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Reduced resilience of a globally distributed coccolithophore to ocean acidification: Confirmed up to 2000 generations

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ABSTRACT

Ocean acidification (OA), induced by rapid anthropogenic CO_2 rise and its dissolution in seawater, is known to have consequences for marine organisms. However, knowledge on the evolutionary responses of phytoplankton to OA has been poorly studied. Here we examined the coccolithophore *Gephyrocapsa oceanica*, while growing it for 2000 generations under ambient and elevated CO_2 levels. While OA stimulated growth in the earlier selection period (from generations ~700 to ~1550), it reduced it in the later selection period up to 2000 generations. Similarly, stimulated production of particulate organic carbon and nitrogen reduced with increasing selection period and decreased under OA up to 2000 generations. The specific adaptation of growth to OA disappeared in generations 1700 to 2000 when compared with that at 1000 generations. Both phenotypic plasticity and fitness decreased within selection time, suggesting that the species' resilience to OA decreased after 2000 generations under high CO_2 selection.

1. Introduction

The oceans are rapidly taking up anthropogenic CO_2 with increasing atmospheric CO_2 concentrations. Dissolution of CO_2 in seawater increases pCO_2 and decreases pH, leading to ocean acidification (OA, Sabine et al., 2004). OA is known to affect a number of marine organisms, including primary producers (Riebesell and Tortell, 2011; Gao et al., 2012a,b; Beardall et al., 2014), but knowledge on the effects of multiple stressors as well as evolutionary responses has been little documented (Collins and Bell, 2004; Lohbeck et al., 2012; Jin et al., 2013a; Schaum et al., 2013; Riebesell and Gattuso, 2015). Consequently, the possibility of the evolutionary rescue of phytoplankton from OA has been gaining increasing concern (Lohbeck et al., 2012; Schaum et al., 2013; Schlüter et al., 2014; Scheinin et al., 2015).

Coccolithophores, a pan-global group of oceanic phytoplankton, produce platelets of calcium carbonate (called coccoliths) and play fundamental roles in the global carbon cycle through their direct effects on surface ocean chemistry and through ballasting of organic carbon fluxes to the deep sea, having a major feedback effect on global climate (Hutchins, 2011). OA-associated changes in carbonate chemistry can cause physiological, biochemical and photobiological influences on coccolithophores, by altering their calcification (Riebesell et al., 2000; Iglesias-Rodriguez et al., 2008; Gao et al., 2009; Sett et al., 2014), photosynthesis (Feng et al., 2008; Fiorini et al., 2011; Jin et al., 2013b), as well as elemental composition (Müller et al., 2010; Rickaby et al., 2010). The coccolithophore, *Emiliania huxleyi*, is shown to have adapted to long term OA conditions by restoring calcification and growth rates (Lohbeck et al., 2012); its particulate inorganic carbon (PIC) and particulate organic carbon (POC) production becomes significantly higher after a selection of 2000 generations under combined rising CO_2 and temperature conditions (Schlüter et al., 2014). Another cosmopolitan coccolithophore, *Gephyrocapsa oceanica*, after having been grown under OA conditions for ~700 generations, shows a moderate evolutionary response with increased growth, cellular POC and nitrogen contents, but decreased C:N ratios (Jin et al., 2013a).

Coccolithophores, in spite of their energy-driven calcification process, exhibit obvious characteristics similar to other popular phytoplankton groups, such as short generation time and large population sizes (Reusch and Boyd, 2013; Collins et al., 2014), which allow the experimenter to follow evolution in real time in response to global changes such as rising CO_2. The coccolithophores display both plastic and evolutionary responses to OA, with the ability of a genotype to produce distinct phenotypes when exposed to changing environments (Pigliucci, 2005). However, little has been documented as to how these two processes respond to OA along different time scales. Since coccolithophores and other fast growing phytoplankton, such as diatoms, can grow up to 10,000–30,000 generations by the end of this century, more generations might address possible adaptive responses (Lohbeck et al., 2012., Schaum et al., 2013).

Here, we took an experimental evolutionary approach to investigate the responses of the globally distributed coccolithophore, *G. oceanica*, to OA, while growing it for approximately 2000 generations at high

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(1000 μatm, HC) and ambient (390 μatm, LC) CO_2 concentrations, aiming to look into both the phenotypic plasticity and evolutionary responses within different selected time scales.

2. Materials and methods

2.1. Culture conditions

G. oceanica (NIES-1318), originally isolated from the East China Sea, was obtained from the National Institute for Environmental Studies (Japan). This strain was originally calcifying, but had lost its calcifying capacity before we performed the long-term selection experiment as described in Jin et al. (2013a). Part of the rationale of using non-calcifying species is documented in our previous study (Jin et al., 2013a). In addition, naked coccolithophores are normally reported in the sea (Paasche, 2002), and they have been widely used to represent the clones with noncalcifying capacity when compared with the calcifying cells (Paasche, 2002 and references therein). Three independent cultures were run in closed 1 L polycarbonate bottles and were semi-continuously diluted with CO_2-equilibrated seawater at either HC or LC pCO_2. To maintain a stable carbonate chemistry in the cultures (Table 1), the initial cell concentration was set at 100 cells mL^{-1} and the medium was partially renewed every 6 or 7 days (maximal cell densities at the end of each dilution were about 1.4×10^5 cells mL^{-1}) to restore the cell density to its initial level as described previously (Jin et al., 2013a). The cultures were maintained under 100 μmol photons m^{-2} s^{-1} photosynthetically active radiation with a light–dark cycle of 12:12 in a plant growth chamber (GXZ, Ruihua, Wuhan, China) at a constant temperature of 20 °C. For assays, both the HC and LC selected lines under 1000, 1700 and 2000 generations were transferred to media pre-equilibrated to the high or ambient CO_2 concentrations to acclimate for 10 generations which resulted in four treatments: HC selected lines assayed at HC environment (HC × HC), HC selected lines assayed at LC environment (HC × LC), LC selected lines assayed at HC environment (LC × HC) and LC selected lines assayed at LC environment (LC × LC). The initial cell concentration was also 100 cells mL^{-1} as in the long-term selection tests.

2.2. Growth rate determination

Cell concentration was measured every 6 or 7 days before and after the dilution of the medium, using a particle counter (Z2, Beckman instruments, Florida, USA). The specific growth rate (μ) was calculated as $\mu = (\ln C_1 - \ln C_0) / (t_1 - t_0)$, where C_1 and C_0 were the cell densities at time t_1 and t_0 ($t_1 - t_0 = 6$ or 7 days), respectively.

2.3. C and N analysis

Samples (300 mL) taken from each replicate at the middle of the light period were filtered onto pre-combusted (500 °C for 5 h) Whatman GF/F filters (25 mm) and frozen at −20 °C. For particulate organic carbon (POC) analysis, filters were fumed over HCl for 24 h to remove all inorganic carbon. The samples were analyzed using a Perkin Elmer Series II CHNS/O Analyzer 2400 (Perkin Elmer, USA) for the contents of POC and particulate organic nitrogen (PON).

Rates of POC or PON production were calculated as: P = specific growth rate μ (d^{-1}) × cellular POC or PON content (pg $cell^{-1}$).

2.4. Estimation of changes in carbonate chemistry

The pH in the cultures was measured with a pH meter (Benchtop pH 510, Oakton) that was calibrated with National Bureau of Standards (NBS) buffer solution (Hanna). The concentration of DIC was measured before and after the renewal of the culture using a DIC analyzer (AS-C3, Apollo Scitech) that employed an infrared gas detector (Li-Cor 7000, Li-Cor). Other related parameters of the carbonate chemistry were estimated based on known values of pH, DIC, nutrients and pCO_2 using the software CO_2SYS (Lewis and Wallace, 1998). The equilibrium constants (K_1 and K_2) of carbonic acid dissociation (Roy et al., 1993) were used for all calculations.

2.5. Plasticity and fitness responses

Changes in plasticity were calculated from the POC production rate (P) in the cells grown under the low or high CO_2 levels, as follows:

$$\frac{|P(HC) - P(LC)|}{P(LC)}.$$

Although plasticity responses are normally calculated from net photosynthetic oxygen evolution (Schaum et al., 2013), and this trait was not recorded in the present study, the trait of POC content production rate could be the proxy for net photosynthetic oxygen evolution, since it reflects carboxylation efficiency and real primary productivity.

For fitness responses, μ replaced POC production in the above formula. Corresponding measurements were carried out after 1000, 1700 and 2000 generations of selection.

2.6. Direct and correlated responses

Growth rates were used to calculate the direct and correlated responses. Here, the direct responses refer to traits of HC evolved lines measured at 1000 μatm CO_2 relative to growth rates of LC evolved lines measured at 1000 μatm CO_2. Correlated responses refer to traits of HC evolved lines measured at 390 μatm CO_2 relative to phenotypes of LC evolved lines measured at 390 μatm CO_2. Measurements of the related parameters were carried out in 1000, 1700 and 2000 generations of selection.

2.7. Data analysis

A two or three-factor nested random effects model and paired t-tests were used to establish differences among the treatments ($p = 0.05$). The interactive effects of CO_2 treatment and replicate were statistically analyzed using two-way ANOVA with three independent replicates being introduced as a random factor, nested within each selection treatment. The relationship between direct response or fitness and selection generations was fitted using linear regression.

Table 1
Parameters of the seawater carbonate system under high (1000 μatm, HC) and low (390 μatm, LC) pCO_2 levels in *Gephyrocapsa oceanica* cultures at representative 1000, 1700 and 2000 generations. Data are the means ± SD of 3 measurements.

	Treatment	pCO_2 (μatm)	pH_{NBS}	DIC (μmol kg^{-1})	HCO_3^- (μmol kg^{-1})	CO_3^{2-} (μmol kg^{-1})	Total alkalinity (μmol kg^{-1})
1000 generations	HC	1000	7.80 ± 0.03	2047.2 ± 146.2	1925.0 ± 133.8	89.9 ± 12.3	2144.1 ± 158.4
	LC	390	8.15 ± 0.03	1852.6 ± 124.6	1667.2 ± 103.0	172.8 ± 21.6	2095.5 ± 150.4
1700 generations	HC	1000	7.82 ± 0.03	2129.6 ± 151.4	2000.3 ± 138.1	97.0 ± 13.4	2237.2 ± 167.5
	LC	390	8.17 ± 0.02	1929.5 ± 73.4	1731.0 ± 60.5	185.9 ± 12.9	2188.2 ± 88.8
2000 generations	HC	1000	7.82 ± 0.04	2116.0 ± 205.0	1987.6 ± 186.8	96.1 ± 18.2	2222.3 ± 226.8
	LC	390	8.16 ± 0.03	1916.5 ± 144.8	1719.9 ± 120.0	184.0 ± 25.3	2172.7 ± 174.9

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