

## The plastid genomes of nonphotosynthetic algae are not so small after all

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### ABSTRACT

The thing about plastid genomes in nonphotosynthetic plants and algae is that they are usually very small and highly compact. This is not surprising: a heterotrophic existence means that genes for photosynthesis can be easily discarded. But the loss of photosynthesis cannot explain why the plastomes of heterotrophs are so often depauperate in noncoding DNA. If plastid genomes from photosynthetic taxa can span the gamut of compactness, why can't those of nonphotosynthetic species? Well, recently we showed that they can. The free-living, heterotrophic green alga *Polytoma uvella* has a plastid genome boasting more than 165 kilobases of noncoding DNA, making it the most bloated plastome yet found in a heterotroph. In this addendum to the primary study, we elaborate on why the *P. uvella* plastome is so inflated, discussing the potential impact of a free-living vs. parasitic lifestyle on plastid genome expansion in nonphotosynthetic lineages.

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The evolutionary forfeiting of photosynthesis is typically followed by severe reduction or outright loss of the plastid genome.<sup>1,2</sup> Indeed, the smallest plastid DNAs (ptDNAs) observed to date all come from nonphotosynthetic plants and algae. For instance, the nonphotosynthetic parasite *Helicosporidium* sp. has by far the most miniscule ptDNA (37.4 kb) ever found in a green alga.<sup>3</sup> Even tinier ptDNAs have been uncovered in apicomplexan parasites, such as *Plasmodium falciparum* (34.2 kb)<sup>4</sup> and *Eimeria tenella* (34.7 kb),<sup>5</sup> as well as in heterotrophic land plants, including the orchid *Epipogium roseum* (19 kb)<sup>6</sup> and the holoparasite *Pilostyles aethiopica* (11.4 kb).<sup>7</sup>

The diminutive ptDNAs of nonphotosynthetic species are a consequence of 2 distinct processes: (i) Plastid gene loss, which mostly pertains to the disappearance of genes connected to photosynthesis, but can also involve the loss, or plastid-to-nucleus transfer, of genes unrelated to photosynthesis.<sup>1</sup> And (ii) the widespread erosion of noncoding ptDNA, including intergenic and intronic regions.<sup>3</sup>

Taken to its ultimate extreme, process (i) can eventually result in the complete forfeiture of a plastid genome—an extremely rare event, but one that is thought to have

occurred in the holoparasitic land plant *Rafflesia lagascae*<sup>8</sup> and in members of the nonphotosynthetic green algal genus *Polytomella*.<sup>9</sup> Conversely, the contraction of noncoding ptDNA in nonphotosynthetic taxa should not have a direct impact on whether a plastid genome is lost or maintained. In fact, one would expect there to be extensive plastid gene loss following the transition from a photosynthetic to nonphotosynthetic lifestyle, but it is not obvious why such a transition should promote the wholesale deletion of noncoding DNA from a plastid genome. One might also ask: why don't heterotrophs dispose of their plastids entirely? The reason being that plastids are a hub for many essential biochemical pathways apart from photosynthesis, such as isoprenoid biosynthesis.<sup>10</sup>

Like their counterparts in heterotrophic species, the plastid genomes of photosynthetic eukaryotes can also contain very little noncoding DNA, as exemplified by some red algae (e.g., *Cyanidioschyzon merolae*)<sup>11</sup> and prasinophytes (e.g., *Ostreococcus tauri*).<sup>12</sup> But the genomes from photosynthetic plastids can be bloated as well, as seen in some chlamydomonadalean green algae, such as *Volvox carteri* (~525 kb with >80% noncoding).<sup>13</sup> If the ptDNAs from photosynthetic species can span the full gamut of compactness then why can't those

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from nonphotosynthetic lineages? Well, it turns out that they can.

A recent analysis of the nonphotosynthetic chlamydomonadalean *Polytoma uvella* revealed an uncharacteristically large and inflated plastid genome: ~230 kb, 75% noncoding DNA.<sup>14</sup> What's more, the ptDNA of *P. uvella* is 60 kb larger than that of one of its closest photosynthetic relatives, *Chlamydomonas leiostraca*—a trend that had not previously been observed in any other close photosynthetic-nonphotosynthetic duo. *P. uvella* is also a close relative of *Polytomella*, but each of these 2 lineages lost photosynthesis independently of one another and, at first glance, their plastid genomic architectures could not be more different: expansion and complete deletion, respectively.

Despite its large size, the *P. uvella* plastid genome has, like other nonphotosynthetic ptDNAs, undergone significant gene loss, shedding all coding regions for photosynthetic pathways. However, unlike the ptDNAs from other nonphotosynthetic species, that of *P. uvella* does not appear to have experienced rampant contraction of its noncoding regions—rather, the opposite: its intergenic spacers are expanded relative to those of *C. leiostraca*.<sup>14</sup> But why?

Perhaps the contraction of noncoding DNA in the plastids of heterotrophic species has less to do with the loss of photosynthesis and more to do with another life-history feature: parasitism. Most of the known heterotrophic plastid-bearing lineages are parasitic.<sup>1,2,15</sup> And, with some exceptions,<sup>16,17</sup> the transition from a free-living to a parasitic existence is associated with genomic compaction—a trend that has been recorded across all domains of life and for all types of genomes, particularly the genomes of obligate parasites.<sup>18,19</sup> *P. uvella*, however, is not a parasite. It is free living and there is no reason to believe that it had a recent parasitic ancestor.<sup>10</sup> Thus, the lack of genomic compaction in this colourless alga might partly be a consequence of it not being a parasite.

The absence of parasitism certainly does not preclude a plastid genome from being compact, be it in a nonphotosynthetic or a photosynthetic species. There are examples of nonparasitic colourless plants and algae with very little noncoding DNA in their plastid genomes.<sup>20,21</sup> But the switch to a parasitic lifestyle, in many cases, probably contributes to the extreme genomic compaction found in some ptDNAs.<sup>22</sup> It is worth noting that parasitic algae are nearly always endobiotic whereas nonphotosynthetic land plants are typically ectoparasites, surviving via myco-heterotrophy or through the direct parasitism of other plants. Nevertheless, both endo- and ecto-parasitic modes of existence have been associated with extreme plastomic reduction.<sup>6,3</sup>

*Polytomella* species are free living and have likely lost their plastid genomes,<sup>13</sup> which might seem to be a contradiction to some of the arguments made above. But, again, the contraction (or expansion) of noncoding DNA is not the driving force for plastid genome loss, and there is no reason to think that the ancestral ptDNA of *Polytomella* spp. did not have an expanded architecture before being jettisoned. In fact, the order to which both *Polytomella* spp. and *P. uvella* belong—the Chlamydomonadales—has a propensity for plastid genomic inflation, with at least 6 members known to have ptDNAs in excess of 250 kb.<sup>9,23</sup>

There has been much debate about the forces driving organelle genomic expansion, with some arguing that it might be a consequence of random genetic drift, mutation rate, and/or inefficient and finicky DNA maintenance processes.<sup>24</sup> The identification of an inflated ptDNA in a heterotrophic alga only adds a further layer of complexity to the already complicated conundrum of genome size evolution. If anything, the *P. uvella* plastid genome reinforces the idea that no type of chromosome is immune to genomic expansion, even those that exist in the dark.

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