LETTER

Highest plasticity of carbon-concentrating mechanisms in earliest evolved phytoplankton

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Scientific Significance Statement

Photosynthesis evolved in oceans of a distant past, when CO_2 partial pressure was high. Over time, pCO_2 dropped while O_2 levels increased. Consequently, phytoplankton required carbon-concentrating mechanisms (CCMs) to actively supply their carbon-fixing enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase with sufficient inorganic carbon. Earlier evolved phytoplankton groups were shown to possess more active CCMs, because they had to deal with decreasing pCO_2 and increasing O_2 levels. In this study, we examined whether these earlier evolved groups are also more plastic in their CCMs than later evolved groups. Our analysis shows that earlier evolved groups, i.e., cyanobacteria and dinoflagellates, exhibit a high CCM plasticity toward elevated pCO_2, whereas the more recently evolved haptophytes and diatoms do not. These findings improve our understanding of the evolution of CCMs and support predictions of phytoplankton group responses toward elevated pCO_2.

Abstract

Phytoplankton photosynthesis strongly relies on the operation of carbon-concentrating mechanisms (CCMs) to accumulate CO_2 around their carboxylating enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Earlier evolved phytoplankton groups were shown to exhibit higher CCM activities to compensate for their RuBisCO with low CO_2 specificities. Here, we tested whether earlier evolved phytoplankton groups also exhibit a higher CCM plasticity. To this end, we collected data from literature and applied a Bayesian linear meta-analytic model. Our results show that with elevated pCO_2, photosynthetic CO_2 affinities decreased

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strongest and most consistent for the earlier evolved groups, i.e., cyanobacteria and dinoflagellates, while CO_2-dependent changes in affinities for haptophytes and diatoms were smaller and less consistent. In addition, responses of maximum photosynthetic rates toward elevated $p CO_2$ were generally small and inconsistent across species. Our results demonstrate that phytoplankton groups with an earlier origin possess a high CCM plasticity, whereas more recently evolved groups do not, which likely results from evolved differences in the CO_2 specificity of RuBisCO.

Nearly all primary production on earth relies on the key enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) that catalyzes the fixation of CO_2. This enzyme is characterized by a generally low affinity for CO_2 and a competing reaction with O_2. To compensate for the poor catalytic properties of RuBisCO, phytoplankton evolved various mechanisms to increase the CO_2 concentration at the site of carboxylation (Badger et al. 1998). These carbon-concentrating mechanisms (CCMs) include active transport of CO_2 and/or HCO_3^- into the cell, the prevention of leakage out of the cell, as well as the expression of carbonic anhydrase (CA), an enzyme accelerating the interconversion between CO_2 and HCO_3^- (Sültemeyer et al. 1993; Raven et al. 2011). Together, these mechanisms determine the ability of phytoplankton to optimize carboxylation and suppress oxygenation.

Phytoplankton groups evolved RuBisCO types with different specificities, which describe the preference for CO_2 over O_2, i.e., carboxylation over oxygenation (Watson and Tabita 1997; Badger et al. 1998). Groups with an earlier origin (e.g., cyanobacteria and dinoflagellates) evolved during periods with high CO_2 and low O_2 levels (Fig. 1; Berner 2006). Earlier groups were shown to have a lower RuBisCO specificity for CO_2 as compared to more recently evolved groups

(e.g., haptophytes and diatoms; Tortell 2000). These early groups were also found to possess higher CCM activities, i.e., they more strongly increase the CO_2 concentration around RuBisCO (Fig. 1). More specifically, the intracellular accumulation of inorganic carbon (C_i) in cyanobacteria and dinoflagellates was higher than in haptophytes and diatoms (Badger et al. 1998; Tortell 2000).

Various phytoplankton species were shown to down-regulate their CCMs with elevated $p CO_2$ (Raven et al. 2011). Yet, it is unclear whether this CCM plasticity depends on the RuBisCO specificity and associated CCM activity, and thus differs between phytoplankton groups. Here, we hypothesized that the phytoplankton groups with a high CCM activity (i.e., cyanobacteria and dinoflagellates) also exhibit a high CCM plasticity. To test this hypothesis, we first collected published C_i acquisition parameters, including half-saturation-concentrations for CO_2 ($K_{1/2}$) and maximum photosynthetic rates (V_{max}) of phytoplankton acclimated to low or ambient and high $p CO_2$. We subsequently calculated the log response ratio (RR) to describe the change in $K_{1/2}$ and V_{max} for each tested phytoplankton species at elevated $p CO_2$ relative to ambient or low $p CO_2$. Thus, RR describes the relative regulation of C_i acquisition in response to elevated $p CO_2$. Lastly, we tested these responses for each phytoplankton group available in our dataset, i.e., cyanobacteria, dinoflagellates, haptophytes, and diatoms, using a Bayesian linear meta-analytic model (Hadfield and Nakagawa 2010).

Methods

For collecting data from literature, we searched the ISI Web of Science using search terms "photosynthesis" or "carbon acquisition," "phytoplankton" or "algae," and "CO_2" or "ocean acidification" and extracted studies with distinct CO_2 treatments, i.e., low or ambient (50–430 μatm) and high (750–5000 μatm) $p CO_2$, tested with mono-clonal cultures. In addition, we tracked back publications from reference lists. From these studies, we selected those where light and nutrients were in ample supply and information on data variance on the measured parameters was provided. The data and R script presented in this article are archived in the Dryad Digital Repository with doi:10.5061/dryad.j9m4cm6.

All data analyses were conducted in R v3.5.1 (R Core Team 2018). Prior to fitting the model, $\log_e$ RRs were calculated using the means (x) and standard deviations (SD) for $K_{1/2}$ and V_{max}. Specifically, RRs were calculated using the delta method
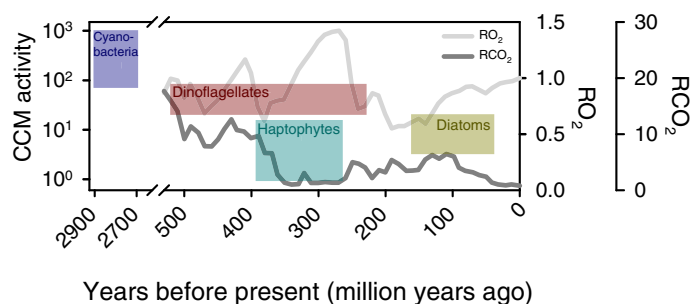

Fig. 1. The evolutionary origin of phytoplankton groups and associated CCM activities. The gray lines describe changes in Phanerozoic atmospheric O_2 and CO_2 concentrations relative to the present (i.e. RO_2 and RCO_2; Berner 2006), and the colored boxes denote the CCM activities as a function of the evolutionary origin of four key phytoplankton groups. The CCM activity expresses the concentration factor of intracellular over extracellular C_i concentration at present-day conditions for cyanobacteria (blue; Badger and Andrews 1982, Volokita et al. 1984), dinoflagellates (red), haptophytes (cyan), and diatoms (yellow; Badger et al. 1998). The evolutionary origin is based on fossil records of cyanobacteria (Nisbet et al. 2007), fossil records and biomarkers of dinoflagellates (Fensome et al. 1996; Moldowan and Talyzina 1998), and molecular clock analyses of haptophytes and diatoms (Young et al. 2012).

for bias correction (Lajeunesse 2015). Both, $K_{1/2}$ and V_{max} are ≥ 0, so that the maximum sample standard deviation (SD_{max}) equals $x\sqrt{N}$, with N being the number of samples. In cases where SD was not reported while SD_{max} was known, we substituted SD_{max} for SD prior to calculation of the RR variance. In total, SD substitution was done for one case in $K_{1/2}$ and five cases in V_{max}. In one case, the reported SD for $K_{1/2}$ exceeded SD_{max} by more than 15 times and was therefore excluded from further analysis. The selection procedure led to calculation of 29 cases for the RR of $K_{1/2}$ and 48 cases for the RR of V_{max} from 27 unique publications.

The resulting RRs were used to fit a Bayesian linear meta-analytic model, estimating the mean and credible intervals of acclimation response for different phyla. This was done using the R package MCMCglmm (Hadfield 2010; Hadfield and Nakagawa 2010) with phylum as a fixed factor and source publication and phylogeny (i.e., genus and species) as random factors to accommodate potential data dependencies due to methodological or phylogenetic dependency. RR was used as the response variable, and its variance was included in the var–covar matrix. Most source publications reported on a limited number of species so that phylogeny and source publication were highly confounded, resulting in poor separation of these variance components. Although discarding phylogeny-related random factors led to similar posterior distributions for fixed factors, they were maintained in the model to accurately reflect the data dependency structure.

Markov chain Monte Carlo (MCMC) was allowed to run for in 5×10^5 iterations with an additional burn-in period of 5×10^4 and a thinning factor of 100. Priors for fixed effects were set to a normal distribution with mean zero and large variance (1×10^{10}) for fixed effects and an inverse-gamma distribution with shape and scale parameters set to 0.001 for residual variance. As random factors were close to zero, we used weakly informative Cauchy priors as recommended by Gelman (2006). MCMC convergence was tested by means of Cramer-von-Mises statistic and Gelmans convergence diagnostic, both provided in the coda package (Plummer et al. 2006). In addition, traces were visually inspected for autocorrelation of MCMC sampling, and effective sampling size was kept above 2500. An Egger's test (Egger et al. 1997) did not show strong evidence for publication biases.

To test for the effect of the relative differences in applied pCO_2 ranges between studies, we performed a Spearman's rank correlation between the RR of V_{max} or $K_{1/2}$ and the $\log_e$ of the pCO_2 in the elevated over ambient pCO_2 treatment for each study reporting CO_2 levels (Sigmaplot version 14, Systat Software).

Results and discussion

The maximum rate, at which CO_2 is fixed, showed differential and generally small responses to elevated pCO_2

(Fig. 2A). For instance, V_{max} decreased (down by 0.4-fold) and increased (up by 2.4-fold) for the cyanobacteria *Calothrix rhizosoleniae* and *Cyanothece* sp., respectively. Consequently, there was no significant response of V_{max} to elevated pCO_2 for any of the tested phytoplankton groups (Fig. 2A). This confirms the operation of effective CCMs in all tested groups, maintaining internal CO_2 close to saturating levels also at low or ambient CO_2 concentrations conditions. We observed consistent and strong increases in $K_{1/2}$ in response to elevated pCO_2 in cyanobacteria and dinoflagellates. Specifically, $K_{1/2}$ increased in the cyanobacteria *Synechococcus* sp. by ~ 5-fold and in *Trichodesmium erythraeum* by ~ 3-fold and in the dinoflagellates *Heterocapsa triquetra* by ~ 17-fold, in *Prorocentrum cordatum* by ~ 13-fold, and in *Protoceratium reticulatum* by ~ 6-fold (Fig. 2B). Consequently, the $K_{1/2}$ of cyanobacteria and dinoflagellates showed a significant increase with elevated pCO_2 of about three- and four-fold, respectively, which indicates a decrease in the overall CO_2 affinity of photosynthesis (Fig. 2B). Despite an increase in $K_{1/2}$ of some haptophyte and diatom species, we observed less consistent and generally small changes in $K_{1/2}$ in these groups (Fig. 2B).

The consistent and strong decreases in CO_2 affinities of cyanobacteria and dinoflagellates in response to elevated pCO_2 reflect a pronounced down-regulation of their CCMs, whereas haptophytes and diatoms exhibit a lower degree of CCM down-regulation (Fig. 2B). CCMs likely evolved during periods in Earth's history characterized by low atmospheric pCO_2 in combination with high pO_2, where low $pCO_2 : pO_2$ ratios (e.g., ~ 300 and ~ 30 Mya; Fig. 1) may particularly favor selection of active CCMs (Giordano et al. 2005; Raven et al. 2008; Raven et al. 2012). The question remains why the ability to operate and regulate CCMs has been maintained during prolonged periods of elevated pCO_2 and low pO_2 levels, particularly as the plasticity of CCMs was shown to rapidly adapt to elevated pCO_2 (Schaum and Collins 2014). Maintenance of CCMs may have resulted from physiological constraints induced by low RuBisCO specificity to CO_2 (Badger et al. 1998; Tortell 2000). The group-specific differences in CCM plasticity described here therefore presumably result from these evolutionary differences in RuBisCO specificity (Tortell 2000).

Next to differences in RuBisCO, various other factors may have also contributed to the observed variation in CCM plasticity. First, phytoplankton species were isolated from different habitats, like coastal waters or open oceans that may affect the responses of CCMs to elevated pCO_2. We tested for the effect of habitat by including it as a fixed factor in our model. This analysis did not show a significant difference in CCM plasticity between habitats across phytoplankton groups (Supporting Information Table S1). Second, the various studies partly applied different ranges of pCO_2, which may have influenced the strength of measured responses. Correlations between the RR for $K_{1/2}$ and the applied pCO_2 range (i.e., the relative difference between elevated and ambient pCO_2) were not significant (Spearman's rank correlation; $n = 26$, $\rho = -0.144$,
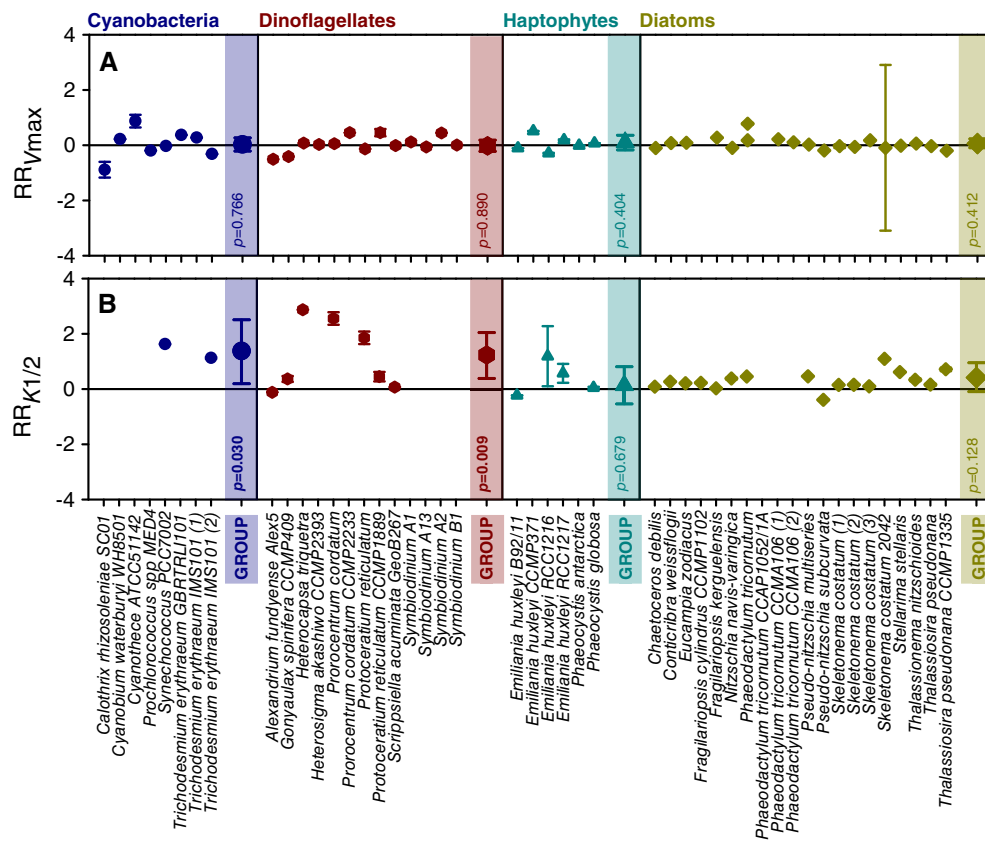
Fig. 2. CCM plasticity of phytoplankton groups, genera, species and strains. Symbols indicate means and standard deviations ($n > 3$; for genera, species, and strains) or means and 95% credible intervals (for groups) of the $\log_e$ RR that describe the change in V_{max} (A) and $K_{1/2}$ (B) at elevated pCO$_2$ relative to ambient or low pCO$_2$. Values are clustered by the four key phytoplankton groups cyanobacteria (blue circles), dinoflagellates (red hexagons), haptophytes (cyan triangles), and diatoms (yellow diamonds). Values in colored boxes denote the Bayesian p values for phytoplankton groups.

$p = 0.479$), whereas the RR for V_{max} showed a significant negative correlation ($n = 41$, $\rho = -0.450$, $p = 0.003$). Thus, differences in the applied pCO$_2$ ranges cannot explain the observed variation in $K_{1/2}$, while it may have played a role in determining the observed variation in V_{max}. Third, CO$_2$ concentrations may also have been drawn down in some of the analyzed experiments and therefore differ from the stated values. Unfortunately, only a limited number of studies indicate the extent of CO$_2$ drawdown, which was insufficient to test for this in our analysis. Lastly, phytoplankton species and groups can differ substantially in their CCM constituents such as the type and associated affinity of CO$_2$ and HCO$_3^-$ transporters, types and locations of CAs, and the presence of a microcompartment containing RuBisCO (Price et al. 2008; Meyer and Griffiths 2013). Differences in such CCM constituents can affect CCM plasticity, and thereby explain part of the variation observed in our results. To test whether these CCM constituents are group specific, however, CCMs needs to be characterized in more species across multiple phytoplankton groups.

Plasticity of traits can evolve through fluctuations in the environment (Agrawal 2001; Schaum et al. 2016). Consequently, the ability to regulate CCMs may possibly also have

been maintained under dynamic CO$_2$ concentrations. CO$_2$ concentrations in the bulk upper ocean waters follow atmospheric pCO$_2$, which varied over geological time scales that are presumably too slow to favor high plasticity over constitutively expressed CCMs. Yet, CO$_2$ concentrations can substantially fluctuate on time scales of days or shorter in cyanobacterial aggregates, mats, and stromatolites (Giordano et al. 2005; Eichner et al. 2017) or during phytoplankton blooms (Hansen 2002). Such fluctuations may favor CCM plasticity over constitutive expression of CCMs, particularly in cyanobacteria and dinoflagellates that most strongly rely on their CCMs for C$_i$ acquisition.

Earlier studies have shown a trade-off between the affinity for CO$_2$ and the maximum CO$_2$ fixation rate by RuBisCO (Savir et al. 2010) as well as by different dinoflagellate species (Eberlein et al. 2014). In our current data analyses, we did not observe such a trade-off in kinetic properties. Specifically, the CO$_2$-driven increase in $K_{1/2}$ (indicating a decrease in CO$_2$ affinity) for cyanobacteria and dinoflagellates was not accompanied by an increase in the maximum CO$_2$ fixation rate (Fig. 2). Elevated pCO$_2$ did not have a consistent effect on the maximum photosynthetic rates, which is in line with the operation of

effective CCMs for all tested phytoplankton groups. Observed variation within groups may have possibly derived from differences in applied pCO$_2$ ranges, or from species-specific differences in CCM constituents (Price et al. 2008; Meyer and Griffiths 2013), as discussed above. In addition, a minimal increase in CO$_2$ fixation rate in a species that is 85–90% CO$_2$ saturated at RuBisCO would be associated to disproportional high energetic costs (Kranz et al. 2015), which may further explain the relatively smaller effects observed for V$_{max}$.

Phytoplankton species with high CCM plasticities may possibly reallocate energy towards other cellular processes. The operation of CCMs is primarily linked to the availability of resources such as nutrients and light (Giordano et al. 2005). With projected increases in atmospheric pCO$_2$, down-regulation of CCMs may result in the reallocation of resources, for instance to nutrient acquisition. Down-regulation of CCMs in response to elevated pCO$_2$ was indeed shown to enhance production rates of organic nitrogen in two dinoflagellate species (Eberlein et al. 2016) and to stimulate N$_2$ fixation rates in some cyanobacteria (Kranz et al. 2009). Low nutrient concentrations limit primary production in many parts of the global oceans (Moore et al. 2013), and nutrients are expected to become increasingly scarce as result of enhanced thermal stratification (Behrenfeld et al. 2006; Boyce et al. 2010). Under such nutrient-limited conditions, elevated pCO$_2$ may allow for the reallocation of energy from CCMs to nutrient acquisition, possibly favoring cyanobacteria and dinoflagellates that possess a high CCM plasticity.

In contrast to growth-promoting effects, down-regulation of CCMs may also reduce the light dissipation potential of phytoplankton. Light availability regulates the cellular demand for inorganic carbon, and thereby induces CCMs (Beardall 1991; Rost et al. 2006). If elevated pCO$_2$ causes down-regulation of the CCMs, however, excess light cannot be dissipated as easily and may thus result in photo-oxidative stress (Rokitta and Rost 2012; Hoppe et al. 2015). Combined exposure of phytoplankton to elevated pCO$_2$ and high light indeed showed lowered primary production in natural phytoplankton communities as well as various diatom species (Gao et al. 2012; Trimborn et al. 2017).

Conclusion

Since their origin, phytoplankton groups have evolved distinct photosynthetic characteristics, despite their functional similarity as primary producers. These characteristics result from their adaptation to changing environmental conditions over geological timescales, for instance, the substantial decrease in atmospheric pCO$_2$ and increase in O$_2$ levels during the Phanerozoic (Fig. 1). Our results suggest that an evolutionarily adapted high CCM activity is accompanied by a high CCM plasticity. Whether the revealed high CCM plasticity in some cyanobacteria and dinoflagellate species will facilitate their success in a high CO$_2$ ocean depends on their ability to

reallocate energy to nutrient acquisition (when nutrient concentrations are low) and their means to dissipate excess energy (when light availabilities are high). Better estimates on the evolved plasticities of physiological key processes not only support our understanding of past and present phytoplankton community dynamics but also improve our predictions on who will benefit from elevated pCO$_2$ in a future ocean.

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