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The monoplastidic bottleneck in algae and plant evolution

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ABSTRACT

Plastids in plants and algae evolved from the endosymbiotic integration of a cyanobacterium by a heterotrophic eukaryote. New plastids can only emerge through fission; thus, the synchronization of bacterial division with the cell cycle of the eukaryotic host was vital to the origin of phototrophic eukaryotes. Most of the sampled algae house a single plastid per cell and basal-branching relatives of polyplastidic lineages are all monoplastidic, as are some non-vascular plants during certain stages of their life cycle. In this Review, we discuss recent advances in our understanding of the molecular components necessary for plastid division, including those of the peptidoglycan wall (of which remnants were recently identified in moss), in a wide range of phototrophic eukaryotes. Our comparison of the phenotype of 131 species harbouring plastids of either primary or secondary origin uncovers that one prerequisite for an algae or plant to house multiple plastids per nucleus appears to be the loss of the bacterial genes *minD* and *minE* from the plastid genome. The presence of a single plastid whose division is coupled to host cytokinesis was a prerequisite of plastid emergence. An escape from such a monoplastidic bottleneck succeeded rarely and appears to be coupled to the evolution of additional layers of control over plastid division and a complex morphology. The existence of a quality control checkpoint of plastid transmission remains to be demonstrated and is tied to understanding the monoplastidic bottleneck.

KEY WORDS: Plastid evolution, Plastid division, MinD/E, FtsZ, Peptidoglycan, Plant embryogenesis

Introduction

Plastids (chloroplasts) define the cytosol of algae and plants like no other compartment. The origin of plastids traces back to the endosymbiotic integration of a cyanobacterium into the cytosol and biochemical pathways of a heterotrophic eukaryote. We know close to nothing about the nature of the protist host, and from which cyanobacterial phyla the primal plastid evolved is also uncertain (Deschamps et al., 2008; Deusch et al., 2008; Criscuolo and Gribaldo, 2011). Based on the overall sequence similarity, the plastid donor appears to belong to a group of cyanobacteria able to fix atmospheric nitrogen (Dagan et al., 2013). Another phylogenetic analysis points to the recently discovered freshwater-dwelling *Gloeomargarita* clade (Ponce-Toledo et al., 2017). What is commonly accepted is that the three archaeplastidal lineages – Glaucophyta, Rhodophyta and Chloroplastida (Zimorski et al., 2014; Archibald, 2015) (Fig. 1A) – arose monophyletically (Rodríguez-Ezpeleta et al., 2005; Jackson and Reyes-Prieto, 2014; Burki, 2014) (see Glossary in Box 1 for specialized terms used

throughout). The Archaeplastida emerged early during eukaryotic evolution (He et al., 2014), probably sometime between the 'upper limit' for the origin of eukaryotes ~1.9 billion years ago (Gya) (Eme et al., 2014) and the fossilization of early red algae some 1.6 to 1.2 Gya ago (Butterfield, 2000; Bengtson et al. 2017). These fossil records display multicellular organisms, in the case of *Bangiomorpha* with branched filaments, indicating that they had already evolved some level of morphological complexity and that single-celled algae – maybe comparable to extant glaucophytes – are older.

The consummated endosymbiotic integration of a prokaryote into a eukaryote is a rare event. There are examples of endosymbiotic integration of prokaryotes in several clades of eukaryotes (Kneip et al., 2008; Nakayama et al., 2014; Nowack, 2014; Bennett and Moran, 2015; Husnik and McCutcheon, 2016), but it was the endosymbiotic integration of the mitochondrion and later the plastid that were key to the evolution of macroscopic life. Among the reasons for why such an integration is a rare event are the challenges of: (1) fusing a prokaryote and an eukaryote genome (Timmis et al., 2004); (2) establishing new transport routes for proteins now encoded in the host nucleus as a consequence of endosymbiotic gene transfer (EGT) (Soll and Schleiff, 2004; Leister, 2016; Garg and Gould, 2016); (3) developing a means of communication such as retrograde signalling (Woodson and Chory, 2008; Chandel, 2015; Singh et al., 2015); and (4) synchronizing prokaryotic fission with the cell cycle of the eukaryotic host (Miyagishima, 2011). The co-evolution of organelle and host depends on the simultaneous and successful implementation of these events. However, some of these events compete, such as EGT and the number of endosymbionts per cell. These conflicts add an additional layer of complexity to the transition from endosymbiont to organelle.

In this Review, we discuss the evolutionary ancestry of plastid division, from the successful integration of the cyanobacterial plastid progenitor into the host, to the additional layers of division control that land plants evolved. We collate information on the mechanisms underlying plastid division and the number of plastids per cell, and examine the implications for organelle evolution and inheritance, before aiming to provide an answer as to why most algae bear only a single plastid per cell.

Endosymbiotic gene transfer: the more the merrier

EGT was a key component of organelle integration during the early stages of algae evolution and is an ongoing process in plants (Matsuo et al., 2005; Cullis et al., 2009). EGT typically occurs in the form of DNA chunks, rather than individual genes (Henze and Martin, 2001; Yuan et al., 2002; Michalovova et al., 2013). This is thought to occur through the uncontrolled lysis of endosymbionts, thus releasing their DNA, of which some can then be randomly incorporated into the nuclear genome (Martin, 2003). Many nuclear genomes of eukaryotes carry evidence for recent gene transfers that are known as nuclear mitochondrial and nuclear plastid DNA sequences (Richly and Leister, 2004; Hazkani-Covo et al., 2010). The number of plastids (or mitochondria) that are present when EGT happens is crucial: if an alga carries only one plastid, then its lysis

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results in the death of the host cell and hence, no progeny will emerge. That is why EGT preferably occurs in the presence of multiple donors per cell, which is true for the cyanobionts that evolved into modern plastids (Barbrook et al., 2006) – both mitochondria and plastids in their current form (Smith 2011a; Smith et al. 2011b) – as well as the complex plastids of secondary endosymbiotic origin (Curtis et al., 2012). Through EGT, the host gains ever more control while the endosymbiont loses its autonomy.

The presence of a single organelle, whether it is a plastid or mitochondrion, significantly slows down the rate of subsequent EGT,

Fig. 1. See next page for legend.

Fig. 1. Plastid evolution and the absence of *minD* and *minE* from plastid genomes of polyplastidic algae and plants. (A) Simplified trajectory of plastid evolution. Archaeplastida (Glaucophyta, Rhodophyta and the Chloroplastida) evolved through primary endosymbiosis (1° ES) and are of monophyletic origin. Algae with complex plastids arose through secondary endosymbiosis (2° ES). Secondary green algae arose by two independent events: once acquiring an alga branching basal in all Chlorophyta in Euglenophyta (chartreuse yellow) and once acquiring an ulvophyte in Chlorarachniophytes (light green). The distantly related Stramenopiles, Hacrobia and Alveolata all harbour a red complex plastid that ultimately traces back to a monophyletic 2° ES event, yet the number and order of subsequent 3° ES (or even 4° ES) events with which the original red complex plastid disseminated, remains a topic of investigation. Names of well-known genera and those discussed throughout the text are provided. (B) Cladograms of the diversity of photosynthetic eukaryotes. Coloured gradients depict the phylogenetic association of the plastids shown in A. Cercozoa include *Paulinella* (cyan), which acquired their chromatophores more recently and independently of the Archaeplastida. The entire plastid dataset of NCBI was screened using a BLASTp and tBLASTn approach and *G. theta* MinD and MinE as queries. Published information on the morphology of the displayed organisms was screened for information on (1) unicellular, morphologically colonial (coenobial) or 'multicellular' growth (including trichal, siphonaceous and parenchymatous), and (2) plastid number (see Table S1 for further details). No plastid-encoded minD or minE homologue was detected for sequenced land plant plastid genomes (five representatives shown) and Chlorophyceae. Note that siphonaceous algae are also labelled as multicellular for simplicity. *Eimeria tenella* (*) forms sporozoites and merozoites that can have more than one apicoplast and highly polyplastidic schizonts and thus represents a case of conditional polyplastidy (Ferguson et al., 2007). Question marks indicate for which morphological characters no reliable reference could be found.

trapping the genomes of the organelles evolutionarily (Barbrook et al., 2006; Curtis et al., 2012). For *Paulinella chromatophora*, it was suggested that horizontal gene transfer (HGT) complemented EGT early during chromatophore origin (Nowack et al., 2016). The frequency of HGTs among eukaryotes is debated (Huang, 2013; Ku et al., 2015), but if true for *P. chromatophora*, HGT ceased with the switch from phagocytosis-based heterotrophy to photoautotrophy (Nowack et al., 2016). HGTs therefore have little, if any, impact on subsequent steps of organelle integration, for which examples are the synchronization of organelle and host division and the transfer of the remaining genetic material of the endosymbiont to the nuclear genome. In summary, the presence of multiple cyanobionts during the early stages of plastid origin appears likely and may be a prerequisite for successful plastid inheritance. Yet, the majority of extant algae appear to have a host cell (nucleus) to plastid number ratio of one and that ratio is rarely altered. As outlined below, we propose that a monoplastidic bottleneck was part of the evolutionary origin of the plastid (Fig. 2).

The monoplastidic bottleneck
A plant or algal cell needs to ensure inheritance of its plastids and mitochondria as the loss of these organelles during cytokinesis is lethal. There are two possible solutions for the control of the inheritance of at least one organelle (of each type) by the offspring. The first is the presence of numerous organelles in the cytosol, which results in a rather passive inheritance based on their stochastic distribution. While this might be feasible for the mitochondria of mammals (Mishra and Chan, 2014), it carries the risk of random failure (Mishra and Chan, 2014). In multicellular organisms, this can be tolerated, but is selected against in single-celled eukaryotes. The second solution is a synchronization of organelle and nuclear division and a controlled distribution of compartments during cytokinesis. If multiple endosymbionts foster the transition of

Box 1. Glossary
Alveolata: a eukaryotic superphylum that, among others, includes the Apicomplexa (including the causal agent of malaria *Plasmodium*).
Archaeplastida: the monophyletic eukaryotic supergroup of plastid-housing organisms that can be traced back to the single primary endosymbiotic event. It unites the Glaucophyta, Rhodophyta and Chloroplastida.
Chlorophyta: one of the two lineages of Chloroplastida, which encompasses unicellular (e.g *Chlamydomonas*) and multicellular green algae (e.g. *Ulva* or sea lettuce).
Chloroplastida: the green archaeplastidal lineage that unites the Chlorophyta and Streptophyta (which includes land plants).
Chromatophore: photosynthetic cyanobiont in *Paulinella* of primary endosymbiotic origin.
CORR hypothesis: co-location for redox regulation; a hypothesis that explains why mitochondria and plastids have retained a genome despite ongoing EGT. In brief, it proposes that certain proteins (especially those of the electron transport chain) are required to remain organelle-encoded as they are part of an *in situ* (*in organello*) gene expression regulation mechanism that is governed by redox-state feedback.
Cryptophyta: a group of algae housing red complex plastids of secondary endosymbiotic origin.
Cyanobionts: symbiotic cyanobacteria.
Embryophyta: the monophyletic lineage of land plants.
Embryoplast: land plant plastids that can differentiate into many different subtypes, such as starch-storing amyloplasts.
Endosymbiotic gene transfer (EGT): the process of gene loss from the organelle to the nuclear genome.
Euglenozoa/Euglenophyta: group of eukaryotes, some of which have a green complex plastid of secondary endosymbiotic origin.
Euphyllophyta: a major clade within the vascular plants that includes ferns (monilophytes) plus gymnosperms and angiosperms (the seed plants).
Glaucophyta: the deepest branching Archaeplastida, includes only a few dozen described species of unicellular algae.Hacrobia: a (debated) lineage with red complex plastids that unites the Cryptophyta and Haptophyta.
Haptophyta: a major group of unicellular algae (with red complex plastids) that includes e.g. the coccolithophore *Emiliana*, ancestors of which contributed to the formation of the white cliffs of Dover.
Horizontal gene transfer (HGT): a process of transfer and integration of genetic material (from one individual to another) that is independent of vertical inheritance (hence independent of species borders and descent) and clearly distinct from EGT.
Lycophytes: the deepest branching lineage of extant vascular plants of which a well-known example is *Selaginella moellendorffii*.
Mono-/bi-/poly-plastidy: presence of one, two or many plastids per cell and/or nucleus.
Muller's ratchet: the irreversible accumulation of deleterious mutations in the absence of sexual reproduction.
Muroplast (formerly known as cyanelle): the plastid of the Glaucophyta, which have retained ancestral traits such as carboxysomes and a thick peptidoglycan layer.
Nucleomorph: a remnant nucleus of an engulfed primary red or green alga (endosymbiont) that became the secondary plastid of some lineages.
Rhodophyta: the red archaeplastidal lineage which encompasses unicellular red algae (e.g. *Cyanidioschyzon* or *Porphyridium*) and multicellular organisms (e.g. *Porphyra*, which is used for wrapping sushi, or *Chondrus*).
Spheroid body: vertically inherited nitrogen-fixing cyanobionts of some diatoms such as *Rhopalodia*.
Stramenopiles (or Heterokonta): eukaryotic superphylum that, among others, includes the (non-plastid bearing) oomycetes (e.g. the phytopathogen *Phytophthora infestans*) and algae with red complex plastids (e.g. diatoms and brown algal kelps).
Streptophyta: one of the two major lineages of Chloroplastida, which encompasses streptophyte algae (also known as charophytes) that range from the unicellular (e.g. *Mesostigma viride*) to multicellular organisms such as *Chara* (stoneworts) and the land plants.
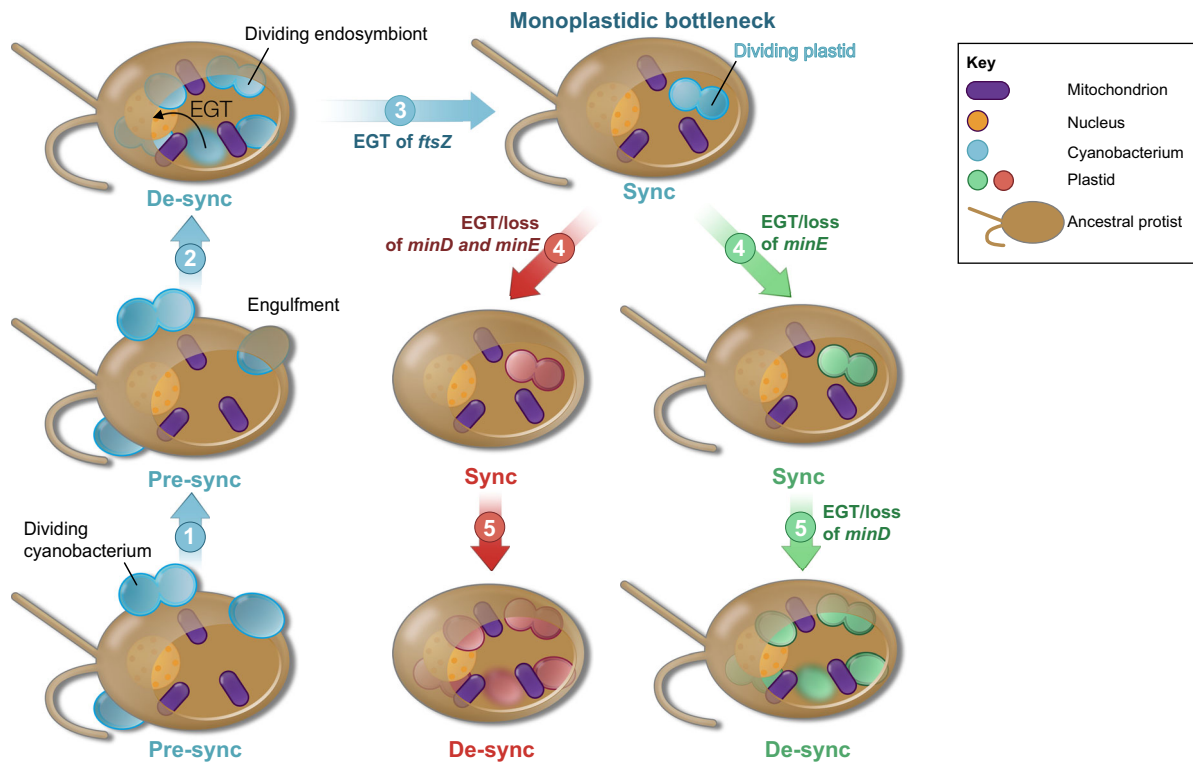
Fig. 2. Plastid origin and the monoplastidic bottleneck. Plastids evolved from free-living cyanobacteria (cyan blue) that were originally engulfed by a heterotrophic protist (the first common ancestor to all Archaeplastida; shown on the bottom right with a nucleus in orange and mitochondria in purple) as a food source. After their phagocytotic uptake and during the early stages of endosymbiont evolution (step 1), the division of the cyanobionts was not yet synchronized ('pre-sync', step 2) with that of the protist host, and thus, multiple endosymbionts were probably present in a host cell. Some of them might have lysed, thereby releasing their DNA and mediating endosymbiotic gene transfer (EGT). At some point during their co-evolution, the division of the plastid became synchronized ('Sync') with that of the host (step 3), through what we call the monoplastidic bottleneck. It is intrinsic to all three lineages (Glaucophyta, Rhodophyta and Chloroplastida) that evolved later (step 4). Representatives of the red and green lineage escaped this monoplastidic bottleneck independently (step 5) and the division of plastids in the cytosol no longer depends on the simultaneous division of the nucleus and host cell. It is conceivable that this de-synchronization is connected to biplastidy (Box 2). The evolution of glaucophyte polyplastidy appears more complicated and is outlined in the text. This situation is reminiscent of the 'Pre-sync' situation early in plastid evolution (steps 1 and 2).

endosymbiont to organelle, because it promotes EGT events (Smith 2011a; Smith et al., 2011b; Barbrook et al., 2006; Curtis et al., 2012), then the first solution appears to be correct. However, this is only the case for the earliest stages of plastid evolution, when plastid-to-nucleus communication and coordination of their division had not yet been established. Basal branching (i.e. an early divergence from the last common ancestor) algae of the red and green lineage are all monoplastidic (Fig. 1A,B), and a few non-vascular land plants are monoplastidic at least during some stages of their life cycle (Brown and Lemmon, 1990; Vaughn et al., 1992). One might even consider some polyplastidic-appearing glaucophytes to be monoplastidic too, but it is difficult to form a definitive conclusion (Box 2) owing to the lack of structural and molecular data available for glaucophytes. Species that harbour two plastids, such as the glaucophyte *Cyanophora*, are worth a second look (Box 2) and may be viewed as a deferred case of monoplastidy.

Monoplastidy is thus the common and ancestral character state of plastid-bearing eukaryotes. It suggests that during the endosymbiont-to-plastid transition, plastid numbers per cell were reduced to one and this monoplastidic bottleneck occurred before the split into the three main archaeplastidal lineages (Fig. 1A). The archaeplastidal ancestor was likely to be a heterotrophic single-celled eukaryote, a protist. Such a heterotrophic protist will have possessed mechanisms for ensuring mitochondrial, but not yet plastid, inheritance. It is conceivable that evolution selected for

monoplastidy to secure proper vertical inheritance of the photosynthetic organelle through the synchronization of the division of plastid and host. The same cannot be said about mitochondria, because their division is not strictly coupled to that of the nucleus; rather, it is coordinated by the endoplasmic reticulum in both animals (Lewis et al., 2016) and plants (Mueller and Reski, 2015). The situation for mitochondria differs because their origin is irrevocably linked to the very origin of eukaryotes and their hallmarks of meiosis, the cell cycle and sex (López-García and Moreira, 2015; Gould et al., 2016; Garg and Martin, 2016).

Support for the theory that monoplastidy was selected for to secure proper plastid inheritance comes from two of the three main archaeplastidal lineages. In both the glaucophyte *C. paradoxa* and the red alga *Cyanidioschyzon merolae* (Fig. 1A), plastid and nucleus division is coordinated in a manner such that mitosis only commences after the successful division of the plastid (Sumiya et al., 2016). The same is observed in eukaryotes that carry plastids of secondary origin (Hashimoto, 2005), which allows us to speculate that the same regulatory machinery (including a means of retrograde communication) which evolved in the primary host to coordinate plastid and nuclear division is now at work in the secondary host, too. Thus, within the archaeplastidal ancestor, we can speculate that a common set of regulatory factors were implemented that orchestrate plastid division and synchronize it with the host cell cycle. Furthermore, along the evolutionary

Box 2. The curious case of biplastidy

Several independent algal groups harbour representatives with two plastids per nucleus, called biplastidy, which could be considered as an intermediate stage between housing strictly one or multiple plastids per cell and nucleus. During cell division, a monoplastidic cell becomes transiently biplastidic to pass on a plastid to its daughter cell. Biplastidy could therefore be the result of a simple shift in plastid division, from shortly prior to cytokinesis to just after cytokinesis. This appears to have occurred multiple times independently: hornworts tend to have one or two plastids per cell (labelled biplastidic in Fig. 1B) (Vaughn et al., 1992) and they branch at the base of the primarily polyplastidic land plants (Wickett et al., 2014). Furthermore, among the secondary red plastid-housing stramenopiles, which contain many polyplastidic species, biplastidy is frequently observed – especially among diatoms (Fig. 1B). A similar situation is observed in a much younger case of plastid acquisition in *P. chromatophora*, a thecate amoeba that has been co-evolving with its cyanobacterial endosymbiont for about 60 Mya (Nowack, 2014). *P. chromatophora* harbours two chromatophores, one of which is transmitted to the daughter cell after cytokinesis when both mother and daughter cell are basically monoplastidic (Nomura et al., 2014; Nowack, 2014). This mode of inheritance also applies to its relative, *P. longichromatophora* (Kim and Park, 2016). What applies to all monoplastidic cells also applies to *Paulinella*: the presence of only one chromatophore would significantly reduce the chances of successful EGTs occurring, trapping *Paulinella* in endosymbiont evolution early on. Therefore, biplastidy might represent a compromise between the synchronization of host and endosymbiont division and the remaining possibility to lose one of the two organelles through lysis (and to hereby facilitate EGT). Biplastidy is far more prevalent than simple chance would suggest and future research is needed to clarify its evolutionary and molecular significance.

trajectory of this synchrony, the number of plastids (or cyanobionts) would have gone from a non-controlled population of many to a single plastid. Next, we inspect the associated components and deduce their role with regards to the evolution of the plastid division machinery.

Plastid division takes two to tango
The contractile ring machinery
Plastids inherited the backbone of their division (i.e. fission) machinery from their cyanobacterial ancestor (Miyagishima and Kabeya, 2010; Dagan et al., 2013; Ponce-Toledo et al., 2017) (Fig. 1A,B). Although cyanobacterial division differs in some components from that of other bacteria, it nevertheless relies on physical constriction of the cell by a contractile ring (Miyagishima et al., 2005), which is formed by the self-assembling GTPase filamentous temperature-sensitive protein Z (FtsZ) (Bi and Lutkenhaus, 1991; de Boer et al., 1992; Osawa et al., 2008; de Boer, 2010). FtsZ is the primary component of the 'Z' ring that forms in the bacterial cytosol and an array of accessory factors either control formation of the Z ring or are recruited to it after the Z ring has formed (Adams and Errington, 2009). Land plant plastids have inherited many of these components, including FtsZ, ARC6 (ACCUMULATION AND REPLICATION OF CHLOROPLASTS 6) and minD/E (also known as ARC11/12) (Strepp et al., 1998; Miyagishima et al., 2014a; Osteryoung and Pyke, 2014; Grosche and Rensing, 2017). They now act in concert with the host cell cycle (Sumiya et al., 2016), not least because most of them are today encoded in the nucleus as a result of EGT (Miyagishima et al., 2012).

During plastid division in Chloroplastida and Rhodophyta (the green and red lineage, respectively), genes that are associated with

plastid division share the same expression pattern (Miyagishima et al., 2012). This includes *ftsZ*, *DRP5B* (dynamin-related protein 5B), which facilitates organelle scission from the cytosolic side of the plastid and the minicell gene *minD*, which regulates the positioning of the FtsZ-based ring on the stromal side of the inner plastid membrane (Fujiwara et al., 2008; Osteryoung and Pyke, 2014). In the case of the chloroplastidian *minD*, the localisation of the gene (i.e. whether in the plastid or nuclear genome) has no impact on its expression pattern, although in the latter case, *minD* expression seemed to be regulated by light rather than the cell cycle (Miyagishima et al., 2012). The plastid division genes (*ftsZ*, *ftsW* and the septum development gene *sepF*) of the glaucophyte *C. paradoxa*, however, experience little fluctuation in expression (Miyagishima et al., 2012). Moreover, both *C. paradoxa* and *C. merolae* only allow mitosis to commence once plastid division is completed (Sumiya et al., 2016). Still, the differences in the expression pattern of division factors might indicate that the plastid division in *Cyanophora* is somewhat distinct from that of the other Archaeplastida. How then could this have occurred?

Glaucophytes such as *Cyanophora* are the most strongly diverging clade of the Archaeplastida (Burki, 2014) (Fig. 1A). Although only 15 glaucophyte species have been described (Guiry, 2012), it is likely that more species exist (Jackson et al., 2015; Takahashi et al., 2016). The most basal-branching glaucophyte genus is *Cyanophora* (Chong et al., 2014). *Cyanophora* cells tend to harbour either one or two plastids (in glaucophytes these are referred to as muroplasts; see Glossary in Box 1); if two are present, then they are semi-connected as if frozen in the act of division (Jackson et al., 2015; Box 2). Other glaucophyte genera, such as *Cyanoptyche*, *Gloeochaete* and *Glaucocystis* are polyplastidic (Jackson et al., 2015), but the muroplast morphology in *Glaucocystis* warrants attention as it has two stellate muroplast clusters within the cell (Schnepf et al., 1966). These clusters consist of individual muroplast lobes that at one end are held together through unknown mechanisms (Schnepf et al., 1966). Therefore, together with the basal position of *Cyanophora* in a clade of polyplastidic algae, these findings further support the idea that harbouring two (physically connected) plastids per cytosol might be an intermediate stage between monoplastidy and polyplastidy. The bundling of plastids in *Glaucocystis* might well be a relic of this transition (Boxes 2 and 3). Glaucophytes are generally considered to have retained ancestral features of the earliest Archaeplastida (Fathinejad et al., 2008; Steiner and Löffelhardt, 2011; Facchinelli et al., 2013), including a thick peptidoglycan (PG) layer between the two membranes of the plastid organelle (Steiner et al., 2001). There is little molecular research being carried out on glaucophytes; however, the retention of some ancient traits and the diversity of their plastid morphology for such a small group could prove useful for future research in light of the recent identification of a PG layer in moss (Hirano et al., 2016).

The ancestral peptidoglycan layer
The cyanobacterial progenitor of the plastid had a layer of PG, which separated its inner and outer membranes. This is evident by the retention of the PG layer – sometimes referred to as a murein layer – by the muroplasts of glaucophytes (Steiner et al., 2001) and the recent identification of a thin PG layer containing D-amino acids that surrounds the plastids of the model moss species *Physcomitrella* (Hirano et al., 2016). Previously, there was a consensus that the murein layer had been lost early during plastid evolution after the green and red lineage had diverged from the glaucophytes. One reason for the loss of the PG layer was the idea

Box 3. Plastid numbers per cell across evolution
Land plants are the best known polyplastidic, multicellular and complex species. Yet, there are many examples for polyplastidic and multicellular organisms that are very distant relatives to land plants, including red seaweeds (rhodophytes, *Gracilaria* or *Choreocolax*; Callow et al., 1979; Schmidt et al., 2010) and brown algae (phaeophytes, *Ectocarpus*; Charrier et al., 2008) (Fig. 1). Monoplastidic, multicellular algae are also found across a broad taxonomic range, including the chlorophytic Palmophyllaceae (Zechman et al., 2010; Lelieart et al., 2016) or the rhodophytes *Porphyra* and *Pyropia* (Sutherland et al., 2011). Polyplastidic, unicellular algae mainly occur among secondary plastid-housing organisms, such as the euglenophyte *Euglenaformis* (Bennett et al., 2014) or the stramenopile *Heterosigma* (Hara and Chihara, 1987). A curious type of polyplastidy occurs in coenocytic (siphonaceous) algae such as *Vaucheria*: they belong to the heterokontophytes, whose complex plastids are surrounded by four membranes, the outermost of which is continuous with the endoplasmic reticulum (Gould et al., 2015). Based on the general morphological description and perception of stramenopiles, one would suspect that their plastids occur in a complex with the nucleus, each complex containing one nucleus and two plastid lobes (Apt et al., 2002). Although a single *Vaucheria* cell can contain thousands of nuclei–plastid complexes, could it be that they are effectively biplastidic (Ott, 1992)? We are not aware of any in-depth analysis on this subject and little molecular work is carried out on siphonaceous algae in general (but see, e.g., Ranjan et al., 2015). Studying nucleus–plastid communication in a coenocytic system might contribute to our understanding of the origins of plastid numbers per cell.

that it interfered with protein import of nuclear-encoded plastid proteins (Steiner and Löffelhardt, 2002). It has always been intriguing that photosynthetic eukaryotes contain genes such as *murE* and *mraY*, which encode enzymes that synthesize PG, and the resulting PG layer is very likely to be associated with plastid division (Machida et al., 2006; Garcia et al., 2008; Takano and Takechi, 2010). The function of MurE diverged in angiosperms and gymnosperms – where it is one of the plastid RNA polymerase-associated proteins (PAPs; Pfalz and Pfannschmidt, 2013) – whereas it has retained its ancestral function in the moss *Physcomitrella* (Garcia et al., 2008; Lin et al., 2017). This raises the question of whether the retention of even only a thin murein layer could be associated with the regulation of plastid division.

Independent lines of evidence connect the PG layer with the regulation of plastid division. For instance, PG-inhibiting antibiotics, such as ampicillin or fosfomycin, affect the division and morphology of plastids in streptophyte algae (Matsumoto et al., 2012), lycophytes (Izumi et al., 2003) and mosses (Katayama et al., 2003). Additionally, plastid division is altered in *Physcomitrella* knockout lines for enzymes synthesizing PG (Homi et al., 2009). Such effects are much less pronounced in euphyllophytes (Takano and Takechi, 2010): Izumi and colleagues (Izumi et al., 2008) showed that three different fern species had a reduced number of plastids upon treatment with fosomycin, but not ampicillin, whereas their lycophyte control (*Selaginella nipponica*) responded to both. In addition, fosomycin and ampicillin have no effects on the plastid number of angiosperms (Kasten and Reski, 1997; Izumi et al., 2008). These differences might thus reflect and add to the specific evolutionary changes experienced by the embryoplast (de Vries et al., 2016). A link between the complete loss of the PG layer and the switch from mono- to polyplastidy in embryophytes is feasible, but cannot be its sole reason because of the absence of a PG layer in rhodophytes (Grosche and Rensing, 2017), which are predominantly monoplastidic (Fig. 1B). In bacteria, there is a correlation between the loss of the PG layer and loss of FtsZ

(Miyagishima et al., 2014a), and the division and PG synthesis in bacteria is driven by GTP-dependent treadmilling of FtsZ filaments (Bisson-Filho et al., 2017; Yang et al., 2017). This is in line with the observation that the guidance of synthesis of the PG layer is one of the main functions of FtsZ (de Pedro et al., 1997; Aaron et al., 2007; Typas et al., 2012).

Plastid division involves FtsZ in all Archaeplastida species that have been analysed thus far (Yang et al., 2008; Miyagishima et al., 2012; Chen et al., 2017). Here, two slightly different FtsZ proteins (FtsZ1 and FtsZ2) heteropolymerize to form the inner division ring (Yoshida et al., 2016). Streptophyta and Glaucophyta possess another FtsZ protein, FtsZ3 (Grosche and Rensing, 2017). Localization studies in *P. patens* have shown that FtsZ3 assembles into ring-like structures in the cytosol and plastid (Kiessling et al., 2004). Recently, the loss of the PG-synthesising enzymes (and sensitivity to PG-inhibiting antibiotics) was correlated with the loss of FtsZ3 (Grosche and Rensing, 2017), in line with the aforementioned pattern in bacteria (Miyagishima et al., 2014a). Surprisingly, although FtsZ is clearly involved in plastid division, it is not essential (Schmitz et al., 2009; Miyagishima et al., 2014a). The plastids in *Arabidopsis ftsZ*-knockout lines still divide, but intriguingly, their cells harbour a reduced number of plastids per cell (Schmitz et al., 2009). This suggests that the additional copy of FtsZ3 is strictly connected to a PG-associated function and that whereas the remaining copies of FtsZ are crucial for the fine-tuning of plastid division, they are not essential for the process per se.

Cytosolic forces and the 'inside-first' mechanism of plastid division
Plastid division commences within the organelle (Miyagishima et al., 2014a) because of its cyanobacterial origin. The prokaryotic division machineries act first on the cytosolic face of the plasma membrane (Errington et al., 2003). The cyanobacterial ancestor rests in the cytosol of the host cell, and with the concomitant reduction or even loss of the PG layer as seen in higher land plants, plastids became accessible and amenable to external forces that act within the cytoplasm of the host. The most substantial cytosolic forces are the outer division rings: one is formed by a protein of the dynamin family (Gao et al., 2003; Miyagishima et al., 2003; Yang et al., 2008; Miyagishima and Kabeya, 2010), whose origin and function are connected to the origin and fission of mitochondria (Purkanti and Thattai, 2015; Gould et al., 2016); the other is the plastid division ring (Kuroiwa et al., 1998) that consists of polyglycan filaments in the red alga *C. merolae* (Yoshida et al., 2010).

In eukaryotes, dynamins are conserved GTPases that exert mechanical forces, with some dynamins mediating the contraction and scission process during organelle division (McFadden and Ralph, 2003; Purkanti and Thattai, 2015; Leger et al., 2015). Dynamin-mediated plastid division arose in the common ancestor of Rhodophyta and Chloroplastida, as it is present in all studied members of the red and green lineage, but not glaucophytes (Miyagishima et al., 2014b). Interestingly, mutations in genes coding for the inner division machinery components, such as *ARC6* (a J-domain protein homolog to the cyanobacterial protein Ftn2; Vitha et al., 2003), have a more pronounced influence on plastid number (Fig. 3) than mutations in components acting on the outside (Robertson et al., 1996; Gao et al., 2003; Sakaguchi et al., 2011). Moreover, the division of proplastids – plastids that can differentiate into different types and are in this form only present in land plants – is not affected in dynamin-knockout lines (Robertson et al., 1996). Regardless of whether outer or inner division components are manipulated, proplastid division still occurs (Miyagishima et al., 2014a). Knockout of components such as *ftsZ* in land plants tends to
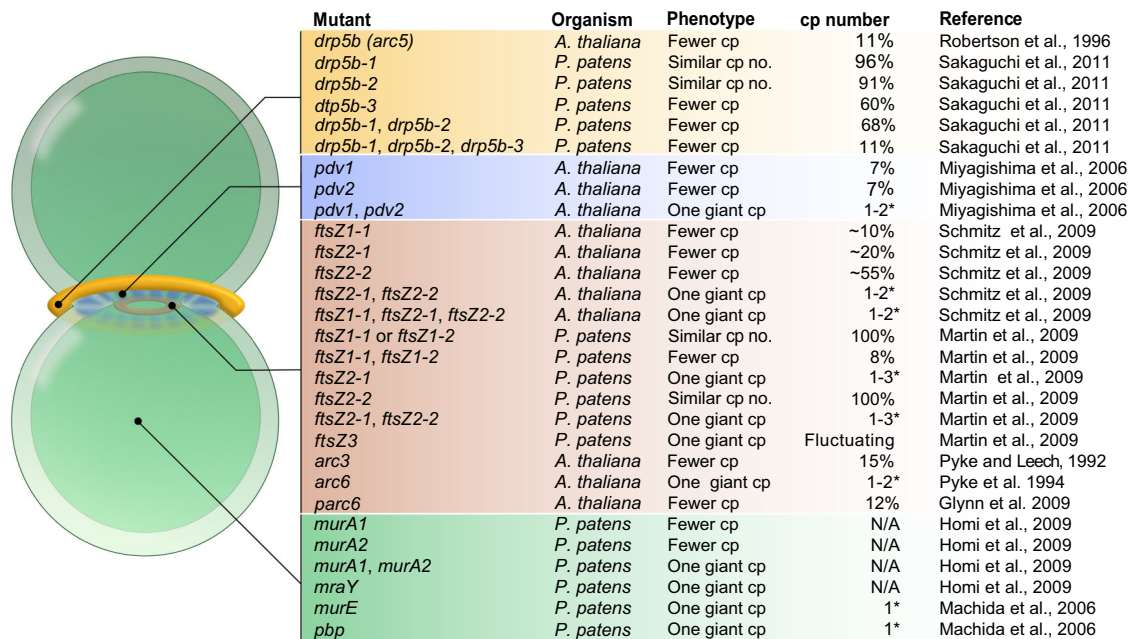
Mutant	Organism	Phenotype	cp number	Reference
drp5b (arc5)	A. thaliana	Fewer cp	11%	Robertson et al., 1996
drp5b-1	P. patens	Similar cp no.	96%	Sakaguchi et al., 2011
drp5b-2	P. patens	Similar cp no.	91%	Sakaguchi et al., 2011
dtp5b-3	P. patens	Fewer cp	60%	Sakaguchi et al., 2011
drp5b-1, drp5b-2	P. patens	Fewer cp	68%	Sakaguchi et al., 2011
drp5b-1, drp5b-2, drp5b-3	P. patens	Fewer cp	11%	Sakaguchi et al., 2011
pdv1	A. thaliana	Fewer cp	7%	Miyagishima et al., 2006
pdv2	A. thaliana	Fewer cp	7%	Miyagishima et al., 2006
pdv1, pdv2	A. thaliana	One giant cp	1–2*	Miyagishima et al., 2006
ftsZ1-1	A. thaliana	Fewer cp	~10%	Schmitz et al., 2009
ftsZ2-1	A. thaliana	Fewer cp	~20%	Schmitz et al., 2009
ftsZ2-2	A. thaliana	Fewer cp	~55%	Schmitz et al., 2009
ftsZ1-1, ftsZ2-2	A. thaliana	One giant cp	1–2*	Schmitz et al., 2009
ftsZ1-1, ftsZ2-1, ftsZ2-2	A. thaliana	One giant cp	1–2*	Schmitz et al., 2009
ftsZ1-1 or ftsZ1-2	P. patens	Similar cp no.	100%	Martin et al., 2009
ftsZ1-1, ftsZ1-2	P. patens	Fewer cp	8%	Martin et al., 2009
ftsZ2-1	P. patens	One giant cp	1–3*	Martin et al., 2009
ftsZ2-2	P. patens	Similar cp no.	100%	Martin et al., 2009
ftsZ2-1, ftsZ2-2	P. patens	One giant cp	1–3*	Martin et al., 2009
ftsZ3	P. patens	One giant cp	Fluctuating	Martin et al., 2009
arc3	A. thaliana	Fewer cp	15%	Pyke and Leech, 1992
arc6	A. thaliana	One giant cp	1–2*	Pyke et al. 1994
parc6	A. thaliana	Fewer cp	12%	Glynn et al., 2008
murA1	P. patens	Fewer cp	N/A	Homi et al., 2009
murA2	P. patens	Fewer cp	N/A	Homi et al., 2009
murA1, murA2	P. patens	One giant cp	N/A	Homi et al., 2009
mraY	P. patens	One giant cp	N/A	Homi et al., 2009
murE	P. patens	One giant cp	1*	Machida et al., 2006
pbp	P. patens	One giant cp	1*	Machida et al., 2006

Fig. 3. Single mutations can cause strong alterations in plastid number per cell. Schematic drawing of a dividing plastid with the outer division components (yellow), the land plant-specific PDV proteins (blue), and the inner division components (red). On the right, the respective division components and published information on the phenotypes of plastid-division mutants are listed (same colour code as on the left). Plastid (cp) number indicates either the (approximate) number of plastids relative to the wild type (in case of the phenotype 'Fewer cp') or the range of macrochloroplast numbers that were observed (indicated with an asterisk). Note that in addition to the macrochloroplast phenotype, Martin et al. (2009) observed various phenotypes for the *P. patens ftsZ3* mutant, including irregular plastid shapes.

reduce the number of plastids (Fig. 3) to a degree that it allows only the proplastids to divide (Schmitz et al., 2009; Miyagishima et al., 2014a). The latter might be facilitated by alternative plastid division processes, such as budding (Miyagishima et al., 2014a), which was proposed to allow plastid division in mutants with a macrochloroplast (Pyke, 2010). Plastid budding has been described for non-chlorophyllous plastids of the *Arabidopsis arc6*, *crumpled leaf* (*crl*; which exhibits a severe phenotype with some cells maybe even lacking plastids altogether; Asano et al., 2004; Chen et al., 2009) and *Solanum lycopersicum* (tomato) *suffulta* plastid division mutants (Forth and Pyke, 2006). Thus, plastid division is evidently quite robust.

We propose that this robustness, including unconventional plastid division mechanisms such as budding, is due to the framework that evolved at the monoplastidic bottleneck. Other layers of additional control, such as the ARC6-interacting PLASTID DIVISION proteins (PDVs) that control the rate of plastid division, (Miyagishima et al., 2006; Glynn et al., 2008), were added to the existing plastid division machinery later in evolution. The use of systematic knockouts to strip these additional layers of control that were implemented to better command plastid division should reveal the ancient molecular chassis that traces back to the time when plastid and nuclear division were synchronized.

Components of the plastid division machinery were lost or modified several times independently during evolution (Miyagishima et al., 2014a). Above, we discussed a handful of genes and properties that might have been crucial for the orchestration of plastid division. Studies have shown that the knockout of single genes such as *ARC6* can transform a polyplastidic embryophyte cell to a cell carrying a single embryoplast (Pyke et al., 1994; Vitha et al., 2003). This raises the question of whether there may be just a few key factors that determine organelle numbers. The mutants mentioned above often harbour a single plastid that is massively increased in size, which is

reminiscent of large algal plastids such as those of *Chlamydomonas*, whose plastid takes up about half of the cell volume (Gaffal et al., 1995). In fact, large single plastids are quite frequently observed when plastid division is impaired, for example in *ftsZ* knockouts in both *A. thaliana* (Schmitz et al., 2009) and *P. patens* (Martin et al., 2009), as well as in *P. patens* knockouts for *murA* and *mraY*, which are involved in PG synthesis (Homi et al., 2009) (Fig. 3). In summary, single gene mutations are capable of reverting polyplastidy back to (macroplastidic) monoplastidy and the list of involved proteins is probably not yet complete.

minD and *minE*: markers for the evolution of (complex) plastids

Plastid division requires the interplay of two genetic compartments: the plastid and the nucleus. Whereas in land plants all plastid division proteins are nucleus encoded, the situation is quite different in algae: their plastid genomes can include *ftsI*, *ftsW*, *sepF*, *minD* and *minE* genes (Miyagishima et al., 2012). Recently, we asked whether the absence of *minD* and *minE* from the plastid genome – now encoded by the nucleus or entirely lost – could be a prerequisite for the evolution of polyplastidy (de Vries et al., 2016). We speculated that EGT of *minD* to the nucleus could be essential to gain more control over plastid function and division. In the context of the monoplastidic bottleneck (Fig. 2), this means that a desynchronization ('de-sync') of plastid and nuclear division was only feasible through additional control mechanisms exercised by the nucleus. This stands in stark contrast to the morphologically similar, but autonomy-based primordial presynchronization ('pre-sync') state of the early endosymbiont inside the host.

We screened all 999 species of Archaeplastida and secondary plastid-bearing lineages for which plastid genomes were available for: (1) the presence of *minD* and *minE* genes in their plastid genomes and (after excluding most of the numerous chlorophyceaen and land plants species with sequenced plastid genomes) the

morphological characters of the remaining 131 species for (2) mono-, bi- or poly-plastidy, and (3) unicellularity, colonial morphology or multicellularity (de Vries et al., 2016) (Fig. 1B). Algae that contain *minD* and/or *minE* in their plastid genome are exclusively monoplastidic and limited to primary green Chlorophyta and secondary red Hacrobia (see Glossary in Box 1). In contrast, secondary green chloroplast genomes of Euglenozoa lack *minD* and *minE*, and the cells are predominantly polyplastidic (Fig. 1A,B). The case of secondary red Hacrobia is peculiar. Only the plastid genomes of Hacrobia (Okamoto et al., 2009; Burki et al., 2012) and *P. chromatophora* encode *minD* and *minE*. In fact, among all Archaeplastida-derived plastids, the plastid genomes of cryptophytes are the only ones to still encode *minE*. The deep branching of cryptophytes among photosynthetic eukaryotes (Stiller et al., 2014; Burki, 2014) and certain traits, such as the coding of plastid protein import machinery components in the nucleomorph and their plastid membrane topology (Gould et al., 2015), suggest they represent an ancestral state to which the presence of *minD* and *minE* in the plastid genome corresponds.

We know that Hacrobia, (e.g. the coccolithophore *Emiliana*), stramenopiles and some Alveolata (including the causal agent of malaria, *Plasmodium*; McFadden, 2011) all harbour a red complex plastid that ultimately traces back to the monophyletic, endosymbiotic incorporation of a rhodophyte by a host of unknown nature (Burki et al., 2012; Zimorski et al., 2014; Archibald, 2015). However, the chronology and number of potential tertiary and maybe even quaternary endosymbiosis events (3° ES and 4° ES, respectively; Fig. 1A) that succeeded the initial secondary ES event, which established the red complex plastid, remain disputed (Burki et al., 2012; Stiller et al., 2014; Gould et al., 2015; Archibald, 2015). It was recently suggested that haptophytes acquired their plastids from ochrophytes (which unite stramenopiles, pelagophytes and kelp) through quaternary ES (Stiller et al., 2014). However, no stramenopile plastid genome contains either a *minD* or *minE* homolog (Fig. 1B) and the nucleus-encoded *minD* and *minE* are furthermore of mitochondrial origin (Leger et al., 2015). This means that haptophytes would have acquired a stramenopile with a plastid that was unlike any of those known today. We cannot formally rule out this possibility, but it does argue against a quaternary endosymbiotic origin of the haptophyte plastid. Besides, if we accept additional layers of endosymbioses (3° and 4° ES), the important question arises: why was the same initial red complex plastid of rhodophyte origin (and similar to the one found in cryptophytes such as *Guillardia theta*) always passed around the individual hosts?

If none of the primary red algae have a *minD* or *minE* homolog in their plastid genome, where does the homolog in the secondary red Hacrobia come from – considering that EGT is genetically and evolutionarily a one-way process (Martin et al., 1998)? The Cyanidiales are on the deepest branch in red algal phylogenies (Yoon et al., 2006). Among the Cyanidiales, *Galdieria sulphuraria* is the only red alga that still harbours some remnants of a *minD* homolog (Leger et al., 2015) (Fig. 1). Using *Galdieria theta minD* as query for a tBLASTn screen versus *Galdieria sulphuraria*, the *Galdieria minD* pseudogene is retrieved with a query coverage of 83% and an average local identity of 46% over two stretches from different reading frames. When the aligned (nucleotide) sequence is used as a query for a BLASTx screen against *G. theta*, it returns *Gt*MinD as the best (and only meaningful) hit. This suggests an orthologous relationship between the two (it is noteworthy that a tBLASTn screen using *Gt*MinD as query against all bacteria in the non-redundant dataset returns *Gloeomargarita lithophora* minD as

the top hit). The most parsimonious assumption is that this putative orthologous relationship is independent of HGT and based on plastid inheritance. Together with the deep-branching position of *G. sulphuraria* (Yoon et al., 2006), these data suggest that secondary red plastids probably stem from an extinct or non-sampled red algal lineage before these genes were lost or transferred to the host nucleus. In summary, *minD* and *minE* are prime candidates for future studies on (1) plastid autonomy with regard to its division, (2) its connection to the transition from mono- to poly-plastidy, and (3) the evolution of red complex plastids.

Multicellularity and polyplastidy are not coupled
Plastids evolved in a unicellular eukaryote. Indeed, in almost any lineage, basal-branching algae are unicellular (Fig. 1B). The Glaucophyta are exclusively unicellular (Jackson et al., 2015). The deepest branching Rhodophyta, the Cyandiales (Yoon et al., 2006), are unicellular, too. In the third lineage, the Chloroplastida, the picture becomes more complicated, with the recent placement of the multicellular Palmophyllaceae (having a mass of unicells in a gel matrix) at the base of all Chlorophyta (Leliaert et al., 2016). Yet, palmophyllaceaen multicellularity probably represents a derived character state, especially because the most basal-branching streptophyte *Mesostigma viride* (Marin and Melkonian, 1999) is unicellular (Fig. 1B). The same pattern applies to monoplastidy: based on the characters of basal-branching streptophytes (*M. viride;* Marin and Melkonian, 1999) and chlorophytes (the prasinophytes; Leliaert et al., 2012), polyplastidy was not a feature of the common ancestor of all Chloroplastida (Leliaert et al., 2011, 2012).

If one draws a simple trajectory of alga and plant evolution, one might get the impression that there was a clear overall increase in morphological complexity, and that polyplastidy evolved as a by-product. This, however, is not the case (Box 3). The green and red lineages evolved multicellularity multiple times independently (Lewis and McCourt, 2004; Leliaert et al., 2012; Cock and Collén, 2015), just as several single algal classes did (e.g. the ulvophytes) based on ancestral character state inferences (Cocquyt et al., 2010). However, multicellularity and polyplastidy do not go hand in hand but rather originated multiple times independently throughout evolution (Box 3). The next section explores what these independent origins imply with regard to proper organelle inheritance.

Lessons from mitochondrial inheritance
Mitochondria and plastids face similar challenges. The genomes of the organelles are in danger of mutational meltdown as a result of Muller's ratchet (see Glossary in Box 1) (Lynch et al., 1993; Lynch, 1996; Martin and Herrmann, 1998), concomitant with an elevated risk of accumulating mutations induced by reactive oxygen species (Aro et al., 1993; Apel and Hirt, 2004; Balaban et al., 2005). One can assume that evolution favoured those organisms that evolved mechanisms that protect the integrity of the rudimentary genomes of these organelles. For mitochondria, many ideas have been formulated and some even experimentally tested (Box 4). Furthermore, the mutational baggage in mitochondrial DNA (mtDNA) accumulating over a lifetime has repeatedly been associated with aging (Harman, 1956, 1972; Balaban et al., 2005).

There are obvious limits in transferring animal-centric information to the analysis of plastids of plants and algae. First, in many plastid-housing eukaryotes, the mutation rates of organelles are quite low (Smith, 2015). This could, however, also be the result of successful genetic mechanisms that assure quality (Box 4); assuming that the mutation rate of a given mtDNA molecule is low would be a type of *post hoc* fallacy. Second, there is lack of an

Box 4. The genetic bottleneck in mitochondrial inheritance

The spread of detrimentally mutated mitochondria in populations could be averted by: (1) preventing organelles from accumulating deleterious mutations in the first place, (2) a mechanism to select for organelles in healthy condition, or (3) a combination of the two. There is some compelling evidence for scenario 1: de Paula and colleagues (de Paula et al., 2013) found that mitochondria in the ovaries, but not sperm cells, of fruit flies and zebrafish stay bioenergetically inactive (i.e. no ATP synthesis through the electron transport chain). This significantly reduces the formation of DNA-damaging reactive oxygen species. It is thought that these inactive mitochondria then serve as templates for the next generation. In scenario 2, there exists a concept of a mitochondrial genetic bottleneck occurring in animal germ cells, after which the intracellular population of mitochondria (mt) and/or mtDNAs is reduced. According to this concept, the mutational load in this reduced set of mtDNAs is thought to have a physiological effect on a given population of germ cells, thus inducing selection that favours germ cells with fit mitochondria (Bergstrom and Pritchard, 1998). However, there is currently no consensus on when and how this genetic bottleneck occurs. A significant reduction in mtDNA at some point during germline development in mice has been described (Cree et al., 2008; Wai et al., 2008), although these data have been challenged (Cao et al., 2009). In *Drosophila*, selective proliferation favours non-mutated mtDNA and endorses selection at the organellar, instead of cellular, level (Hill et al., 2014). Regardless of the details of these controversies, a genetic bottleneck in mitochondrial inheritance probably exists (Stewart and Larsson, 2014).

immediate (i.e. embryonic) separation of germ and soma cells. Third, most algae are unicellular (and monoplastidic) and selection acts immediately. The formulation of models on such a germline-based separation is therefore tricky, although a mechanism that ensures the inheritance of only fit organelles in protists is generally feasible. In budding yeast, especially those mitochondria with a high membrane potential ($\Delta\Psi$), which is indicative of bioenergetic vigour, are passed on to the daughter cell through their localisation to the budding site (Higuchi-Sanabria et al., 2016). Furthermore, the genetic mechanisms discussed in Box 4 also work in unicellular and monoplastidic species such as *Chlamydomonas* that nonetheless bear multiple plastid genome copies (VanWinkle-Swift, 1980; Birky, 2001). But what about such mechanisms in land plants?

Is there a quality control checkpoint for plastid inheritance in land plants?

As for land plants, the number of proplastids in a seed is rather small (~10 versus ~200 chloroplasts found in a mesophyll cell, Possingham, 1980) and that number might be actively reduced (Mogensen, 1996). This raises the question of whether there is a connection between the (ancestral) monoplastidic bottleneck and the genetic bottleneck during organelle inheritance, especially in polyplastidic organisms. Owing to a lack of dedicated studies on the issue this cannot be easily answered, but there are numerous mechanisms to selectively restrict plastid numbers in germ cells; furthermore, plants are generally highly efficient at obtaining homoplasmy (a stage at which all plastid genome copies are identical) after several rounds of cell division (Greiner et al., 2015). Some basal-branching embryophytes are monoplastidic in those cells that are important for spore production (Brown and Lemmon, 1990; Smith 2011a; Smith et al. 2011b) and, in the case of some lycophytes, also in their meristematic tissue (Brown and Lemmon, 1984, 1985). Furthermore, spores and gametes of the multicellular and polyplastidic brown alga *Ectocarpus siliculosus* are also monoplastidic, arguing for

a potentially analogous need for monoplastidy as a quality control checkpoint during the reproductive cycles of *Ectocarpus* (Baker and Evans, 1973; Maier, 1997). Quality control mechanisms to ensure the inheritance of organelles free of mutations established at the early time point in the evolution of the monoplastidic bottleneck would benefit a genetic bottleneck during plastid inheritance. We conclude that the reduction of plastid number in gametes and spores to one is a required reversion back to monoplastidy. However, to what degree some of these functions are conserved, and whether they still act in seed plants remains to be determined – but this surely presents a worthwhile research target.

Conclusions and outlook

The presence of dozens or hundreds of plastids per cell might be perceived as being the norm, but algae and some non-seed plants tell us otherwise. It remains to be investigated how these macrochloroplasts are integrated into signalling pathways that determine the chloroplast compartment size (space and/or volume in a cell that is devoted to chloroplasts) such as the recently described REDUCED CHLOROPLAST COVERAGE (REC) proteins (Larkin et al., 2016). In that context, it is noteworthy that mutants with macrochloroplasts appear to have a similar chloroplast compartment size (Pyke and Leech, 1992). This argues for an additional (possibly REC-dependent) layer of nuclear control over plastid function. What are the benefits of many small versus a few (or one) big plastids in the first place? A larger internal membrane system, for example, for more photosystem complexes, can easily be accommodated in a macrochloroplast. Also, the *in situ* control of gene regulation (in accordance with the CoRR hypothesis, see Glossary in Box 1; Allen, 2015) should be no hurdle, as nucleoids could be distributed along the intermembranes. One of the advantages of having multiple (small) plastids was previously mentioned in the context of EGT: if one organelle lyses for any reason a polyplastidic cell has a back-up. Furthermore, there is some evidence that having a large population of small chloroplasts (rather than a small population of big chloroplasts) allows for more-effective chloroplast relocation-mediated light acclimation and avoidance of photoinhibition (Jeong et al., 2002; Königer et al., 2008), which supports the idea that land plant plastid biology was shaped by stressors typical for terrestrial habitats (cf. de Vries et al., 2016).

There exists a peculiar monoplastidic bottleneck in algae and plant evolution; its reason and extent remains to be fully explored. This monoplastidic bottleneck occurred in the ancestor of Archaeplastida, at a point when the majority of the regulatory mechanisms that govern plastid division (and thereby plastid number) were implemented. Control of plastid number relies upon the modification of the fission machinery that the plastid had encoded within its genome. Subsequently, these genes were complemented by the host through proteins such as those of the dynamin family and the PVDs with the emergence of land plants. To a degree, plastid inheritance follows principles that also apply to mitochondria (rarely studied simultaneously) and both are guided by uniparental inheritance, which is, however, far more involved for organelles of cyanobacterial origin. Whether uniparental inheritance of plastids, akin to mitochondrial inheritance, is associated with the monoplastidic bottleneck is speculative, because a systematic study on the topic is currently lacking. In our opinion, the presence of a monoplastidic checkpoint, even in higher embryophytes (e.g. in the zygote), cannot entirely be ruled out. If such a checkpoint indeed exists, but is no longer present in embryophytes, how did they escape monoplastidy? Furthermore, did the same or a radically different solution evolve in those

algae that escaped the monoplastidic bottleneck independently? The occurrence of polyplastidy coincides with a complex and macroscopic morphology, both in rhodophytes and streptophytes, underscoring the impact of this transition. A testable prediction from our rationale is that the molecular regulation of plastid division in those monoplastidic plant cells is homologous to that established at the monoplastidic bottleneck event. Comparative studies hold the potential to uncover the molecular basis established during the monoplastidic bottleneck, and to what degree it continues to influence and define plastid inheritance in land plants.

Supplementary information
Supplementary information available online at
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Table S1. List of species referred to in this study

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