Biogeosciences Discuss., 11, C2444–C2448, 2014
www.biogeosciences-discuss.net/11/C2444/2014/

Biogeosciences Discussions Open Access

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.

S. A. Krueger-Hadfield et al.

dsch@mba.ac.uk

Received and published: 13 June 2014

We would like to thank both reviewers for their overwhelming support for our paper. Both have, however, raised a few interesting points of discussion, to which we have responded as follows:

Reviewer #2

"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."

We did a thorough and exhaustive review of the current body of phytoplankton literature in which microsatellites were used to assess population genetic structure. The gold standard appears to be the Rynearson et al. body of work, in particular the use of Psex in determining the status of repeated genotypes as clones (this technique is used for higher plants and in macroalgae, yet has not been used for microalgae). However, this current study is not an exhaustive review of previous microsatellite work in a wide variety of phytoplankton species. In particular, the reviewer inquires as to why we did not discuss the work of Nagai et al. (2007, JPhycol) on Alexandrium tamarense, in which the authors did find some repeated genotypes in populations in four different bays. There is no information on whether these repeated MLGs were assessed as to their clonal status (i.e., Psex) or the actual numbers as this information is not provided. Thus, we compared our results with the work of Rynearson et al. We do agree that a review of the literature is needed to compare and contrast phytoplankton species which differ in variety of ways (e.g., the occurrence of resting stages, initiation of sexual reproduction, etc.). However, this is the not purpose of our current study. We feel that we have reviewed the literature and used examples where appropriate.

"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.

BGD
11, C2444–C2448, 2014

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."

The dilution-to-extinction method is a well-established methodology for isolating single celled organisms (Anderson's book entitled "Algal Culturing Techniques" Academic Press 2005), hence we did not feel the need to describe it in any detail. What is of importance with respect to Ehux is that upon dilution where one or two cells are left in the highest dilution, Ehux establishes itself in the form of a colony at the bottom of the culture vessel. This, therefore, means any dividing cells are restricted to the colony. It is this colony that we go on to extract and, thus, select as a clonal isolate, thereby making it comparable to the "picking" method. In fact our results support that our multiple genotypes often came from different sample stations and therefore different starting material (Table 2). Moreover, the MLG3-Geo is made up of three isolates from Chile (PVD-CH6, -CH112 & -CH47) that were isolated using AFC.

"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."

Citing an authority is not an unusual practice but we can omit this in the final version.

"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

BGD
11, C2444–C2448, 2014

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."

We can allay the reviewer fears here regarding the magnifications used. Fig.3 is not the typical magnification used to assess morphotype. We will include the settings in the final version. Moreover, an additional 62 micrographs on the whole population environmental samples were used to determine the nature of the morphotype present (p9, line 23). We are of the opinion that this is enough and thus representative. With respect to CMM type and morphotype relationship, we demonstrate clearly a distinct relationship between the two broader morphotype groupings of A &R, with CMM I, III & IV; and B, B/C & C with CMM II, which is backed with statistical rigor.

Specific comments Pg.4364 L19-21: Normally, whole microsatellite sequences are deposited in GENBANK, including the flanking sequences around the repeat motif. However, the original authors of the Ehux microsatellites only deposited the forward and reverse sequences. As these are microsatellite, only blastn can be used. This was used against all transcripts and did not filter out low complexity regions.

Pg4365 L27: We will edit this section to include the following information: “A positive and negative control was electrophoresed with each set of samples run on the sequencer. After optimization, a subset of known genotypes was transferred to SourceBioScience Nottingham for fragment analysis on a 3730xL DNA analyser run on a 50 cm capillary array. For all clonal isolates, 7 μL of each PCR product was sent to SourceBioScience, including positive and negative controls for each sequencer run.”

Pg4375 L18-20: We will edit to read as follows “CMM I in a homozygous state was also found in other geographic strains, seven were of Chilean and two of Norwegian origins (Table 1).”

Pg4378 L2-5: The figures are the result of typesetting by Biogeosciences Discussions. We will seek to rectify this for the final version. Similarly, we can look at Table 2 again.

BGD
11, C2444–C2448, 2014

Pg4369 L5: The following is directly from Hinz' thesis draft: "Microsatellite loci vary greatly in their mutation rates, and those for the microsatellite loci used in this study are not known, however, using the typical range of 10-2 to 10-6 mutations per locus per generation (Li et al., 2002), the number of mutations per locus per generation during the 15 years in culture is expected to be between 7 x 10-3 and 142." This is the result after 15 years in culture, not in a single generation. We will rephrase this sentence in the final version.

Pg4375 L1-4: This sentence must be read in context with the accompanying text of this section. We are referring to the two morphotype groups as revealed by our data. Hagino et al. and Bendif et al. are suggesting different groupings based on the plastid or mitochondrial genes.

Pg4385 L4-8: Just because syngamy has yet to be reproduced in culture does not mean that it does not occur. Similarly, it is well established that mitochondrial inheritance follows the maternal line. Why should this not be the case for Ehux? If the reviewer insists, we can remove the maternal line option thereby simply emphasizing the role of selection pressure.

Pg4395-4398: Again this was a single table as we submitted it, but during typesetting the table was split up. We will review this for the final version.

P4407-4408: Please see previous comments, but figures were typeset differently than we had submitted them.

Supp Mat: Please see Table 2 as each microsatellite genotype was provided.

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