytes. Science 309:134–137

Gockel G, Hachtel W (2000) Complete map of the plastid genome of the non-photosynthetic euglenoid flagellate *Astasia longa*. Protist 151:347–351

Gould SB, Waller RF, McFadden GI (2008) Plastid Evolution. Annu Rev Plant Biol 59:491–517

Hagopian JC, Reis M, Kitajima JP, Battacharya D, de Oliveira MC (2004) Comparative analysis of the complete plastid genome sequence of the red alga Gracilaria tenuistipitata var. liui provides insight into the evolution of rhodoplasts and their relationship to other plastids. J Mol Evol 59:464–477

Hanaoka M, Kanamaru K, Fujiwara M, Takahashi H, Tanaka K (2005) Glutamyl-tRNA mediates a switch in RNA polymerase use during chloroplast biogenesis. EMBO Rep 6:1–6

Hausner G, Olson R, Simon D, Johnson I, Sanders ER, Karol KG, McCourt RM, Zimmerly S (2006) Origin and evolution of the chloroplast *trnK* (*matK*) intron: a model for evolution of group II intron RNA structures. Mol Biol Evol 23:380–391

Hibberd JM, Bungard RA, Press MC, Jeschke WD, Scholes JD, Quick WP (1998) Localization of photosynthetic metabolism in the parasitic angiosperm *Cuscuta reflexa*. Planta 205:506–513

Hübschmann T, Hess WR, Börner T (1996) Impaired splicing of the *rps12* transcript in ribosome-deficient plastids. Plant Mol Biol 30:109–123

Jansen RK, Raubeson LA, Boore JL, dePamphilis CW, Chumley TW, Haberle RC, Wyman SK, Alverson AJ, Peery R, Hernam SJ, Fourcade HM, Kuehl JV, McNeal J, Leebens-Mack J, Cui L (2005) Methods for obtaining and analyzing whole chloroplast genome sequences. Methods Enzymol 395:348–384

Jansen RK, Cai Z, Raubeson LA, Daniell H, de Pamphilis CW, Leebens-Mack J, Müller KF, Guisinger-Bellian M, Haberle RC, Hansen AK, Chumley TW, Lee S-B, Peery R, McNeal J, Kuehl JV, Boore JL (2007) Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. Proc Natl Acad Sci USA 104:19369–19374

Kanevski I, Maliga P (1994) Relocation of the plastid rbcL gene to the nucleus yields functional ribulose-1, 5-bisphosphate carboxylase in tobacco chloroplasts. Proc Natl Acad Sci USA 91:1969–1973

Knauf U, Hachtel W (2002) The genes encoding subunits of ATP synthase are conserved in the reduced plastid genome of the heterotrophic alga *Prototheca wickerhamii*. Mol Genet Genomics 267:492–497

Krause K, Maier RM, Kofer W, Krupinska K, Herrmann RG (2000) Disruption of plastid-encoded RNA polymerase genes in tobacco: expression of only a distinct set of genes is not based on selective transcription of the plastid chromosome. Mol Gen Genet 263:1022–1030

Krause K, Berg S, Krupinska K (2003) Plastid transcription in the holoparasitic plant genus Cuscuta: parallel loss of the rrn16 PEP-promoter and of the rpoA and rpoB genes coding for the plastid-encoded RNA polymerase. Planta 216:815–823

Legen J, Kemp S, Krause K, Profanter B, Herrmann RG, Maier RM (2002) Comparative analysis of plastid transcription profiles of entire plastid chromosomes from tobacco attributed to wild-type and PEP-deficient transcription machineries. Plant J 31:171–188

Lohan AJ, Wolfe KH (1998) A subset of conserved tRNA genes in plastid DNA of non-green plants. Genetics 150:425–433

Lusson NA, Delavault PM, Thalouarn PA (1998) The *rbcL* gene from the non-photosynthetic parastite *Lathraea clandestina* is not transcribed by a plastid-encoded RNA polymerase. Curr Genet 34:212–215

Madey E, Nowack LM, Thompson JE (2002) Isolation and characterization of lipid in phloem sap of canola. Planta 214:625–634

Matsuzaki M, Kuroiwa H, Kuroiwa T, Kita K, Noyaki H (2008) A cryptic algal group unveiled: a plastid biosynthesis pathway in the oyster parasite *Perkinsus marinus*. Mol Biol Evol 25:1167–1179

McNeal JR, Leebens-Mack JH, Arumuganathan K, Kuehl JV, Boore JL, dePamphilis CW (2006) Using partial genomic fosmid libraries for sequencing complete organellar genomes. Biotechniques 41:69–73

McNeal JR, Arumuganathan K, Kuehl JV, Boore JL, dePamphilis CW (2007a) Systematics and plastid genome evolution of the cryptically photosynthetic parasitic plant genus Cuscuta (Convolvulaceae). BMC Biol 5:55

McNeal JR, Kuehl JV, Boore JL, dePamphilis CW (2007b) Complete plastid genome sequences suggest strong selection for retention of photosynthetic genes in the parasitic plant genus Cuscuta. BMC Plant Biol 7:57

Moore RB, Obornik M, Janouskovec J, Chrudimsky T, Vancova M, Green DH, Wright S, Davies NW, Bolch CJS, Heiman K, Slapeta J, Hoegh-Gildberg O, Logsdon JM Jr, Carter DA (2008) A photosynthetic alveolate closely related to apicomplexan parasites. Nature 451:959–963

Morden CW, Wolfe KH, dePamphilis CW, Palmer JD (1991) Plastid translation and transcription genes in a non-photosynthetic plant: intact, missing and pseudo genes. EMBO J 10:3281–3288

Neuhaus HE, Emes MJ (2000) Nonphotosynthetic metabolism in plastids. Annu Rev Plant Physiol Plant Mol Biol 51:111–140

Ohyama K, Fukuzawa H, Kohchi T, Shirai H, Sano T, Sano S, Umesono K, Shiki Y, Takeuchi M, Chang Z, Aota S, Inokuchi H, Ozeki H (1986) Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha*. Nature 322:572–574

Race HL, Herrmann RG, Martin W (1999) Why have organelles retained genomes? Trends Genet 15:364–370

Revill MJW, Stanley S, Hibberd JM (2005) Plastid genome structure and loss of photosynthetic ability in the parasitic genus Cuscuta. J Exp Bot 56:2477–2486

Sanchez-Puerta MV, Lippmeier JC, Apt KE, Delwiche CF (2007) Plastid genes in a non-photosynthetic dinoflagellate. Protist 158:105–117

Schwender J, Goffman F, Ohlrogge JB, Schachar-Hill Y (2004) Rubisco without the Calvin cycle improves the carbon efficiency of developing green seeds. Nature 432:779–782

Sheveleva EV, Giordani NV, Hallick RB (2002) Identification and comparative analysis of the chloroplast α-subunit gene of DNA-dependent RNA polymerase from seven *Euglena* species. Nucleic Acids Res 30:1247–1254

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chungwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M (1986) The complete nucleotide seuence of the tobacco chloroplast genome: its gene organization and expression. EMBO J 5:2043–2049

Siemeister G, Hachtel W (1990) Structure and expression of a gene encoding the large subunit of ribulose-1, 5-bisphosphate carboxylase (rbcL) in the colorless eugleoid flagellate *Astasia longa*. Plant Mol Biol 14:825–833

Stefanovic S, Olmstead RG (2005) Down the slippery slope: plastid genome evolution in Convolvulaceae. J Mol Evol 61:292–305

Tartar A, Boucias DG (2004) The non-photosynthetic, pathogenic green alga *Helicosporidium sp*. has retained a modified, functional plastid genome. FEMS Microbiol Lett 233:153–157

Thalouarn P, Theodet C, Russo N, Delavault P (1994) The reduced plastid genome of a non-photosynthetic angiosperm *Orobanche hederae* has retained the rbcL gene. Plant Physiol Biochem 32:233–242

van der Kooij TAW, Krause K, Dörr I, Krupinska K (2000) Molecular, functional and ultrastructural characterisation of plastids from six species of the parasitic flowering plant genus Cuscuta. Planta 210:701–707

van der Kooij TAW, Krupinska K, Krause K (2005) Tocochromanol content and composition in different species of the parasitic flowering plant genus Cuscuta. J Plant Physiol 162:777–781

Vogel J, Hübschmann T, Börner T (1997) Splicing and intron-internal RNA editing of trnK-matK transcripts in barley plastids: support of matK as an essential splice factor. J Mol Biol 270:179–187

Wickett NJ, Yhang Y, Hansen SK, Roper JM, Kuehl JV, Plock SA, Wolf PG, dePamphilis CW, Boore JL, Goffinet B (2008) Functional gene losses occur with minimal size reduction in the plastid genome of the parasitic liverwort *Aneura mirabilis*. Mol Biol Evol 25:393–401

Williamson DH, Gardner MJ, Preiser P, Moore DJ, Rangachari K, Wilson RJM (1994) The evolutionary origin of the 35 kb circular DNA of *Plasmodium falciparum*: new evidence supports a possible rhodophyte ancestry. Mol Gen Genet 243:249–252

Wilson RJM, Denny PW, Preiser PR, Rangachari K, Roberts K, Roy A, Whyte A, Strath M, Moore DJ, Moore PW, Williamson DH (1996) Complete gene map of the plastid-like DNA of the malaria parasite *Plasmodium falciparum*. J Mol Biol 261:155–172

Wolf PG, Rowe CA, Sinclair RB, Hasebe M (2003) Complete nucleotide sequence of the chloroplast genome from a leptosporangiate fern, *Adiantum capillus-veneris*. DNA Res 10:59–65

Wolfe AD, Morden CW, Palmer JD (1992) Function and evolution of a minimal plastid genome from a non-photosynthetic plant. Proc Natl Acad Sci USA 89:10648–10652

Wolfe AD, dePamphilis CW (1997) Alternate pathways of evolution for the photosynthetic gene rbcl in four non-photosynthetic species of Orobanche. Plant Mol Biol 33:965–977

Wolfe AD, dePamphilis CW (1998) The effect of relaxed functional constraints on the photosynthetic gene rbcL in photosynthetic and non-photosynthetic parasitic plants. Mol Biol Evol 15:1243–1258

Woodson JD, Chory J (2008) Coordination of gene expression between organellar and nuclear genomes. Nat Rev Genet 9:383–395

Yosef I, Irihimovitch V, Knopf JA, Cohen I, Orr-Dahan I, Nahum E, Keasar C, Shapira M (2004) RNA binding activity of the Ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit from *Chlamydomonas reinhardtii*. J Biol Chem 279:10148–10156

Zerges W (2002) Does complexity constrain organelle evolution? Trends Plant Sci 7:175–182