ARTICLES
PUBLISHED ONLINE: 8 APRIL 2012 | DOI: 10.1038/NGEO1441
nature geoscience

Adaptive evolution of a key phytoplankton species to ocean acidification

Kai T. Lohbeck[1,2], Ulf Riebesell[2] and Thorsten B. H. Reusch[1]*

Ocean acidification, the drop in seawater pH associated with the ongoing enrichment of marine waters with carbon dioxide from fossil fuel burning, may seriously impair marine calcifying organisms. Our present understanding of the sensitivity of marine life to ocean acidification is based primarily on short-term experiments, in which organisms are exposed to increased concentrations of CO_2. However, phytoplankton species with short generation times, in particular, may be able to respond to environmental alterations through adaptive evolution. Here, we examine the ability of the world's single most important calcifying organism, the coccolithophore *Emiliania huxleyi*, to evolve in response to ocean acidification in two 500-generation selection experiments. Specifically, we exposed *E. huxleyi* populations founded by single or multiple clones to increased concentrations of CO_2. Around 500 asexual generations later we assessed their fitness. Compared with populations kept at ambient CO_2 partial pressure, those selected at increased partial pressure exhibited higher growth rates, in both the single- and multiclone experiment, when tested under ocean acidification conditions. Calcification was partly restored: rates were lower under increased CO_2 conditions in all cultures, but were up to 50% higher in adapted compared with non-adapted cultures. We suggest that contemporary evolution could help to maintain the functionality of microbial processes at the base of marine food webs in the face of global change.

At present, the ocean takes up about one-third of fossil fuel CO_2 emissions and eventually will sequester up to 90% of anthropogenic CO_2 (refs 1,2). When CO_2 dissolves in sea water, it forms carbonic acid, which increases seawater acidity and decreases carbonate ion concentration and carbonate saturation[2]. These changes in seawater chemistry, dubbed ocean acidification[3], increasingly impact marine organisms[4,5] and ecosystems[6–8]. Most prominently affected are species that build their cell walls, shells, scales or skeletons from calcium carbonate[1,4,6]. A case in point are coccolithophores (Prymnesiophyceae), a group of unicellular microalgae thriving in the sunlit surface layer of the ocean, that are among the most productive calcifying organisms in the sea[9]. Under favourable light and nutrient conditions, coccolithophores may form extensive blooms. Those are even visible from space, because light reflection from their delicate calcite platelets (coccoliths) turns the surface ocean milky. Vast areas of the ocean floor covered with coccolith-derived sediments are testimony to their long-term role in the oceanic carbon cycle[9]. According to one prominent hypothesis, coccoliths ballast organic aggregates and fecal pellets, thereby accelerating their sinking to deeper waters and thus critically contribute to carbon export from surface waters to the ocean interior[10]. Like many other marine calcifiers, coccolithophores are sensitive to ocean acidification, with most studies showing a decline in growth and calcification rate and an increase in coccolith malformation at increased CO_2 levels[11–13]. Most studies on the effects of ocean acidification on marine organisms, including coccolithophores, have been short term (<1 yr) and none tested for evolutionary adaptation[14], a major unknown when attempting to predict future impacts of ocean acidification on marine life[15,16]. As populations of coccolithophores reproduce quickly and have large population sizes, they should be particularly prone to respond to ocean changes through adaptive evolution[16–19]. Such rapid evolutionary adaptation has previously been shown in microbial selection experiments on genetic model species exposed to new environmental conditions[20–22].

E. huxleyi single- and multiclone selection experiment

To test whether marine phytoplankton can adapt to ocean acidification, we conducted laboratory selection experiments with the coccolithophore *E. huxleyi*, a bloom-forming microalgae found in mid- to high latitudes in both hemispheres[9]. We designed two experiments, one with replicated populations assembled from equal contributions of six clones or genotypes (multiclone experiment) and one based on replicates founded by a single genotype (single-clone experiment). The multiclone experiment was designed to provide standing genetic variation that would be readily available to genotypic selection[23], whereas in the single-clone experiment starting with one haphazardly chosen genotype, evolutionary adaptation requires new mutations. Both experiments used freshly isolated genotypes from Bergen, Norway, and ran in batch cultures over ~500 asexual generations under continual slow rotation at ambient (400 µatm), medium (1,100 µatm) and high (2,200 µatm) levels of CO_2 partial pressure (p_{CO_2}). The medium-CO_2 treatment represented a level projected for the beginning of the next century[24]. The high level served as a proof of principle representing a sufficiently strong selective pressure. Although falling outside the range of projected oceanic CO_2 concentrations, it is within the range of values occurring temporarily in coastal areas under upwelling of O_2-deficient water[25]. Well before reaching the stationary phase, exactly 10^5 cells were transferred to the next batch cycle to keep the mutational target large and genetic-drift effects low. Maximal cell densities at the end of each five-day batch cycle were 1.5×10^5 ml^{-1}, which corresponded to a maximal drawdown of dissolved inorganic carbon (DIC) of 6.5%.

[1]Evolutionary Ecology of Marine Fishes, Helmholtz-Centre for Ocean Research Kiel (GEOMAR), Düsternbrooker Weg 20, 24105 Kiel, Germany, [2]Biological Oceanography, Helmholtz-Centre for Ocean Research Kiel (GEOMAR), Düsternbrooker Weg 20, 24105 Kiel, Germany. *e-mail: treusch@geomar.de.

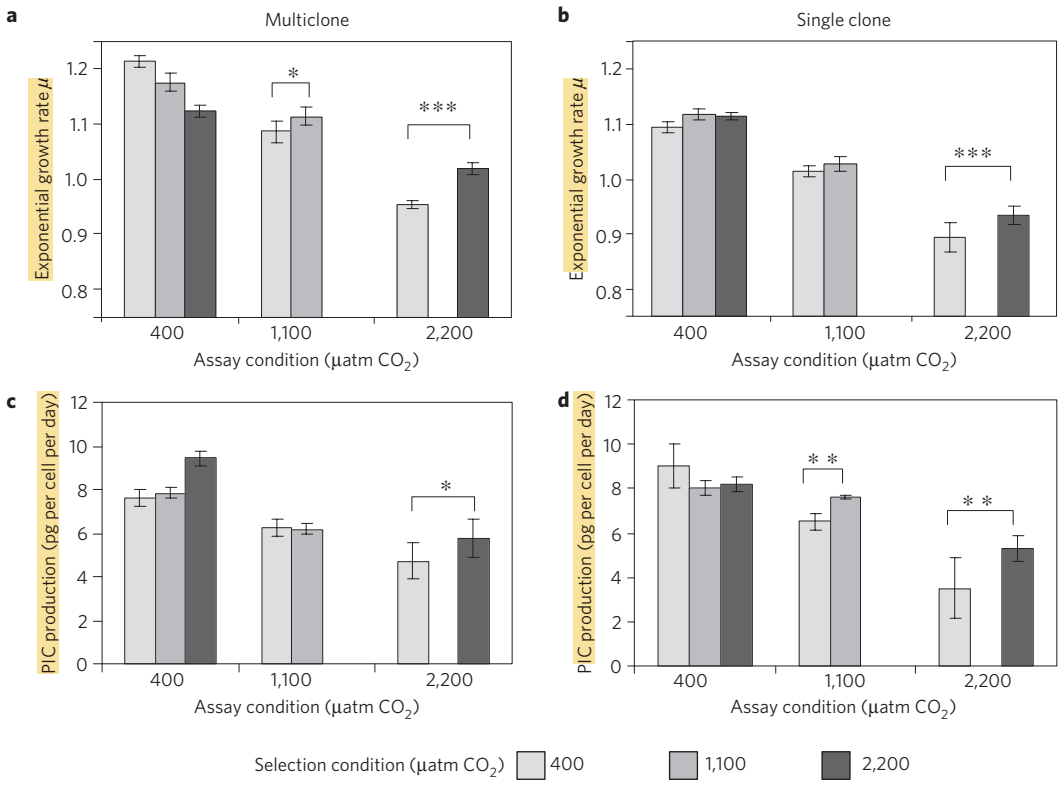

Figure 1 | Phenotypic responses to ~500 generations of selection in *E. huxleyi*. Replicate cultures ($n = 5$) were either founded by six clones (left panels) or a single clone (right panels). **a**,**b**, Mean exponential growth rate (±1 standard deviation). **c**,**d**, Mean production rate of particulate inorganic carbon (PIC). Adaptation to medium (1,100 μatm) and high (2,200 μatm) p_{CO_2} were assessed in two-way ANOVAs (selection x assay conditions), followed by planned contrasts among CO_2-selected versus ambient-selected population after one full batch cycle of acclimation. Contrasts were carried out only under the assay conditions of increased CO_2 and when the interaction selection × assay condition was significant. For PIC production in the single-clone experiment, a Welch ANOVA was carried out owing to unequal variances, followed by a Wilcoxon planned comparison. $^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

Evolutionary responses of exponential growth rates

Increased CO_2 levels resulted in sustained lower growth and calcification rates in line with previous findings[11–13]. Lower population growth rates translated into ~530 asexual generations at ambient-, 500 generations at medium- and 430 generations at high-CO_2 levels during the experimental time interval of ~1 yr. As any freshly isolated microbial population will be subject to selection to general laboratory conditions, phenotypic changes in response to selection were always tested relative to populations selected at ambient CO_2 (refs 20,21), rather than comparing phenotypes at the start and end of the experiment. These reciprocal assay experiments were conducted under non-competitive exposure to CO_2 enrichment. The salient test for adaptation involved the comparison of populations adapted to increased CO_2 conditions with those grown for long term under ambient CO_2 (the selection conditions), both tested under increased CO_2 (the assay condition). To control for physiological acclimation, an entire batch cycle of ~8 asexual generations was carried out in the assay conditions before assessing adaptation.

In both experiments, *E. huxleyi* populations adapted to ocean acidification and showed significantly increased exponential growth rates under increased CO_2 compared with controls that were propagated for the same time under ambient CO_2 (Fig. 1a,b). As algal growth was terminated well before reaching the stationary phase in our experiments, exponential growth rates are directly related to Darwinian fitness, that is, the number of offspring produced[22]. In nature, for example under competitive conditions of a phytoplankton bloom, fitness will contain additional components[17]. Accordingly, multiclonal populations selected under increased CO_2 showed fitness increases (as quotient of the exponential growth rate[22]) of 2.6% (medium-CO_2 selection) and 7% (high-CO_2 selection). Note that owing to its logarithmic properties, a 7% fitness difference would translate into a threefold difference in cell numbers after 14 days of exponential growth, for example during a phytoplankton bloom. However, the experimental period of ~1 yr (500 asexual generations) was apparently insufficient to fully restore growth rates to values found under ambient CO_2 through adaptive evolution (Fig. 1a,b).

Next, correlated responses to selection were examined, that is, the performance of populations selected at increased CO_2 when exposed back to their ancestral environment. Accordingly, we compared high-CO_2-selected populations with those selected at ambient CO_2, both subjected to ambient assay conditions. We observed negative correlated responses to selection, with medium- and high-CO_2-selected lines growing worse under ambient conditions (Fig. 1a; Analysis of variance (ANOVA), significant interaction selection × assay condition: $F_{1,16} = 21.6$, $P = 0.0002$ (data set ambient + medium CO_2); $F_{1,16} = 322.7$, $P < 0.0001$ (data set ambient + high CO_2)). The presence of the six experimental clones throughout the experiment was examined semiquantitatively using microsatellite genotyping and diverged consistently among treatments (Fig. 2). Clone no. 75 remained in the ambient-, clone nos 75 and 41 in the medium- and clone no. 62 in the high-CO_2 treatment, whereas all other clones fell below our detection threshold. The likelihood that such consistent clone sorting would happen by chance was very small ($P < 0.001$). One mechanism of adaptive change was thus selection among genotypes, the type of standing genetic variation present within asexually reproducing populations.

Interestingly, in the single-clone experiment, high-CO_2-selected populations performed better under all CO_2 environments, as

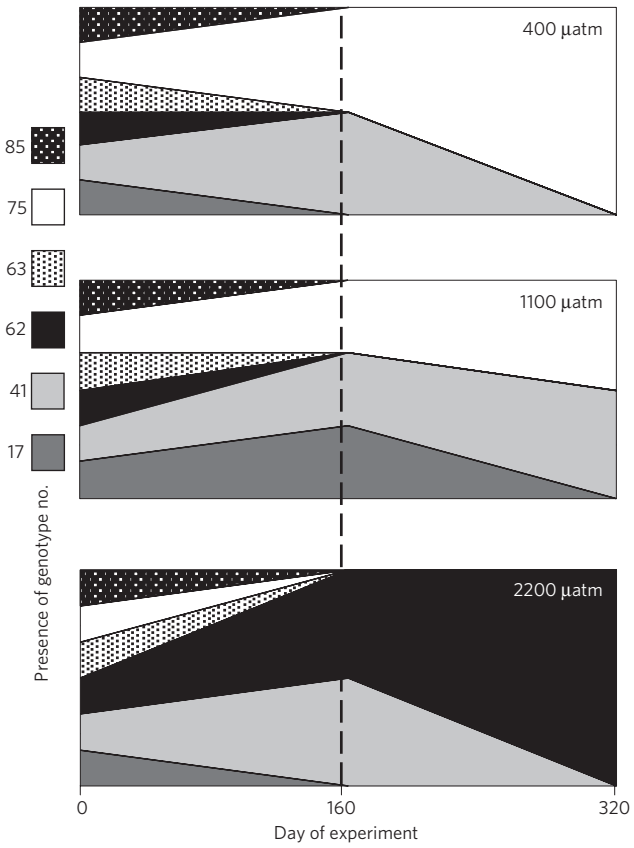

Figure 2 | Time course of the presence of *E. huxleyi* genotypes in the multiclone experiment. All three CO_2 treatments started with the same mixture of six genotypes. Genotypes were identified through polymerase chain reaction of diagnostic alleles at five microsatellite marker loci[35]. For each genotype, at least one diagnostic microsatellite allele was present. As the threshold, 5% of the peak height of the respective allele at the experimental start was chosen. The combinatorial likelihood for a single (ambient + high CO_2), or the same two genotypes (medium CO_2), remaining in all five replicates owing to a random process was $P < 0.0001$.

demonstrated by a non-significant interaction selection × assay condition and a significant positive effect of CO_2 selection (Fig. 1b; ANOVA, $F_{1,16} = 16.5$, $P = 0.0009$, (data set ambient + medium CO_2); $F_{1,16} = 17.3$, $P = 0.0007$ (data set ambient + high CO_2)). Here, mean fitness increase was 1.8 and 3.3% for the populations selected at medium- and high-CO_2 conditions, respectively, compared with the respective controls. That populations originating from a single clone and selected in a new environment also perform better under ancestral conditions compared with controls, that is, positive correlated responses to selection, is well known from evolution experiments[26]. Several processes may be responsible including universal fitness benefits of mutations under the new selection regime through pleiotropy[27] and non-transitive competitive interactions among two or more mutant genotypes (clonal interference)[28].

Correlated traits responding to selection at increased CO_2

Although we selected on exponential growth rates as one important fitness component, another prime interest in oceanography and biogeochemistry is how production rates of particulate inorganic carbon (PIC) present in the coccoliths changed during selection, given its importance for particle ballasting[10]. One expectation was that coccolith formation under increased CO_2 becomes more costly[29,30], leading to its gradual reduction either through genetic decay or direct selection for lower calcification rates. On the contrary, both multi- and single-clone populations selected at increased CO_2 revealed higher calcification rates than control populations when tested in a high-CO_2 environment. Calcification rates were thus partly restored, with 22% (multiclone assay, 2,200 µatm p_{CO_2} selection) and 17 and 51% (single-clone assay at 1,100 and 2,200 µatm p_{CO_2} selection, respectively) more PIC production compared with ambient-CO_2-selected controls (Fig. 1c,d). Between both experiments, the sign of the correlated response in PIC production differed, with CO_2 selection having a general positive effect in the multiclone experiment (Fig. 1c, ANOVA, $F_{1,16} = 8.2$, $P = 0.011$ (data set ambient + medium CO_2), $F_{1,16} = 5.41$, $P = 0.03$ (data set ambient + high CO_2)). In contrast, PIC production correlated negatively in the single-clone experiment (Fig. 1d, Welch ANOVA for unequal variances, subsequent pairwise comparisons of increased- versus ambient-CO_2-selected populations in reciprocal condition, all $P \leq 0.0127$). That we observed negative and positive correlations of the same trait (calcification rate) as response to selection for increased CO_2 among subexperiments seems puzzling. However, as the influence of many versus single founding genotypes is confounded among both experiments, we cannot decide whether genotype identity or diversity *per se* was responsible for the differences observed in the correlated response. Notwithstanding, it is noteworthy that in three out of four cases, there were significant increases of calcification rate compared with non-adapted controls under CO_2 enrichment (Fig. 1c,d). Interestingly, when tested in their respective selection environment, PIC production between the 400 (ambient) and 1,100 µatm p_{CO_2} selection treatment was not statistically significant in the single-clone experiment (Welch ANOVA, pairwise Wilcoxon test, $Z = 1.46$, $P = 0.143$), indicating a complete restoration of PIC production in the single- but not the multiclone experiment. The outcome of our selection experiment also indicates a functional importance of calcification for growth rates and competitive fitness in *E. huxleyi*[12,31].

Responses of correlated traits to selection, such as cell size, particulate organic carbon (POC) per cell and the PIC:POC ratio were more complex, but all measured traits responded either generally to selection at increased CO_2, or selection responses depended on the assay environment (Fig. 3a–h). Cell size suffered a sustained decrease under high-CO_2 conditions. This decrease was partly reverted under adaptation to high- (multiclone) and medium- (single-clone) CO_2 selection conditions, always compared with the respective controls (Fig. 3a,b, Supplementary Tables S1, S2). The multiclone cultures selected at increased CO_2 even completely restored their cell size relative to cultures selected under ambient conditions when tested under increased CO_2. PIC on a per-cell basis mostly increased in adapted populations relative to non-adapted controls, as a response to high- (multiclone) and medium- and high- (single-clone) CO_2 selection conditions (Fig. 3c,d). When assayed at both 1,100 and 2,200 µatm, cells fixed more POC per cell under increased CO_2 irrespective of the previous selection environment. Those rates were only slightly affected by adaptation to increased CO_2, but more so by pronounced correlated responses of evolved lines exposed to the ancestral ambient environment (Fig. 3e,f). Overall, there was a general positive effect of selection to restore PIC:POC-ratios in high-CO_2 levels in both experiments relative to controls adapted to ambient CO_2, although this effect was statistically significant only at 2,200 µatm (Fig. 3g,h, Supplementary Tables S1, S2). As ocean acidification possibly increases the energetic cost of biogenic calcification[4], a partial restoration of calcification rate imposes energetic trade-offs on coccolithophore cells that may not be visible in a nutrient- and light-saturated laboratory environment. Longer sustained experiments with additional limiting factors and careful consideration of possible trade-offs are needed to reveal the

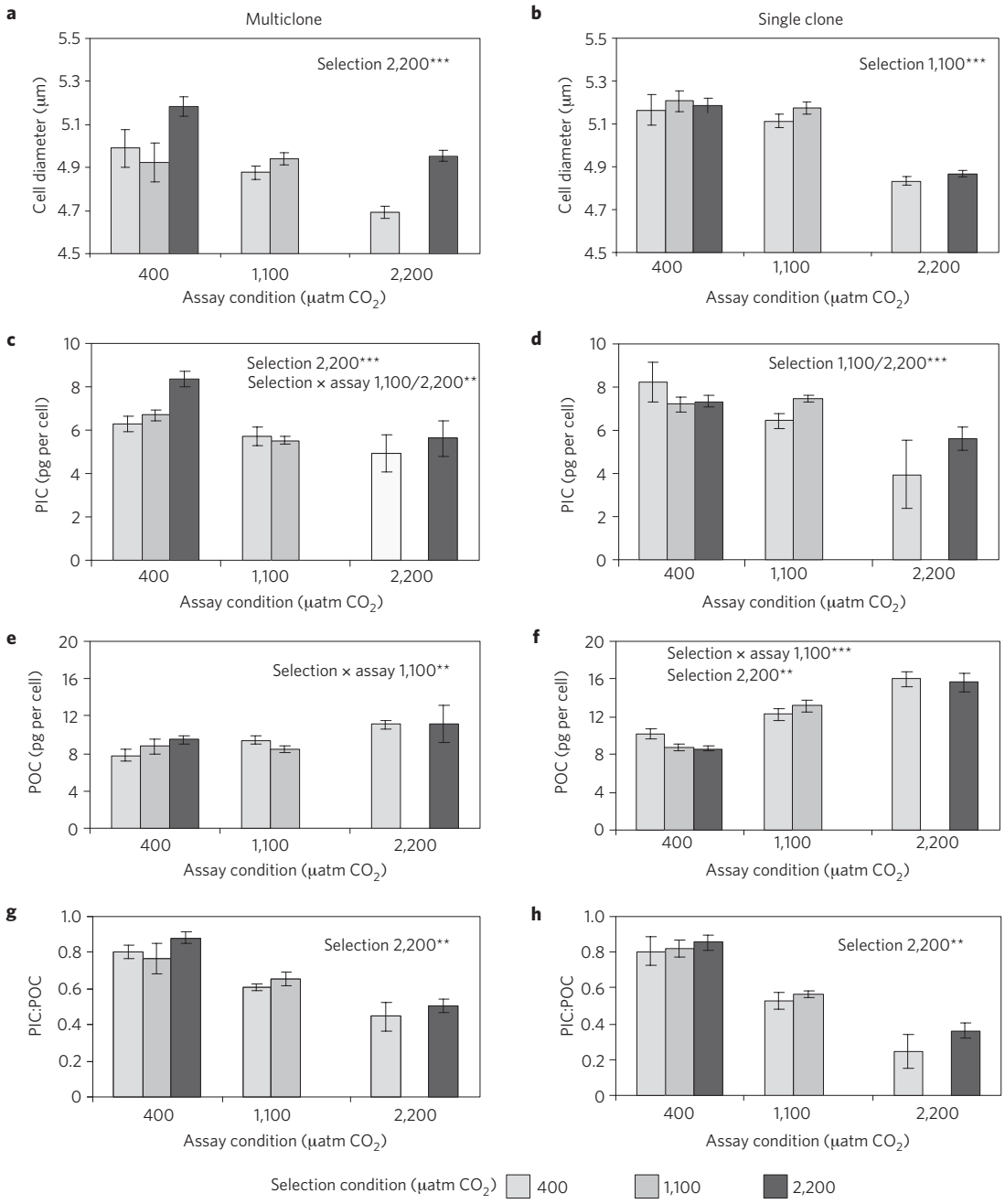

Figure 3 | Response of correlated traits after selection to increased CO_2 levels in the coccolithophore *E. huxleyi*. **a**,**b**, Mean cell diameter (mean ±1 standard deviation, $n = 5$) in the multi- and single-clone experiment. **c**,**d**, PIC per cell. **e**,**f**, POC per cell. **g**,**h**, PIC:POC ratio. Statistically significant results of main and interaction effects of 500 generations of selection under the respective CO_2 environment (1,100 or 2,200 µatm) are given in each panel (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$). For further statistical details see Supplementary Tables S1, S2.

absolute amount of possible phenotypic evolution as a response to ocean acidification.

Mechanisms of adaptation to ocean acidification

Previous studies found no adaptive responses of the haploid freshwater algae *Chlamydomonas reinhardtii* to selection at increased CO_2. Rather, as a correlated response, reduced growth was found when exposing high-CO_2-selected populations back to the ambient environment, indicating a degeneration of carbon-concentrating mechanisms by conditionally neutral mutations[20]. In contrast, our study identified direct, positive adaptation to increased CO_2 levels in a calcifying marine phytoplankton species. We identified genotypic selection as one immediate mechanism of population-level adaptation in the multiclone experiment. The adaptive responses observed in the single-clone experiment can only be explained by the emergence and partial fixation of advantageous new mutations. The rate of adaptation was in line with other laboratory selection experiments in diploid microbes that were conducted over some hundreds of asexual generations (Supplementary Table S3) and match population genetic expectations (Supplementary Information). It has been argued that evolution is slow in diploid microalgae compared with haploid species because mutations must be partially or completely dominant to produce a phenotype in diploid *E. huxleyi*[14,32]. However, a direct comparison of adaptation rates in haploid and diploid yeast strains revealed only small, non-significant differences

under comparable population sizes and generation times as employed in our single-clone experiment[32,33] (Supplementary Table S3). Interestingly, replicates within treatments evolving under increased CO_2 were not more divergent in terms of their phenotypes than those under ambient conditions. Accordingly, variances in growth rates were statistically not different from each other (Bartlett test of variance homogeneity, all $P > 0.1$). Nevertheless, the beneficial mutations underlying adaptation in the replicate lines probably have a different genetic basis[21]. Such phenotyic convergence is often observed in microbial evolution experiments, in particular if there are functional constraints to adaptation[34]. Interestingly, clone no. 62, which by chance was selected as founder for the single-clone experiment (Fig. 2), prevailed in the multiclone experiment under high-CO_2 selection conditions. As the presence of five other genotypes constitutes a different selection environment within the multi- compared with the single-clone experiment, it is not surprising that mean values of growth and calcification vary due to competition and clonal interference[21,27].

Ecological and biogeochemical implications

We deliberately chose a simple selection protocol and exposed asexually reproducing populations immediately into new, CO_2-enriched environments. Our intention was to make the results as comparable as possible with the many other microbial selection experiments and short-term physiological assessments of ocean acidification[13]. It is clear that the genetic diversity of natural phytoplankton populations will by far exceed the diversity in our multiclone experiment[35,36], let alone in the single-clone approach that started from a single founding genotype. Moreover, sexual reproduction will probably further enhance within-population additive genetic variance and thus, the possible rate of evolutionary adaptation[37]. Although the frequency of sexual reproduction and recombination is unknown for natural *E. huxleyi* populations, the diversity of genotypes even in bloom situations[35] indicates abundant sexual reproduction in the ocean. Coccolithophores play an important role for ocean productivity and the export of particulate carbon to the deep sea[9,10,15,31]. Hence, the swift adaptation processes observed here have the potential to affect food-web dynamics and biogeochemical cycles on timescales of a few years, thus surpassing predicted rates of ongoing global change including ocean acidification. As experimental evolution experiments reveal only the potential for adaptation, they need to be scrutinized against field observations. A recent study reports surprisingly high coccolith mass in an *E. huxleyi* population off Chile in high-CO_2 waters[38] that indicates across-population variation in calcification, in line with findings of rapid microevolution identified here. With the macro-evolutionary trends and associated ecological niches among the major eukaryotic phytoplankton groups being reasonably well understood[39], our results provide a starting point to examine the potential of within-species adaptive evolution in marine microbes.

Methods

Experimental cultures were founded with freshly isolated *E. huxleyi* clones and grown in sterile filtered artificial seawater media under continuous rotation at 15 °C and 150±10 µmol m^{-2} s^{-1} photon flux density under a 16:8 light:dark cycle.

The seawater carbonate system was set up by bicarbonate addition and subsequent aeration using a controlled CO_2-gas-mixing system. Carbonate chemistry was determined by DIC and total alkalinity measurements. Average culture p_{CO_2} values were calculated from DIC and total alkalinity measurements and drawdown estimates.

The multiclonal selection experiment was initiated with an equal contribution of 1.67×10^4 cells from six genotypes, whereas the single-clone experiment received 10^5 cells from one randomly chosen genotype. Both experiments were run in parallel by serial transfer of 10^5 cells every five days. Cell counts were carried out in triplicate after each batch cycle and exponential growth rates were calculated for each replicate. We tested for adaptation to increased CO_2 concentrations after 320 days (~500 asexual generations). Populations grown at ambient CO_2 were compared with populations selected at high CO_2 in both the ambient- and increased-CO_2 assay environments. As response variables, growth rates, cell diameter, PIC and POC per cell and their production rates were assessed. Genotype presence in the multiclone experiment was assessed with genotype-specific alleles in at least one microsatellite locus, using five *E. huxleyi* specific primers[35] after 0, 160 and 320 days. Statistical analyses used two-way ANOVA, in combination with planned contrasts or Welch ANOVA with subsequent Wilcoxon tests when variances were heterogeneous.

Received 8 July 2011; accepted 12 March 2012; published online 8 April 2012; corrected after print 20 November 2012

References

1. Orr, J. C. *et al.* Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437,** 681–686 (2005).
2. Feely, R. A. *et al.* Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science* **305,** 362–366 (2004).
3. Caldeira, K. & Wickett, M. E. Oceanography: Anthropogenic carbon and ocean pH. *Nature* **425,** 365–365 (2003).
4. Fabry, V. J., Seibel, B. A., Feely, R. A. & Orr, J. C. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J. Mar. Sci.* **65,** 414–432 (2008).
5. Frommel, A. Y. *et al.* Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. *Nature Clim. Change* **2,** 42–46 (2012).
6. Hoegh-Guldberg, O. *et al.* Coral reefs under rapid climate change and ocean acidification. *Science* **318,** 1737–1742 (2007).
7. Fabricius, K. E. *et al.* Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nature Clim. Change* **1,** 165–169 (2011).
8. Hall-Spencer, J. M. *et al.* Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* **454,** 96–99 (2008).
9. Westbroek, P., Young, J. R. & Linschooten, K. Coccolith production (biomineralization) in the marine alga *Emiliania huxleyi*. *J. Eukaryot. Microbiol.* **36,** 368–373 (1989).
10. Armstrong, R. A., Lee, C., Hedges, J. I., Honjo, S. & Wakeham, S. G. A new, mechanistic model for organic carbon fluxes in the ocean based on the quantitative association of POC with ballast minerals. *Deep-Sea Res. II* **49,** 219–236 (2001).
11. Riebesell, U. *et al.* Reduced calcification of marine plankton in response to increased atmospheric CO_2. *Nature* **407,** 364–367 (2000).
12. Zondervan, I. The effects of light, macronutrients, trace metals and CO_2 on the production of calcium carbonate and organic carbon in coccolithophores—a review. *Deep-Sea Res. II* **54,** 521–537 (2007).
13. Riebesell, U. & Tortell, P. D. in *Ocean Acidification* (eds Gattuso, J-P. & Hansson, L.) 99–121 (Oxford Univ. Press, 2011).
14. Collins, S. Comment on Effects of long-term high CO_2 exposure on two species of coccolithophores by Müller *et al.* (2010). *Biogeosci. Disc.* **7,** 2673–2679 (2010).
15. Riebesell, U., Körtzinger, A. & Oschlies, A. Sensitivities of marine carbon fluxes to ocean change. *Proc. Natl Acad. Sci. USA* **106,** 20602–20609 (2009).
16. Joint, I., Doney, S. C. & Karl, D. M. Will ocean acidification affect marine microbes? *ISME J.* **5,** 1–7 (2011).
17. Collins, S. Many possible worlds: Expanding the ecological scenarios in experimental evolution. *Evol. Biol.* **38,** 3–14 (2011).
18. Hoffmann, A. A. & Sgro, C. M. Climate change and evolutionary adaptation. *Nature* **470,** 479–485 (2011).
19. Reusch, T. B. H. & Wood, T. E. Molecular ecology of global change. *Mol. Ecol.* **16,** 3973–3992 (2007).
20. Collins, S. L. & Bell, G. Phenotypic consequences of 1,000 generations of selection at elevated CO_2 in a green alga. *Nature* **431,** 566–569 (2004).
21. Elena, S. F. & Lenski, R. E. Evolution experiments with microorganisms: The dynamics and genetic bases of adaptation. *Nature Rev. Genet.* **4,** 457–469 (2003).
22. Lenski, R., Rose, M., Simpson, S. & Tadler, S. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. *Am. Nat.* **138,** 1315–1341 (1991).
23. Becks, L., Ellner, S. P., Jones, L. E. & Hairston, N. G. Jr Reduction of adaptive genetic diversity radically alters eco-evolutionary community dynamics. *Ecol. Lett.* **13,** 989–997 (2010).
24. IPCC *Climate Change 2007: Impacts, Adaptation and Vulnerability* (eds Parry, M. L., Canziani, O. F., Palutikof, J. P., van der Linden, P. J. & Hanson, C. E.) (Cambridge Univ. Press, 2007).
25. Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D. & Hales, B. Evidence for upwelling of corrosive 'acidified' water onto the continental shelf. *Science* **320,** 1490–1492 (2008).
26. Bennett, A. F. & Lenski, R. E. Evolutionary adaptation to temperature. II thermal niches of experimental lines of *Escherichia coli*. *Evolution* **47,** 1–12 (1993).
27. Lenski, R. E. *et al.* Evolution of competitive fitness in experimental populations of *E. coli*: What makes one genotype a better competitor than another? *Anton. van Leeuw.* **73,** 35–47 (1998).

28. Gerrish, P. & Lenski, R. The fate of competing beneficial mutations in an asexual population. *Genetica* **102–103,** 127–144 (1998).
29. Zondervan, I., Zeebe, R. E., Rost, B. & Riebesell, U. A time series study of silica production and flux in an eastern boundary region: Santa Barbara Basin, California. *Glob. Biogeochem. Cycles* **15,** 507–516 (2001).
30. Mackinder, L. *et al*. Expression of biomineralization-related ion transport genes in *Emiliania huxleyi*. *Environ. Microbiol.* **13,** 3250–3265 (2011).
31. Paasche, E. A review of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions. *Phycologia* **40,** 503–529 (2002).
32. Zeyl, C., Vanderford, T. & Carter, M. An evolutionary advantage of haploidy in large yeast populations. *Science* **299,** 555–558 (2003).
33. Desai, M. M., Fisher, D. S. & Murray, A. W. The speed of evolution and maintenance of variation in asexual populations. *Curr. Biol.* **17,** 385–394 (2007).
34. Travisano, M., Vasi, F. & Lenski, R. E. Long-term experimental evolution in *Escherichia coli*. III. Variation among replicate populations in correlated responses to novel environments. *Evolution* **49,** 189–200 (1995).
35. Iglesias-Rodriguez, M. D., Schofield, O. M., Batley, J., Medlin, L. K. & Hayes, P. K. Intraspecific genetic diversity in the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae): The use of microsatellite analysis in marine phytoplankton population studies. *J. Phycol.* **42,** 526–536 (2006).
36. Langer, G., Nehrke, G., Probert, I., Ly, J. & Ziveri, P. Strainspecific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry. *Biogeosciences* **6,** 2637–2646 (2009).
37. Kaltz, O. & Bell, G. The ecology and genetics of fitness in *Chlamydomonas*. XII: Repeated sexual episodes increase rates of adaptation to novel environments. *Evolution* **56,** 1743–1753 (2002).
38. Beaufort, L. *et al*. Sensitivity of coccolithophores to carbonate chemistry and ocean acidification. *Nature* **476,** 80–83 (2011).
39. Falkowski, P. G. & Oliver, M. J. Mix and match: How climate selects phytoplankton. *Nature Rev. Microbiol.* **5,** 813–819 (2007).

Acknowledgements

We thank J. Meyer, A. Zavišić, K. Beining, A. Ludwig, S. Fessler and P. Fritsche for laboratory assistance; J. Czerny and C. Eizaguirre for advice on the experimental design, H. Schulenburg, J. Olsen and O. Roth for comments on earlier drafts; L. Bach, S. Febiri, T. Großkopf, L. Mackinder, D. Haase and K. Schulz for support during the experiments. T.B.H.R. and U.R. received financial support for this project from the German Federal Ministry of Education and Research (BMBF; project BIOACID).

Author contributions

T.B.H.R. conceived the project, all authors designed the experiment and K.T.L. carried out the experiment. All authors analysed and interpreted the data and wrote the manuscript.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/naturegeoscience. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence should be addressed to T.B.H.R. and requests for materials should be addressed to K.T.L.

CORRIGENDUM

Adaptive evolution of a key phytoplankton species to ocean acidification

Kai T. Lohbeck, Ulf Riebesell and Thorsten B. H. Reusch

Nature Geoscience **5,** 346–351 (2012); published online 08 April 2012; corrected after print 20 November 2012.

In the version of this article originally published, the *y* axis scales of Fig. 3c–f were incorrect. This has been corrected in the PDF and HTML versions.