Letters

The enigmatic loss of light-independent chlorophyll biosynthesis from an Antarctic green alga in a light-limited environment

Introduction

Chlorophyll production is a complicated, multifaceted process. Indeed, the cyanobacterial progenitor of chloroplasts bestowed eukaryotic plants and algae with two distinct nonhomologous enzymes for reducing protochlorophyllide to chlorophyllide (the penultimate step of chlorophyll *a* (chl*a*) biosynthesis): light-dependent (LPOR) and light-independent protochlorophyllide oxidoreductase (DPOR) (Armstrong, 1998; Fujita & Bauer, 2003; Reinbothe *et al.*, 2010). The former, which is encoded by the nuclear gene *por*, is employed by all photosynthetic eukaryotes explored to date (Hunsperger *et al.*, 2015) and, as its name implies, is only active when its pigment substrate (protochlorophyllide) absorbs light (Griffiths *et al.*, 1996; Shui *et al.*, 2009). DPOR, conversely, is encoded in the chloroplast genome by the genes *chlL*, *chlN*, and *chlB* (Suzuki & Bauer, 1992; Li *et al.*, 1993), has been lost multiple times independently throughout eukaryotic evolution (Fujita & Bauer, 2003; Ueda *et al.*, 2014; Hunsperger *et al.*, 2015; Kim *et al.*, 2017), and can facilitate chlorophyll synthesis in the dark (Shui *et al.*, 2009; Reinbothe *et al.*, 2010).

The evolutionary origins of LPOR and DPOR are reflected in how they function today. For instance, DPOR, which predates LPOR, is believed to have evolved from a nitrogenase-like enzyme in anoxygenic photosynthetic bacteria (Reinbothe *et al.*, 1996; Fujita & Bauer, 2003), and is why this enzyme is oxygen sensitive (Yamazaki *et al.*, 2006; Yamamoto *et al.*, 2009; Stolárik *et al.*, 2017). Conversely, LPOR first arose in cyanobacteria (Suzuki & Bauer, 1995) whose oxygenic mode of photosynthesis probably provided strong selective pressures for an enzyme that works well in oxygen-rich conditions, which it does (Reinbothe *et al.*, 1996; Yamazaki *et al.*, 2006; Shui *et al.*, 2009). These two enzymes also differ in their sensitivities to light. Again, LPOR's ability to function is contingent on the absorption of light energy by protochlorophyllide (Griffiths *et al.*, 1996), which has maximum absorbances in both the blue and red regions of the visible light spectrum (Koski & Smith, 1948). But research suggests that LPOR is three to seven times more efficient when protochlorophyllide absorbs red light (647 nm) relative to blue light (407 nm) (Hanf *et al.*, 2012), which penetrates deeper into the water column. Unlike LPOR, the efficiency of DPOR is not impaired by differing wavelengths of visible light. DPOR, however, is dependent on iron for constructing iron–sulfur clusters (Fujita & Bauer, 2000), which is not true of the iron moiety-lacking LPOR.

The various differences between LPOR and DPOR can help explain why both enzymes have been maintained in a wide range of photosynthetic eukaryotes for hundreds of millions of years. Nevertheless, DPOR has been lost on multiple occasions. For example, angiosperms and some gymnosperms have surrendered light-independent chlorophyll biosynthesis (Skribanek *et al.*, 2008; Solymosi & Schoefs, 2010; Ueda *et al.*, 2014), as have various algae, with examples from species with primary plastids as well as from those with complex plastids, which are derived from one eukaryote engulfing another (Hunsperger *et al.*, 2015; Kim *et al.*, 2017). The reasons why some lineages have forfeited DPOR while others have retained it are poorly understood. It has been hypothesized that for algae inhabiting iron-depleted environments maintenance of DPOR could be metabolically disadvantageous and, therefore, such conditions might contribute to its loss (Behrenfeld *et al.*, 2006; Bowler *et al.*, 2010; Hunsperger *et al.*, 2015). But for algae living in deep or turbid waters, with limited availability of red light, or those spending extended periods in darkness, having DPOR would seem to be an asset, and could partly account for its widespread conservation across photosynthetic life (Fong & Archibald, 2008; Ueda *et al.*, 2014).

One lineage in which the maintenance of DPOR is particularly prevalent is the chlorophycean class of green algae (Hunsperger *et al.*, 2015; Turmel & Lemieux, 2018). The ability to carry out light-independent chlorophyll production is a reoccurring theme throughout this monophyletic group of mostly freshwater flagellates, including in the model species *Chlamydomonas reinhardtii*, *Volvox carteri*, and *Dunaliella salina* (Turmel & Lemieux, 2018). Therefore, it was surprising when a chlorophycean that has lost DPOR was recently identified, a finding made all the more interesting given the environment from which this alga comes.

A DPOR-less *Chlamydomonas*

In the McMurdo Dry Valleys of Antarctica sits the perennially ice-covered Lake Bonney, which is home to a diversity of microbial life, despite the harsh conditions (Bielewicz *et al.*, 2011; Kong *et al.*, 2014; Dolhi *et al.*, 2015; Li *et al.*, 2016), including the polyextremophilic green alga *Chlamydomonas* sp. UWO241 (hereafter UWO241) (Possmayer *et al.*, 2016; Cvetkovska *et al.*, 2017). Lake Bonney is not for the faint-hearted photosynthesizer. Situated *c.* 17 m below its surface, UWO241 is exposed to continuous cold (*c.* 5°C year round), high salinity (0.7 M), reduced levels of phosphorus, seasonal extremes in photoperiod (e.g. 24-h darkness during the peak austral winter), and perpetual low irradiance

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(< 50 μmol photons m^{-2} s^{-1}), which is biased in the blue-green spectrum (450–550 nm) (Neale & Priscu, 1995). UWO241 is an obligate cold extremophile (psychrophile) and is unable to grow ≥ 18°C. Accordingly, it has evolved an unconventional photosynthetic apparatus, tailored to work best at *c.* 8°C, but its photosynthetic activity is severely inhibited at moderate temperatures. It can also rapidly repair photosystem II reaction centres, therefore avoiding photoinhibition at low temperatures (Morgan *et al.*, 1998; Pocock *et al.*, 2007; Possmayer *et al.*, 2011). Even more unconventional is the inability of UWO241 to undergo photosynthetic state transitions (from state 1 to state 2), which balance the energy distribution between photosystems I and II (Morgan-Kiss *et al.*, 2002). Instead, UWO241 achieves optimal rates of photosynthesis by maintaining high cyclic electron flow via a novel PSI supercomplex (Szyszka-Mroz *et al.*, 2015). Remarkably, given its light-restricted environment, our present work suggests that this alga has also lost the ability to carry out the light-independent synthesis of chl*a*.

Our sequencing, assembly, and annotation of the entire UWO241 chloroplast genome failed to identify the three genes encoding DPOR (*chlL*, *chlN* and *chlB*) from an otherwise standard and full set of chloroplast coding regions (GenBank accession no. MH590838; Supporting Information Fig. S1; Methods S1). Exhaustive searches of our draft nuclear genome and transcriptome sequences for UWO241 also failed to locate *chlL*, *chlN* or *chlB* (Table S1; Methods S1), indicating that these genes have not migrated to the nuclear compartment, which would have been unprecedented if it were the case. Even a search of the mitochondrial DNA (mtDNA) (GenBank accession no. MH598508; Fig. S2), which in some species can sometimes acquire chloroplast genes, came up empty for the three DPOR-encoding loci. We screened the nuclear genome for other genes encoding enzymes involved in chlorophyll biosynthesis and uncovered a conserved pathway between UWO241 and its close relatives (Table S2).

In some algae that lack DPOR, including certain haptophytes and stramenopiles, the *por* gene is duplicated, and it has been hypothesized that this duplication might compensate for the loss of DPOR, potentially by allowing for the differential regulation of *por* genes (Hunsperger *et al.*, 2015). We found no evidence in the transcriptome or genome data that *por* is duplicated in UWO241. The single *por* gene and its deduced amino acid sequence are complete and similar to their counterparts in other chlorophycean algae (Fig. S3) (e.g. 73.4% pairwise identity with *Chlamydomonas eustigma*). We did, however, uncover two other duplicated genes: GENOMES UNCOUPLED (*GUN4*), encoding a regulatory subunit of Mg-chelatase that enhances chlorophyll biosynthesis and contributes to retrograde signalling (Formighieri *et al.*, 2012; Brzezowski *et al.*, 2014), and chlorophyllide *a* oxygenase (*CAO*), which is responsible for the production of chl*b* (Tanaka *et al.*, 1998; Bujaldon *et al.*, 2016). To the best of our knowledge, this is the first report of duplication of these genes in a green alga. From these data, it is clear that UWO241 has a functional chlorophyll biosynthesis pathway but has lost DPOR and is solely dependent on LPOR for the enzymatic reduction of protochlorophyllide (Fig. S5).

On a side note, the UWO241 ptDNA, at 174 kb, is the second smallest plastome identified from the Chlamydomonadales – a group renowned for harbouring some of the largest plastomes on record, including those of *Volvox carteri* (*c.* 525 kb) (Smith & Lee, 2010) and *Haematococcus lacustris* (1352 kb) (Bauman *et al.*, 2018; Smith, 2018). The comparatively small size of the UWO241 ptDNA reflects its moderate noncoding content (< 50%) rather than the absence of *chlL*, *chlN* and *chlB*, which together represent only a small proportion of the DNA (< 5 kb) of plastomes. It has been argued that the energy limitations from living in a low-light environment contributed to the evolutionary reduction of chloroplast genome size (Marcelino *et al.*, 2016), making the reduced size of UWO241 plastome all the more interesting. The UWO241 mitochondrial genome, however, is the largest and most bloated mitosome observed to date from the Chlamydomonadales (Del Vasto *et al.*, 2015), measuring 59.9 kb and containing more than 75% noncoding DNA (Fig. S2).

There are other clear-cut examples of the loss of DPOR in green algae, including from prasinophytes *Ostreococcus tauri* and *Micromonas pusilla* (Hunsperger *et al.*, 2015) but, to the best of our knowledge, this is the first concrete case from the Chlorophyceae (Table S3). Plastome sequencing has suggested that DPOR was abandoned in the chlorophyceans *Hafniomonas laevis* and *Neochloris aquatica* (Lemieux *et al.*, 2015; Fučíková *et al.*, 2016), as well as certain ulvophytes and pedinophyceans (e.g. *Pedinomonas minor*) (Turmel *et al.*, 2017); however, nuclear and mitochondrial genome analyses are needed to confirm these findings. It is notable that *N. aquatica* has been isolated in multiple places on mainland Antarctica, including frozen ponds (Campbell & Claridge, 1987). The absence of DPOR has also been documented in algae with complex plastids, such as the cryptophyte *Guillardia theta*, the stramenopile *Phaeodactylum tricornutum*, and the haptophyte *Emiliania huxleyi* (Fong & Archibald, 2008; Hunsperger *et al.*, 2015; Kim *et al.*, 2017). Most of these algae are marine phytoplankton that are often found in iron-depleted ocean environments where the costs of producing an iron-requiring DPOR protein may outweigh its benefits (Behrenfeld *et al.*, 2006; Bowler *et al.*, 2010). Understanding the loss of light-independent chlorophyll biosynthesis from UWO241 – an alga from a light-limited environment – is not so straightforward. To understand the absence of DPOR in this alga we need to carefully examine its extreme environment.

A closer look at Lake Bonney

Living in a permanently ice-covered Antarctic lake with continual shading and long periods of sustained darkness would appear to be an ideal place to have DPOR (Ueda *et al.*, 2014). Moreover, the fact that the light penetrating the waters of Lake Bonney is skewed towards the blue-green spectrum where LPOR is thought to be less efficient (Koski & Smith, 1948; Hanf *et al.*, 2012) would seem to make DPOR all the more valuable – not to mention that UWO241 survival depends on blue light, as it reverts to a downregulated photochemical state and is unable to grow in the presence of red light (Morgan-Kiss *et al.*, 2005).

So, why has UWO241 lost this important enzyme? The levels of iron in Lake Bonney at the depth at which UWO241 is found are potentially quite low (Ward *et al.*, 2003; Mikucki *et al.*, 2004). But if an iron deficiency contributed to the loss of DPOR in this species then one might also expect other iron-dependent proteins to have been lost or substituted, particularly the replacement of ferredoxin with flavodoxin, which has occurred in certain algae (La Roche *et al.*, 1993, 1995). This is not the case here: UWO241 has two near-identical copies of the ferredoxin gene, and accumulates high amounts of functional ferredoxin protein, which is thought to be an adaptation to the cold (Cvetkovska *et al.*, 2018). That said, UWO241 does display an iron-stressed phenotype and can show signs of iron stress even under conditions that are iron replete (Cook, 2018). Moreover, iron stress can be exacerbated by high salinity (Tripathi *et al.*, 2018) and, as already noted, the depth at which UWO241 was isolated is hypersaline.

However, Lake Bonney does have one striking feature that could shed some light on the loss of DPOR in UWO241. The dissolved oxygen concentration over the first 15 m is very high (> 1000 μM), *c.* 250–350% higher than would be predicted if the lake was not ice covered and saturated with air above its surface (Morgan-Kiss *et al.*, 2006), and it remains high (*c.* 200% air saturation) at 17 m, where UWO241 is found. This is significant because, as noted earlier, the Achilles' heel of DPOR is its oxygen-sensitive iron−sulfur cluster (Yamazaki *et al.*, 2006; Ueda *et al.*, 2014; Stolárik *et al.*, 2017), whereas LPOR is insensitive to oxygen. If the high dissolved oxygen content of Lake Bonney inhibits the functioning of DPOR, then one would presume that there would be no deleterious effect resulting from a mutation that knocked out the DPOR pathway. This nonadaptive scenario could explain why we did not find the *chlL*, *chlN* or *chlB* genes in UWO241: their sequences have slowly eroded through the steady accumulation of neutral mutations. Of course, if this hypothesis is correct, then the DPOR from not just UWO241 but from other photosynthetic species within Lake Bonney should also be rendered nonfunctional by the high oxygen concentrations of the water above the chemocline and therefore be susceptible to knockout. This reasoning fits well with previous hypotheses arguing that certain land plants and algae have lost DPOR because present-day atmospheric oxygen levels are incompatible with the oxygen-sensitive DPOR enzyme (Reinbothe *et al.*, 1996; Schoefs & Franck, 2003; Hunsperger *et al.*, 2015). Finally, there also is some evidence that DPOR might be cold-sensitive, at least in land plants (Muramatsu *et al.*, 2001).

It is also highly possible that the loss of DPOR was not brought about by the conditions of Lake Bonney and that it did not specifically occur in UWO241 or in the ancestral 'Lake Bonney' lineage that gave rise to UWO241. Rather, DPOR may have already been absent in the ancestral 'high-light' *Chlamydomonas* population that colonised Antarctica and eventually led to the present-day UWO241 strain inhabiting Lake Bonney. Support for this hypothesis comes from phylogenetic analyses showing that the closest known relatives of UWO241 are marine species (e.g. *Chlamydomonas parkeae*) known to exist in shallow water (Possmayer *et al.*, 2016), which is typically an environment rich in red light and, therefore, one favoring LPOR over DPOR. Currently, there are no available chloroplast genome sequences for any close marine relatives of UWO241. The closest relative for which there are ptDNA data is *Chlamydomonas moewusii*, a freshwater species encoding DPOR (Boudreau *et al.*, 1994).

Other potential examples from Lake Bonney and beyond

Lake Bonney harbours a diversity of photosynthetic eukaryotes, including chlorophytes, cryptophytes, haptophytes, and stramenopiles, which have been shown to be vertically stratified within the water column (Bielewicz *et al.*, 2011; Kong *et al.*, 2014; Dolhi *et al.*, 2015; Li *et al.*, 2016). Cryptophytes, for instance, dominate the nutrient-deficient shallower water (6–10 m), whereas haptophytes and stramenopiles occupy the mid-depths (*c.* 13 m), and chlorophytes reside in the deepest layers (15–20 m) of the photic zone (Bielewicz *et al.*, 2011), below which the lake becomes anoxic. UWO241 is currently the only photosynthetic protist from Lake Bonney to have its chloroplast genome completely sequenced. Therefore, it is not known if other eukaryotic algae in the lake (or any of the other lakes in the McMurdo Dry Valleys) have lost DPOR. One of the most prolific stramenopiles within Lake Bonney belongs to the genus *Nannochloropsis* (Kong *et al.*, 2012). It is noteworthy, in this context, that the six available plastome sequences from *Nannochloropsis* species all contain the genes *chlL*, *chlN*, and *chlB* (Wei *et al.*, 2013), but it should be stressed that the presence/absence of DPOR can occur even among members of the same genus.

UWO241 is not the only chlamydomonadalean in Lake Bonney. *Chlamydomonas* sp. ICE-MDV is, in fact, the dominant chlorophyte in the lake (Li *et al.*, 2016). This psychrophile resides at a depth of 13–15 m where the dissolved oxygen concentration is even higher and the iron levels lower than in the deeper photic zone where UWO241 is located. Although data on *Chlamydomonas* sp. ICE-MDV are limited, it has been shown that it can grow under a broad range of light intensities but cannot grow in the dark in the presence of organic carbon (Li *et al.*, 2016). Given all of this, it will be especially interesting to see if *Chlamydomonas* sp. ICE-MDV has forfeited DPOR, but this information will only be useful alongside detailed phylogenetic data on this species and on its relationship to UWO241. Its namesake, *Chlamydomonas* ICE-L (arguably the best studied psychrophilic green alga) does encode DPOR, but unlike UWO241 and ICE-MDV, it is found on Antarctic sea ice where the oxygen concentrations are not extremely high, and it hails from a different chlamydomonadalean clade (the Monadinia) than that of UWO241 (the Moewusinia) (Zhang *et al.*, 2018).

The physiological consequences of an unusual chlorophyll biosynthesis pathway

Whatever the evolutionary explanation for jettisoning DPOR, surely its absence in UWO241 has impacted this alga's ability to produce chlorophyll and efficiently perform photosynthesis in a light-limited environment. In addition to the loss of DPOR, our genomic characterization of the chlorophyll biosynthesis pathway in UWO241 revealed the duplication of *CAO* (Figs S4, S5), encoding an indispensable regulatory gene for chl*b* production.

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Previous work showed that UWO241 has a normal complement of photosynthetic pigments but exhibits a low chl*a* : *b* ratio (*c.* 1.8–2.2) compared with other green algae (> 3). Moreover, chl*b* is exclusively associated with the light-harvesting antenna around PSII (Pocock *et al.*, 2007; Szyszka *et al.*, 2007). Chl*b* absorbs blue light efficiently, and higher amounts of this pigment are typically associated with shade adaptation (Falkowski & Owens, 1980). It is tempting to speculate that the duplication of *CAO* could be responsible for the constitutively increased levels of chl*b* in UWO241 and could be an adaptation to maximize light absorption in the depths of Lake Bonney, but a detailed functional analysis of the CAO enzymes will be needed to make such assertions.

Recent field experiments on the acclimation of natural algal populations and transplanted samples of UWO241 within Lake Bonney demonstrated that seasonal chl*a* accumulation trends were uncoupled from light availability during the polar night transition and continued to increase as light diminished (Morgan-Kiss *et al.*, 2016). The accumulation of chlorophyll at the end of the growing season may be indicative of photoacclimation to extreme shade (i.e. increase in chlorophyll amounts per cell), but exactly how UWO241 achieves this, particularly without the use of DPOR, remains to be determined. This work led to the development of a model suggesting that during light−dark transitions, UWO241 undergoes a cascade of physiological and molecular alterations to the photosynthetic apparatus, keeping it in a downregulated but functional form that can be rapidly reactivated by sunlight (Morgan-Kiss *et al.*, 2006, 2016). Such a strategy could be advantageous in polar environments where the growing season is short. Developing methodologies to study chlorophyll production, retention and degradation in UWO241 and related algae from Lake Bonney during transition to complete darkness would provide the experimental evidence to support this theory.

These seasonal trends in chl*a* abundance will become all the more interesting once there are data on the presence−absence of DPOR from additional Lake Bonney phytoplankton. Further work may show that UWO241 is not unique in its inability to carry out light-independent chlorophyll biosynthesis, but there should be some caution in assuming that what is true for UWO241 is also true for its photosynthetic counterparts in Lake Bonney and beyond, especially with respect to photosynthesis. As already mentioned, UWO241 is unique among all explored natural photosynthetic eukaryotes in that it cannot undergo photosynthetic state transitions (i.e. it is permanently locked in state 1) (Morgan-Kiss *et al.*, 2002). Undoubtedly, the more we learn about this cold-loving biflagellate, the more atypical it turns out to be, even compared with other extremophilic *Chlamydomonas* species. For now, we will have to wait and see just how unconventional the loss of DPOR is in the context of the oxygen-rich lakes of the McMurdo Dry Valleys and to close marine relatives of UWO241. But, hopefully, we will not be in the dark for long.

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Author contributions

DRS, MC and NPAH conceived and planned the experiments and wrote the initial draft of the manuscript. SO helped assemble the chloroplast genome. All authors contributed to writing and revising the manuscript.

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References

Armstrong GA. 1998. Greening in the dark:light-independent chlorophyll biosynthesis from anoxygenic photosynthetic bacteria to gymnosperms. *Journal of Photochemistry and Photobiology B: Biology* **43**: 87–100.

Bauman N, Akella S, Hann E, Morey R, Schwartz AS, Brown R, Richardson TH. 2018. Next-generation sequencing of *Haematococcus lacustris* reveals an extremely large 1.35-megabase chloroplast genome. *Genome Announcements* **6**: e00181-18.

Behrenfeld MJ, Worthington K, Sherrell RM, Chavez FP, Strutton P, McPhaden M, Shea DM. 2006. Controls on tropical Pacific Ocean productivity revealed through nutrient stress diagnostics. *Nature* **442**: 1025.

Bielewicz S, Bell E, Kong W, Friedberg I, Priscu JC, Morgan-Kiss RM. 2011. Protist diversity in a permanently ice-covered Antarctic lake during the polar night transition. *The ISME Journal* **5**: 1559.

Boudreau E, Otis C, Turmel M. 1994. Conserved gene clusters in the highly rearranged chloroplast genomes of *Chlamydomonas moewusii* and *Chlamydomonas reinhardtii*. *Plant Molecular Biology* **24**: 585–602.

Bowler C, Vardi A, Allen AE. 2010. Oceanographic and biogeochemical insights from diatom genomes. *Annual Review of Marine Science* **2**: 333–365.

Brzezowski P, Schlicke H, Richter A, Dent RM, Niyogi K, Grimm B. 2014. The GUN4 protein plays a regulatory role in tetrapyrrole biosynthesis and chloroplast-to-nucleus signalling in *Chlamydomonas reinhardtii*. *The Plant Journal* **79**: 285–298.

Bujaldon S, Kodama N, Rappaport F, Subramanyam R, de Vitry C, Takahashi Y, Wollman F-A. 2016. Functional accumulation of antenna proteins in chlorophyll *b*-less mutants of *Chlamydomonas reinhardtii*. *Molecular Plant* **10**: 115–130.

Campbell IB, Claridge GGC. 1987. *Antarctica: soils, weathering processes and environment. Development in soil science, vol. 16.* Amsterdam, the Netherlands: Elsevier Science.

Cook GP. 2018. *Antarctic* Chlamydomonas *strains c. sp. UWO241 and ICE-MDV exhibit differential restructuring of the photosynthetic apparatus in response to iron.* MSc thesis, MIAMI University, Oxford, OH, USA.

Cvetkovska M, Hüner NPA, Smith DR. 2017. Chilling out: the evolution and diversification of psychrophilic algae with a focus on Chlamydomonadales. *Polar Biology* **40**: 1169–1184.

Cvetkovska M, Szyszka-Mroz B, Possmayer M, Pittock P, Lajoie G, Smith DR, Hüner NPA. 2018. Characterization of photosynthetic ferredoxin from the Antarctic alga *Chlamydomonas* sp. UWO241 reveals novel features of cold adaptation. *New Phytologist* **219**: 588–604.

Del Vasto M, Figueroa-Martinez F, Featherston J, Gonzalez MA, Reyes-Prieto A, Durand PM, Smith DR. 2015. Massive and widespread organelle genomic expansion in the green algal genus *Dunaliella*. *Genome Biology and Evolution* **7**: 656–663.

Dolhi JM, Teufel AG, Kong W, Morgan-Kiss RM. 2015. Diversity and spatial distribution of autotrophic communities within and between ice-covered Antarctic lakes (McMurdo Dry Valleys). *Limnology and Oceanography* **60**: 977–991.

Falkowski PG, Owens TG. 1980. Light–shade adaptation: two strategies in marine phytoplankton. *Plant Physiology* **66**: 592–595.

Fong A, Archibald JM. 2008. Evolutionary dynamics of light-independent protochlorophyllide oxidoreductase genes in the secondary plastids of cryptophyte algae. *Eukaryotic Cell* **7**: 550–553.

Formighieri C, Ceol M, Bonente G, Rochaix J-D, Bassi R. 2012. Retrograde signalling and photoprotection in a gun4 mutant of *Chlamydomonas reinhardtii*. *Molecular Plant* **5**: 1242–1262.

Fučíková K, Lewis PO, Lewis LA. 2016. Chloroplast phylogenomic data from the green algal order Sphaeropleales (Chlorophyceae, Chlorophyta) reveal complex patterns of sequence evolution. *Molecular Phylogenetics and Evolution* **98**: 176–183.

Fujita Y, Bauer CE. 2000. Reconstitution of light-independent protochlorophyllide reductase from purified BchL and BchN-BchB subunits *in vitro* confirmation of nitrogenase-like features of a bacteriochlorophyll biosynthesis enzyme. *Journal of Biological Chemistry* **275**: 23583–23588.

Fujita Y, Bauer CE. 2003. The light-independent protochlorophyllide reductase: a nitrogenase-like enzyme catalyzing a key reaction for greening in the dark. In: Guilard R, Kadish K, Smith KM, eds. *The porphyrin handbook*. Amsterdam, the Netherlands: Academic Press, 109–156.

Griffiths WT, McHugh T, Blankenship RE. 1996. The light intensity dependence of protochlorophyllide photoconversion and its significance to the catalytic mechanism of protochlorophyllide reductase. *FEBS Letters* **398**: 235–238.

Hanf R, Fey S, Schmitt M, Hermann G, Dietzek B, Popp J. 2012. Catalytic efficiency of a photoenzyme—an adaptation to natural light conditions. *ChemPhysChem* **13**: 2013–2015.

Hunsperger HM, Randhawa T, Cattolico RA. 2015. Extensive horizontal gene transfer, duplication, and loss of chlorophyll synthesis genes in the algae. *BMC Evolutionary Biology* **15**: 16.

Kim JI, Moore CE, Archibald JM, Bhattacharya D, Yi G, Yoon HS, Shin W. 2017. Evolutionary dynamics of cryptophyte plastid genomes. *Genome Biology and Evolution* **9**: 1859–1872.

Kong W, Li W, Romancova I, Prášil O, Morgan-Kiss RM. 2014. An integrated study of photochemical function and expression of a key photochemical gene (*psbA*) in photosynthetic communities of Lake Bonney (McMurdo Dry Valleys, Antarctica). *FEMS Microbiology Ecology* **89**: 293–302.

Kong W, Ream DC, Priscu JC, Morgan-Kiss RM. 2012. Diversity and expression of RubisCO genes in a perennially ice-covered Antarctic lake during the polar night transition. *Applied and Environmental Microbiology* **78**: 4358–4366.

Koski VM, Smith JH. 1948. The isolation and spectral absorption properties of protochlorophyll from etiolated barley seedlings. *Journal of the American Chemical Society* **70**: 3558–3562.

La Roche J, Geider RJ, Graziano LM, Murray H, Lewis K. 1993. Induction of specific proteins in eukaryotic algae grown under iron-, phosphorus-, or nitrogen-deficient conditions. *Journal of Phycology* **29**: 767–777.

La Roche JL, Murray H, Orellana M, Newton J. 1995. Flavodoxin expression as an indicator of iron limitation in marine diatoms. *Journal of Phycology* **31**: 520–530.

Lemieux C, Vincent AT, Labarre A, Otis C, Turmel M. 2015. Chloroplast phylogenomic analysis of chlorophyte green algae identifies a novel lineage sister to the Sphaeropleales (Chlorophyceae). *BMC Evolutionary Biology* **15**: 264.

Li J, Goldschmidt-Clermont M, Timko MP. 1993. Chloroplast-encoded *chlB* is required for light-independent protochlorophyllide reductase activity in *Chlamydomonas reinhardtii*. *Plant Cell* **5**: 1817–1829.

Li W, Podar M, Morgan-Kiss RM. 2016. Ultrastructural and single-cell-level characterization reveals metabolic versatility in a microbial eukaryote community from an ice-covered Antarctic lake. *Applied and Environmental Microbiology* **82**: 3659–3670.

Marcelino VR, Cremen MC, Jackson CJ, Larkum AA, Verbruggen H. 2016. Evolutionary dynamics of chloroplast genomes in low light: a case study of the endolithic green alga *Ostreobium quekettii*. *Genome Biology and Evolution* **8**: 2939–2951.

Mikucki JA, Foreman CM, Sattler B, Lyons WB, Priscu JC. 2004. Geomicrobiology of Blood Falls: an iron-rich saline discharge at the terminus of the Taylor Glacier, Antarctica. *Aquatic Geochemistry* **10**: 199–220.

Morgan RM, Ivanov AG, Priscu JC, Maxwell DP, Hüner NPA. 1998. Structure and composition of the photochemical apparatus of the Antarctic green alga, *Chlamydomonas subcaudata*. *Photosynthesis Research* **56**: 303–314.

Morgan-Kiss RM, Ivanov AG, Hüner NPA. 2002. The Antarctic psychrophile, *Chlamydomonas subcaudata*, is deficient in state I–state II transitions. *Planta* **214**: 435–445.

Morgan-Kiss RM, Ivanov AG, Pocock T, Król M, Gudynaite-Savitch L, Hüner NPA. 2005. The Antarctic psychrophile, *Chlamydomonas raudensis* Ettl (UWO241) (Chlorophyceae, Chlorophyta), exhibits a limited capacity to photoacclimate to red light. *Journal of Phycology* **41**: 791–800.

Morgan-Kiss RM, Lizotte MP, Kong W, Priscu JC. 2016. Photoadaptation to the polar night by phytoplankton in a permanently ice-covered Antarctic lake. *Limnology and Oceanography* **61**: 3–13.

Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, Hüner NPA. 2006. Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiology and Molecular Biology Reviews* **70**: 222–252.

Muramatsu S, Kojima K, Igasaki T, Azumi Y, Shinohara K. 2001. Inhibition of the light-independent synthesis of chlorophyll in pine cotyledons at low temperature. *Plant and Cell Physiology* **42**: 868–872.

Neale PJ, Priscu JC. 1995. The photosynthetic apparatus of phytoplankton from a perennially ice-covered Antarctic lake: acclimation to an extreme shade environment. *Plant and Cell Physiology* **36**: 253–263.

Pocock TH, Koziak A, Rosso D, Falk S, Hüner NPA. 2007. *Chlamydomonas raudensis* (UWO 241), Chlorophyceae, exhibits the capacity for rapid D1 repair in response to chronic photoinhibition at low temperature. *Journal of Phycology* **43**: 924–936.

Possmayer M, Berardi G, Beall BF, Trick CG, Hüner NPA, Maxwell DP. 2011. Plasticity of the psychrophilic green alga *Chlamydomonas raudensis* (UWO 241) (Chlorophyta) to supraoptimal temperature stress. *Journal of Phycology* **5**: 1098–1109.

Possmayer M, Gupta RK, Szyszka-Mroz B, Maxwell DP, Lachance MA, Hüner NPA, Smith DR. 2016. Resolving the phylogenetic relationship between *Chlamydomonas* sp. UWO 241 and *Chlamydomonas raudensis* SAG 49.72 (Chlorophyceae) with nuclear and plastid DNA sequences. *Journal of Phycology* **52**: 305–310.

Reinbothe C, El Bakkouri M, Buhr F, Muraki N, Nomata J, Kurisu G, Fujita Y, Reinbothe S. 2010. Chlorophyll biosynthesis: spotlight on protochlorophyllide reduction. *Trends in Plant Science* **15**: 614–624.

Reinbothe S, Reinbothe C, Apel K, Lebedev N. 1996. Evolution of chlorophyll biosynthesis—the challenge to survive photooxidation. *Cell* **86**: 703–705.

Schoefs B, Franck F. 2003. Protochlorophyllide reduction: mechanisms and evolution. *Phytochemistry and Photobiology* **78**: 543–557.

Shui J, Saunders E, Needleman R, Nappi M, Cooper J, Hall L, Kehoe D, Stowe-Evans E. 2009. Light-dependent and light-independent protochlorophyllide oxidoreductases in the chromatically adapting cyanobacterium *Fremyella diplosiphon* UTEX 481. *Plant and Cell Physiology* **50**: 1507–1521.

Skribanek A, Solymosi K, Hideg É, Böddi B. 2008. Light and temperature regulation of greening in dark-grown ginkgo (*Ginkgo biloba*). *Physiologia Plantarum* **134**: 649–659.

Smith DR. 2018. *Haematococcus lacustris*: the makings of a giant-sized chloroplast genome. *AoB Plants* **10**: ply058.

Smith DR, Lee RW. 2010. Low nucleotide diversity for the expanded organelle and nuclear genomes of *Volvox carteri* supports the mutational-hazard hypothesis. *Molecular Biology and Evolution* **27**: 2244–2256.

Solymosi K, Schoefs B. 2010. Etioplast and etio-chloroplast formation under natural conditions: the dark side of chlorophyll biosynthesis in angiosperms. *Photosynthesis Research* **105**: 143–166.

Stolárik T, Hedtke B, Šantrůček J, Ilík P, Grimm B, Pavlovič A. 2017. Transcriptional and post-translational control of chlorophyll biosynthesis by dark-operative protochlorophyllide oxidoreductase in Norway spruce. *Photosynthesis Research* **132**: 165–179.

Suzuki JY, Bauer CE. 1992. Light-independent chlorophyll biosynthesis: involvement of the chloroplast gene *chlL* (*frxC*). *Plant Cell* **4**: 929–940.

Suzuki JY, Bauer CE. 1995. A prokaryotic origin for light-dependent chlorophyll biosynthesis of plants. *Proceedings of the National Academy of Sciences, USA* **92**: 3749–3753.

Szyszka B, Ivanov AG, Hüner NPA. 2007. Psychrophily is associated with differential energy partitioning, photosystem stoichiometry and polypeptide phosphorylation in *Chlamydomonas raudensis*. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* **1767**: 789–800.

Szyszka-Mroz B, Pittock P, Ivanov AG, Lajoie G, Hüner NPA. 2015. The Antarctic psychrophile *Chlamydomonas* sp. UWO 241 preferentially phosphorylates a photosystem I-cytochrome b6/f supercomplex. *Plant Physiology* **169**: 717–736.

Tanaka A, Ito H, Tanaka R, Tanaka NK, Yoshida K, Okada K. 1998. Chlorophyll *a* oxygenase (CAO) is involved in chlorophyll *b* formation from chlorophyll *a*. *Proceedings of the National Academy of Sciences, USA* **95**: 12719–12723.

Tripathi DK, Singh S, Gaur S, Singh S, Yadav V, Liu S, Singh VP, Sharma S, Srivastava P, Prasad SM *et al.* 2018. Acquisition and homeostasis of iron in higher plants their probable role in abiotic stress tolerance. *Frontiers in Environmental Science* **5**: 86.

Turmel M, Lemieux C. 2018. Evolution of the plastid genome in green algae. *Advances in Botanical Research* **85**: 157–193.

Turmel M, Otis C, Lemieux C. 2017. Divergent copies of the large inverted repeat in the chloroplast genomes of ulvophycean green algae. *Scientific Reports* **7**: 994.

Ueda M, Tanaka A, Sugimoto K, Shikanai T, Nishimura Y. 2014. *chlB* requirement for chlorophyll biosynthesis under short photoperiod in *Marchantia polymorpha* L. *Genome Biology and Evolution* **6**: 620–628.

Ward BB, Granger J, Maldonado MT, Wells ML. 2003. What limits bacterial production in the suboxic region of permanently ice-covered Lake Bonney, Antarctica? *Aquatic Microbial Ecology* **31**: 33–47.

Wei L, Xin Y, Wang D, Jing X, Zhou Q, Su X, Jia J, Ning K, Chen F, Hu Q *et al.* 2013. *Nannochloropsis* plastid and mitochondrial phylogenomes reveal organelle diversification mechanism and intragenus phylotyping strategy in microalgae. *BMC Genomics* **14**: 534.

Yamamoto H, Kurumiya S, Ohashi R, Fujita Y. 2009. Oxygen sensitivity of a nitrogenase-like protochlorophyllide reductase from the cyanobacterium *Leptolyngbya boryana*. *Plant and Cell Physiology* **50**: 1663–1673.

Yamazaki S, Nomata J, Fujita Y. 2006. Differential operation of dual protochlorophyllide reductases for chlorophyll biosynthesis in response to environmental oxygen levels in the cyanobacterium *Leptolyngbya boryana*. *Plant Physiology* **142**: 911–922.

Zhang Z, An M, Miao J, Gu Z, Liu C, Zhong B. 2018. The Antarctic sea ice alga *Chlamydomonas* sp. ICE-L provides insights into adaptive patterns of chloroplast evolution. *BMC Plant Biology* **18**: 53.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article:

Fig. S1 Genetic map of the *Chlamydomonas* sp. UWO241 chloroplast genome.

Fig. S2 Genetic map of the *Chlamydomonas* sp. UWO241 mitochondrial genome.

Fig. S3 Multiple alignment of the predicted DPOR amino acid sequences from *Chlamydomonas* sp. UWO241 and other closely related green algae.

Fig. S4 Multiple sequence alignment of the two predicted chlorophyllide *a* oxygenase proteins from *Chlamydomonas* sp. UWO241 (CAO-A; CAO-B) and closely related green algae.

Fig. S5 A model for the chlorophyll biosynthesis pathway in *Chlamydomonas* sp. UWO241.

Methods S1 Growth, genome and transcriptome sequencing, and genomic analyses of *Chlamydomonas* sp. UWO241.

Table S1 Masurca hybrid assembly statistics of *Chlamydomonas* sp. UWO241 Illumina and PacBio sequencing data.

Table S2 Genes encoding for proteins involved in chlorophyll biosynthesis present in the nuclear genome of *Chlamydomonas* sp. UWO241.

Table S3 Presence/absence of *chlL*, *chlN*, and *chlB* in complete or near-complete plastid genome sequences from chlorophycean green algae.

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Key words: *Chlamydomonas* sp. UWO241, chlorophyll, DPOR, photosynthesis, psychrophile.

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