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Interactive comment on “Genotyping an *Emiliania huxleyi* (Prymnesiophyceae) bloom event in the North Sea reveals evidence of asexual reproduction” *by* S. A. Krueger-Hadfield et al.

Anonymous Referee #2

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Dear Editor,

The article by Krueger-Hadfield et al. “Genotyping an Emiliania huxleyi (Prymnesiophyceae) bloom event in the North Sea reveals evidence of asexual reproduction” is interesting and well written. I recommend this article be accepted with some essential revision. I call these "major revisions" to emphasise their importance.

As the title suggests, the most important observation is the finding of repeated multilocus genotypes, representing potential clones, in the plankton. Before population genetics on phytoplankton started, it was expected that phytoplankton blooms would be formed of a small number of clones, but repeatedly microsatellite studies on other

groups did not find this. Among the most striking observations are to be found in the extensive publications of Rynearson et al. describing populations of the diatom Ditylum brightwellii in a northern Pacific fjord, where potential multilocus genotypes were very rare even in this water body that is relatively much more confined. This pattern was found again in many other eukaryotic phytoplankton. The observations by Krueger-Hadfield are very different, and raise the question what differs about E. huxleyi compared to the diatoms D. brightwellii, Pseudo-nitzschia, Skeletonema marinoi and some other phytoplankton.

The authors do not discuss the work of Nagai on Alexandrium species and other dinoflagellates in Japan coast, where multi-locus genotypes using 9 or 11 markers were also found, and should do a more thorough and quantitative review of this work and others along with the micro satellite studies they do cite.

The authors need to describe much more carefully the protocol for isolating the clones used. They did not use direct single-cell isolation as the studies on diatoms and dinoflagellates used. The protocol they used is very difficult to understand with a too-brief description. The dilution-to-extinction regime could potentially allow time for a cell to divide and then the same clone could be isolated twice, from same water sample, over-estimating the occurrence of the clone in nature. Even if insufficient time was given for cells to divide, this protocol would appear to be a higher selective pressure than direct single-cell isolation. It is difficult to rule out that the original clonal diversity was not much higher, but that only a portion of the clones were in a physiological state or had needed pre-adaptations to be able to grow under their selection conditions of dilution-to-extinction. This is a particular concern as in most cases the same MLGs arose from the same water sample.

Much clearer description of isolation protocol is needed, and also authors should address these technical concerns. Also, they should include comparison more quantitatively of the chance of finding multilocus genotypes to that seen in other studies. It may be necessary to compare direct single-cell isolation with their protocol on one sample.

But perhaps they have the data and arguments to address this major concern.

Other general comments: In several cases the authors cite “P. von Dassow, personal communication” saying some E. huxleyi cells do not produce haploid phase. This has not been published anywhere and is not proven. Absence of evidence is not equal to evidence of absence. It also does not seem necessary to their conclusion, as it would normally be expected that it would not be hard to find repeatedly certain clones of a plankton cell dividing mostly asexually, whether or not it has sex rarely or not at all. They note this on p. 22 (4380) lines 20-23. I suggest not weakening or distracting from their argument with unnecessary citation to unpublished and unclear observation.

Would like more clarity about CMM and GPA and morphotype. Also, 90 micrographs from all clonal isolates (p. 9, line 23) seems very few were taken per isolate. How is that enough to do proper analysis of morphotype of each clone??? Not clear. At what magnification was this made? Not sure if accurate measurements could be made if taken at relatively low mag as in Fig. 3. Relationship of CMM type to morphotype seems very important, but is not coming out very clearly still.

I commend the authors for generally very careful reanalysis of performance of the microsat markes previously developed, including in good supplementary material. This is very important and they made a contribution improving the ability to use these micro satellite on an important plankton species.

Specific comments: p. 6 (4364) lines 19-21: “(i) Ten polymorphic microsatellite sequence primer pairs (AJ487304-17; AJ494737-42, Table 1) were blasted against the CCMP1516 genome (Read et al., 2013) in order to verify the amplification of a single site within the genome.” How blasted?? Blasted by blastn against unmasked reads, masked reads? All scaffolds? Only “diploid” scaffolds? Including or not including unplaced reads? What thresholds required?

p. 7 (4365) line 27: “Biosystems, Foster City, CA, USA). The loading mix was denatured at 92 C for 3min. Following optimization and with appropriate controls for

any sequencer to sequencer base pair shifts, 7 μL of each PCR product was sent to SourceBioScience” Please describe appropriate controls and optimization.

p. 17 (4375), lines 18-20: “CMM I in a homozygous state was also found in other geographic strains. Seven and two strains were from Chilean and Norwegian origin, respectively (Table 1).” I do not understand the second sentence. . .

p. 20 (4378), lines 2-5: “5). In addition, the morphotypes did not cluster together on the basis of microsatellite genetic distance, notably the four B/C morphotypes from the cooler Australian waters were dispersed between other morphotypes (Fig. 6).” This is very difficult to see. . . please re-make Fig. 6, or perhaps an additional figure, to make this possible to see.

p. 21 (4369), line 5: “estimated the number of mutations per microsatellite locus per generation during a 15 year culture period to be between $7\times10{-}3$ to 142”. I think “142” is an error number. . . how could the number of mutations per locus per generation be greater than 1??

p. 27 (4375) lines 1-4: “Interestingly, the plastid gene tufA (Cook et al., 2011) supports the division of E. huxleyi into two main subgroups or varieties (Cook et al., 2013), while the mitochondrial (mtDNA) cox1b-ATP4 genes (Hagino et al., 2011) found that no genetic distinction could be made. “ This does not appear to be correct. I think Hagino et al. 2011, Beaufort et al. 2011, and Bendif et al. 2014 all found the mitochondrial genes divide E. huxleyi into two main clades.

p. 27 (4385) lines 4-8: “The most parsimonious explanation for this apparent discrepancy is that the chromosomal (CMM) and plastid (tufA) alleles are under different selection pressure, possibly as a function of their individual attributes to fitness, while the mtDNA genes provide an insight into the ancestral history of this species through their maternal line.” What is the “maternal line” in E. huxleyi? Plastid and mitochondrial inheritance patterns are unknown in these organisms. In fact, syngamy has never even been observed! This explanation is unwarranted.

p. 37-40 (4395-4398). Tables 2a, 2b, 2c... why not Table 2, Table 3, Table 4?? Also, no table legend is given. Please fix.

p. 49-50 (4407-4408), figures 6-7. Very very difficult to read and follow. Please increase legend size so can be visible without increasing to 200% or 300% magnification. Better labeling of figures needed. Please re-make the figures completely so they can be more easily read and interpreted.

Would like them to also include the actual genotypes observed (alleles at each locus) in supplementary material please.

Interactive comment on Biogeosciences Discuss., 11, 4359, 2014.