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Discussions**

Interactive comment on "Comment on "Effects of long-term high CO$_2$ exposure on two species of coccolithophores" by Müller et al. (2010)" *by* S. Collins

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Comment on
"Effects of long-term high CO_2 exposure on two species of coccolithophores" by Müller et al. (2010)

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Abstract

Populations can respond to environmental change over tens or hundreds of generations by shifts in phenotype that can be the result of a sustained physiological response, evolutionary (genetic) change, shifts in community composition, or some combination of these factors. Microbes evolve on human timescales, and evolution may contribute to marine phytoplankton responses to global change over the coming decades. However, it is still unknown whether evolutionary responses are likely to contribute significantly to phenotypic change in marine microbial communities under high pCO$_2$ regimes or other aspects of global change. Recent work by Müller et al. (2010) highlights that long-term responses of marine microbes to global change must be empirically measured and the underlying cause of changes in phenotype explained. Here, I briefly discuss how tools from experimental microbial evolution may be used to detect and measure evolutionary responses in marine phytoplankton grown in high CO_2 environments and other environments of interest. I outline why the particular biology of marine microbes makes conventional experimental evolution challenging right now and make a case that marine

microbes are good candidates for the development of new model systems in experimental evolution. I suggest that "black box" frameworks that focus on partitioning phenotypic change, such as the Price equation, may be useful in cases where direct measurements of evolutionary responses alone are difficult, and that such approaches could be used to test hypotheses about the underlying causes of phenotypic shifts in marine microbe communities responding to global change.

One of the major tasks faced by biologists today is to understand how future populations of marine phytoplankton may differ from contemporary ones. But how far away are these future populations? The answer must be decades, since most DIC manipulation experiments use projected CO_2 levels from about the turn of the next century (Barry et al., 2010). Microbes have large population sizes and reproduce quickly, which ensures a more than adequate supply of mutations for evolutionary change over decades, either from standing genetic variation, or from novel mutations. If some component of global change exerts selection pressure, there is also scope for adaptation by natural selection in phytoplankton. That genetic change will occur is inevitable; the question is whether evolutionary change will be an important contributor to phenotypic shifts that arise in marine algae during long-term responses to ocean acidification. This question is being addressed by at least two separate groups of researchers working in two separate paradigms, with surprisingly little dialogue between them: biological oceanographers and microbial experimental evolutionary biologists (for examples see Falkowski and Oliver, 2007; Rost et al., 2008; Bell and Collins, 2008).

The study by Müller et al. (2010) marks an important step towards explicitly incorporating the possibility of genetic evolution into empirical biological oceanography. As an evolutionary biologist, I read this paper with great interest, though probably with a different agenda than the intended audience. Since Müller and colleagues set up their

cultures as a mini selection experiment (long term growth of replicate populations that were initially genetically identical under novel and control environmental conditions), I read it looking for indications of evolutionary change after a few dozen generations of growth under rising pCO$_2$.

The goal of the experiment by Müller et al. (2010) was not to measure evolutionary change, but to ask whether short-term physiological responses scale up. While the data show convincingly that physiological responses to DIC enrichment measured over a few generations in two coccolithophore species predict the physiology seen after tens or hundreds of generations, the authors note that the possibility of genetic change cannot be ruled out. The usual way to check for an evolutionary response to selection is to either measure the ability of an evolved genotype to outcompete its own ancestor (Elena and Lenski, 2003), or to compare components of fitness, such as growth rates, in both high pCO$_2$ and low pCO$_2$ of strains grown under both long-term high pCO$_2$ and long-term low pCO$_2$ (Collins and Bell, 2004). While the first comparison is not possible in this particular experiment since the genetic tools to transform coccolithophores are not yet available, the second could be carried out by simply returning the high pCO$_2$ selection lines to low pCO$_2$ at the end of the experiment, and doing the opposite with the low pCO$_2$ selection lines. Genetic tools exist for some other marine phytoplankton, however, such that future experiments using other taxa may be able to directly check for the presence of a direct evolutionary response to high pCO$_2$ or other environmental variable of interest (Hallmann 2007). A second tool used in experimental evolution is to measure correlated responses (Travisano et al., 1995), which are heritable changes in fitness or physiology in non-selected environments (corresponding to the pheno-type of the high pCO$_2$ selected cultures under any conditions other than the ones they experienced during selection). Correlated responses would be expected if, for example, long-term growth at high pCO$_2$ favoured types that were somehow specialized for growth at high pCO$_2$. For instance, long-term growth at elevated pCO$_2$ may affect the ability of strains to deal with nutrient limitation or changes in temperature. Although the authors never claim to be doing experimental evolution, basic tools from experi-

mental evolution could be applied in this type of experiment to systematically support or disprove the conclusion that primarily a sustained physiological response is being observed, and to verify whether or not a systematic genetic response has occurred.

The experiment by Müller et al. (2010) raises the question of how evolutionary effects might be measured in marine phytoplankton. Many marine microbes commonly used for physiology or ecology studies are far from ideal organisms for experimental evolution. Coccolithophores, for example, are grown in culture as asexual diploids, making it unlikely that novel or rare mutations will be selected unless they are expressed in het-erozygotes. Recessive mutations cannot be brought together in homozygotes since we do not yet know how to mate coccolithophores in culture. This impedes the action of natural selection in laboratory cultures (Colegrave, 2002; Zeyl et al., 2003). In addition, marine algal cultures must be grown at relatively low cell densities, and it is logistically difficult to grow the number of independent replicate cultures usually used in microbial experimental evolution to gain the statistical power to detect small changes in fitness – typically tens of cultures in each environment. For example, a fitness differ-ence on the order of 0.01 between populations that have been exposed to high pCO$_2$ over the short vs. long times could easily be ecologically important, and is probably a reasonable effect size for selection in a relatively benign environment, including el-evated CO$_2$ (Kassen and Bataillon, 2006; Perfeito et al., 2007). Growth rate is often a reasonable proxy for fitness in batch culture experiments such as the one done by Müller et al. (2010), so a fitness difference of 0.01 may be measured as a difference in growth rates of around 1%. However, the number of replicates needed to detect such a difference even given a very small standard deviation in fitness between replicate populations (say 0.001) is 17 independent replicates per group for a simple t-test. Fur-thermore, much of the power of traditional microbial experimental evolution lies in being able to have a living "fossil record" of evolving populations where samples are placed in suspended animation (usually in a freezer where growth is stopped completely or on a petri dish where growth is minimal) at several timepoints during the experiment. This allows the fitness (either growth rate or competitive ability) and phenotypes of the

same evolving strain at several timepoints (including the ancestral version) to be measured at exactly the same time under common conditions, allowing direct comparisons (Elena and Lenski, 2003; for an extensive treatment of microbial selection experiments see Bell, 2008). Finally, high DIC alone probably does not impose strong selection on marine algae – when a decrease in growth rate is seen, it is small, indicating that adaptive evolution may be slow, or that neutral evolutionary change may be responsible for phenotypic shifts (Collins and Bell, 2004). Because of these limitations, detecting evolutionary effects seems unlikely in marine microbes.

There are, of course, limits that all biologists face when using laboratory models to understand natural microbial populations. One of the obvious hurdles to applying the results of laboratory experiments to natural populations is that, even for common and well-characterised microbial model systems, we know shockingly little about the life cycles of microbes in nature. This is also true of marine phytoplankton, where studies of ploidy, as well as the prevalence and importance of sex and resting stages of natural populations, are still in their infancy (de Vargas and Probert 2004; Frada et al., 2009; Kremp et al., 2009) . Because of this, it is difficult to know how measurements made in cultures of one life history stage (for example an asexual diploid) of what is presumably a more complex life cycle inform us about responses of natural populations. The only way to remedy this is to do more basic biology, which is much simpler for me to write here than it is for anyone to do. A second hurdle that any experimental evolution in marine microbes will have in common with other laboratory-based work is that the entire point of laboratory experiments is to simplify, speed up (usually), and scale down the real world so that we can understand it better. After we do this successfully, we then have to contextualise our results by scaling up, slowing down and adding complications. Here, insights from ecological evolution studies can explain the systematic effects of some simplifications used in laboratory work, such as speeding up environmental change (Collins and de Meaux 2009), using a stable environment even though we know that most natural environments fluctuate (Lande 2007), using homogenous environments instead of patchy ones (Rainey and Travisano 1998),

looking at responses to changes in single instead of multiple environmental variables (Barrett et al. 2005), and using single strains rather than communities where groups interact (Rueffler et al. 2006; Collins 2010).

I do not intend to paint a grim picture or to discourage attempts to detect evolutionary change in marine algae. On the contrary, ecological concerns as well as curiosity about the basic biology of the world around us demand that we use extant tools to ask (and answer) evolutionary questions about marine microbes. Marine microbes also have many characters that make them (at least for me) tempting candidates for new model systems in experimental evolution, and that make them immediately accessible for basic evolution experiments right now. In particular, many marine microbes grow relatively rapidly, can be cultured in the laboratory, and have several variants available in culture collections. Genetic tools are either available or being developed in many cases (Hallmann 2007), and a sophisticated arsenal of techniques for characterising the physiology and community ecology of marine algae responses to pCO_2 changes are already in place (for example Rost et al., 2008; Barry et al., 2010; Müller et al., 2010). Existing tools that use "black box" approaches, such as the Price equation (Price, 1970; see partition by Collins and Gardner, 2009) or discriminant analysis (Okasha, 2006) could also be used in marine microbes. These methods can be used to partition change in some character of a community into contributions from a sustained physiological response, interactions between lineages, and evolutionary change within lineages, and use data that could be collected with tools that are already available. Here, the outcome of competition between strains, measured as differences in community composition, could be used to uncover evolution within strains, as was done in the worked example in Collins and Gardner (2009) examining the appearance of diuron resistance reported by McClellan et al. (2008). To use a Price equation approach to partition the underlying causes of changes in the bulk phenotype (such as carbon uptake) of a community, the covariance of a character of interest (such as carbon uptake) with fitness (growth rate) must be known or measured for each member of the community, and changes in community composition must be reported quantitatively and at the

same level of taxonomy (species, genus, functional group etc.) as the trait and fitness measurements. For example, if community composition is reported at the level of functional groups, then the covariance between carbon uptake and growth rate must be reported as an average for each functional group for all terms in the partitioned Price equation. Some of the data needed are not commonly measured or reported in studies of marine phytoplankton, which was noted in Collins and Gardner (2009), where a specific list of data required to use a Price equation partition to describe carbon uptake in marine phytoplankton in response to carbon enrichment is given.

The Price equation or similar approaches are not "free" solutions - they require that additional data be gathered during experiments. However, it seems likely that such approaches would improve our understanding of why communities change in response to high $p\mathrm{CO}_2$, and would allow at least a direct test of the hypothesis that the main response to high $p\mathrm{CO}_2$ should be changes in community composition owing to changes in the relative fitness of functional groups rather than to adaptive change within functional groups (Riebesell 2004; Falkowski and Oliver 2007). Previous work has demonstrated that selection on interactions between strains or species can act in the opposite direction as selection within strains or species, causing the effects to roughly cancel out, as was the case with the appearance of diuron resistance. This same pattern appears in some cases of multi-strain communities of a single freshwater algal species grown under long-term CO_2 enrichment (Collins, 2010). Here, if only a single physiological response is measured, it will apparently account for the full change in community phenotype, even though evolutionary change has occurred and must be taken into account to correctly understand the underlying basis for observed changes in community function (such as biomass production, carbon uptake, or PIC:POC ratios). To use these approaches, DIC manipulation experiments could be started with genetically diverse communities, either different species or several different strains of a single species such as *E. huxleyii*, so long as the members could be reliably distinguished one from another in mixed culture and changes in strain frequency could be quantified alongside the usual measurements of changes in physiological characters and growth rates. In

this way, evolutionary effects that occur in communities could be indirectly measured. Here, I have commented on a particular experiment carried out using *E. huxleyii*. However, the ideas presented are general and apply to other major planktonic groups such as diatoms and dinoflagellates, as well as to other haptophytes. The discussion of the specific characters of each major group that may present benefits or challenges to studying evolutionary responses in that group is beyond the scope of a short comment, but in principle, any organism that can be cultured for many generations at reasonable population sizes and levels of replication (several independent cultures) could yield information on evolutionary responses.

The time is ripe for collaborations between experimental evolution and biological oceanography, two fields that have developed in near-complete isolation. Collaboration between biological oceanographers and evolutionary biologists on experimental design and data reporting could allow information and understanding from each field to be used more effectively by the other. This would improve investigations in both fields, allow us to build on each other's knowledge, and give us a better chance at answering the question of how and why phytoplankton may respond to global change over the coming decades.

References

Barrett, R. D. H., MacLean, R. C., and Bell, G.: Experimental evolution in Pseudomonas fluorescens in simple and complex environments, Am. Nat., 166, 470-480, 2005.

Barry, J., Tyrrell, T., Hansson, L., and Gattuso, J.-P.: Part 2: Experimental design of pertubation experiments. Chapter 3: Atmospheric CO_2 targets for ocean acidification pertubation experiments, in: Guide to Best Practices in Ocean Acidification Research and Data Reporting, edited by: Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.-P., online available at: http://www.epoca-project.eu/index.php/Home/Guide-to-OA-Research/, 2010.

Bell, G.: Selection: the Mechanism of Evolution, second edition, Oxford University Press, Oxford, 2008.

Bell, G. and Collins, S.: Adaptation, extinction and global change, Evol. Appl., 1, 3–16, 2008.

Colegrave, N.: Sex releases the speed limit on evolution, Nature, 420, 664–666, 2002.

Collins, S. and Bell, G.: Phenotypic consequences of 1000 generations of selection at elevated CO_2 in a green alga, Nature, 431, 566–569, 2004.

Collins, S. and de Meaux, J.: Adaptation to different rates of environmental change in Chlamydomonas, Evolution, 63, 2952-2965, 2009.

Collins, S. and Gardner, A.: Integrating physiological, ecological and evolutionary change: a Price equation approach, Ecol. Lett., 12, 744–757. 2009.

Collins, S.: Competition limits adaptation and productivity in a photosynthetic alga at elevated CO_2, Proc. Roy. Soc. B. in press, 2010.

de Vargas, C. and Probert, I.: New keys to the Past: Current and future DNA studies in Coccolithophores, Micropaleontology, 50, 45-54, 2004.

Elena, S. F. and Lenski, R. E.: Microbial genetics: Evolution experiments with microorgansims: the dynamics and genetic bases of adaptation, Nat. Rev. Genet., 4, 457–469, 2003.

Falkowski, P. G. and Oliver, M. J.: Mix and match: how climate selects phytoplankton, Nat. Rev. Microbiol., 5, 813–819, 2007.

Fradam M., Percopo, I., Young, J., Zingone, A., de Vargas, C. and Probert, I.: First observations of heterococcolithophore-holococcolithophore life cycle combinations in the family Pontosphaeraceae (Calcihaptophycideae, Haptophyta, Mar. Microbiol. 71, 20-27, 2009.

Hallmann, A. Algal Transgenics and Biotechnology, Trans. Plant J., 1, 81-98, 2007.

Kassen, R. and Bataillon, T.: Distribution of fitness effects among beneficial mutations before selection in experimental populations of bacteria, Nat. Genet., 38, 484–488, 2006.

Kremp, A., Rengefors, K. and Montresor, M.; Species-specific encystement patterns in three Baltic cold-water dinoflagellates: The role of multiple cues in resting cyst formation, Limnol. Oceanogr., 54, 1125-1138, 2009.

Lande, R.: Expected relative fitness and the adaptive topography of fluctuating selection, Evolution, 61, 1835-1846, 2007.

McClellan, K., Altenburger, R., and Schmitt-Jansen, M.: Pollution-induced community tolerance as a measure of species interaction in toxicity assessment, J. Appl. Ecol., 45, 1514–1522, 2008.

Müller, M. N., Schulz, K. G., and Riebesell, U.: Effects of long-term high CO2 exposure on two species of coccolithophores, Biogeosciences, 7, 1109–1116, 2010.

Okasha, S.: Evolution and Levels of Selection, Oxford University Press, Oxford, 2006.

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Perfeito, L., Fernandes, L., Mota, C., and Gordo, I.: Adaptive mutations in bacteria: high rate and small effects, Science, 317, 813–815.**year?**

Price, G. R.: Selection and covariance. Nature, 337, 520-521, 1970.

Rainey, P. B. and Travisano, M.: Adaptive radiation in a heterogenous environment, Nature, 408, 69-72, 1998.

Riebesell, U.: Effects of CO_2 Enrichment on Marine Phytoplankton, J. Oceanogr., 60, 719-729, 2004.

Rost, B., Zondervan, I., and Wolf-Gladrow, D.: Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions, Mar. Ecol. Prog. Ser., 373, 227-237, 2008.

Rueffler, C., Van Dooren, T. M. J., Leimar, O., and Abrams, P. A.: Disruptive selection and then what? Trends Ecol. Evol., 21, 238-245, 2006.

Travisano, M., Vasi, F., and Lenski, R.: Long-term experimental evolution in *Escherischia coli*. III. Variation among replicate populations in correlated responses to novel environments, Evolution, 4, 189-200, 1995.

Zeyl, C., Vanderford, T., and Carter, M.: An evolutionary advantage of haploidy in large yeast populations, Science, 299, 555–558, 2003.