A transitional Emiliania huxleyi morphotype

Jorijntje Henderiks

Department of Earth Sciences, Uppsala University, Villavägen 16, 75 236 Uppsala, Sweden – email: jorijntje.henderiks@geo.uu.se

Amos Winter

Department of Marine Sciences, University of Puerto Rico, Mayaguez, PR 00681-9000, Puerto Rico

Manuscript Received 05th May, 2011; Manuscript Accepted 09th March, 2012.

Abstract We recently published on the succession of two distinct morphotypes of *Emiliania huxleyi* (one heavily calcified, one more delicate form) during active upwelling offshore of Namibia (Henderiks *et al.*, 2012). Here, we describe a distinct morphotype (Type T, described herein) of *E. huxleyi*, which represents a transitional form between Type A and Type R. This morphotype is characterized by short and thick I-shaped distal shield elements and heavily calcified inner tube elements that extend irregularly into the central area.

Some coccospheres that showed coccoliths with merged I-elements can be considered an end-member form very closely resembling *E. huxleyi* Type R and *Reticulofenestra* spp. Type T dominated the phytoplankton community in mature upwelled waters offshore Namibia, immediately succeeding coastal-upwelling induced diatom blooms. Type T was encountered in a relatively restricted geographic area and may therefore provide a qualitative test of the reproductive isolation and/or phenotypic plasticity within natural populations of *E. huxleyi* morphotypes.

1. The ABC of morphotypes

The cosmopolitan coccolithophore species, *Emiliania huxleyi* (Lohmann) Hay and Mohler, inhabits tropical to polar waters, covering a wide range of oceanographic conditions (e.g. Brand, 1994; Cubillos *et al.* 2007). Its distribution is supported by high genetic diversity (Medlin *et al.* 1996; Iglesias-Rodriguez *et al.* 2006), as well as a high degree of morphological variability within and among natural populations (e.g. Hagino *et al.*, 2005; Hagino and Okada, 2006; Cubillos *et al.*, 2007). Six different morphotypes of *E. huxleyi* have been described based on different shape, size and degree of calcification of the coccoliths (Type A, B, B/C, C, R; Young *et al.*, 2003, and references therein and Type O; Hagino *et al.* 2011).

Young and Westbroek (1991) were the first to confirm genotypic variation between two different morphotypes (Type A and B) by testing immunological cross-reactions between the coccolith associated polysaccharide (CAP) of each type and antibodies to the CAP. Schroeder et al. (2005) identified a genetic marker for distinguishing strains of E. huxleyi Type A from strains of Type B. More recently, Cook et al. (2011) provided evidence for photosynthetic pigment and genetic differences between two main morphotypes found in the Southern Ocean, Type A and Type B/C. DNA sequencing of plastid gene tufA distinguished morphotypes A, B/C (indistinguishable from B), and R, while little variation was observed within morphotypes (Cook et al., 2011). Minor, but consistent differences in mitochondrial (cox1b-atp4) gene sequences appear to be linked to the biogeography and temperature preference/tolerance (ecotypes) of different E. huxleyi strains (Hagino et al., 2011). The two phylogenetic groups identified by these authors are both dominated by Type A strains (28 out of 39 tested), and therefore seem unrelated to coccolith morphology, despite tight sub-clustering of other tested morphotypes within the clades (3 strains of Type R and 4 strains of Type O, respectively). However, Hagino et al. (2011) did not classify Type A strains by degree of calcification, which is known to vary

between strains and to remain stable in culture, and which is suggestive of distinct calcification physiologies within this common morphotype.

It remains unknown whether different phenotypes and genotypes can occur within interbreeding populations, or whether different morphotypes represent reproductively isolated species. In culture, monoclonal strains (i.e. single genotypes) have never been reported to change from one morphotype to another, despite experimental evidence for significant phenotypic plasticity (i.e. a phenotypic change of a single genotype in response to changing environmental conditions) (Paasche, 2002; Langer et al. 2009). No reports of cross-breeding experiments exist - probably because it is not easy to induce the haploid stage within monoclonal diploid strains and other practical complications. However, the identification of "transitional" morphotypes in natural populations may provide qualitative evidence for the viability and/or frequency of cross-breeding between different morphotypes.

2. The succession of morphotypes

Two distinct morphotypes of E. huxleyi were found to be part of the phytoplankton succession during coastal upwelling offshore of Namibia (Henderiks et al., 2012). Plankton sampling during cruise 48/5 of the R/V Meteor in October 2000 (Alheit, 2000; Figure 1) revealed that a heavily calcified E. huxleyi morphotype, herein described as Type T (but labeled as Type A* in Henderiks et al., 2012; Plate 1d), was the dominant coccolithophore in mature upwelled waters immediately after exhaustion of diatom blooms that were found closer to shore. Type T occurred in high abundance with up to >1 million cells 1-1 at water depths between 0-50m. The highest cell concentrations were encountered at station 579, where they caused a chlorophyll and 19'-hexanoyloxyfucoxanthin maximum at \sim 17m depth. At the same time, a more delicate *E. hux*leyi morphotype (Type B/C; Plate 1a) dominated further offshore, in very late-succession stage upwelling and oceanic waters (Henderiks et al., 2012). Here, we provide

72 Henderiks Winter

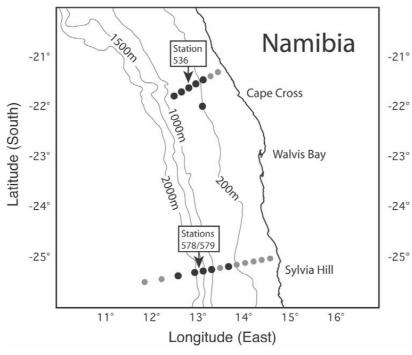


Figure 1: Stations sampled during Cruise 48/5 of the R/V *Meteor*, October 2000. Black symbols: *Emiliania huxleyi* populations (see Plate 1). Grey symbols: no coccolithophores.

additional photographic evidence for the observed morphological variation of *E. huxleyi* offshore Namibia (Plate 1) and describe Type T.

3. Emiliania huxleyi Type T

Coccoliths are medium sized (mean size 2.6-3.1 μ m; range 2-4 μ m), with variable coccosphere diameters (mean diameter 4.9 μ m; range 3.4-8.4 μ m). The distal shield elements are thick and short, with incomplete "hammer-head" terminations that we call "I"-elements, as opposed to the "T"-elements typical for E. huxleyi Type A (cf. Young et al., 2003; Plate 1b). Often the distal shield I-elements are loosely arranged, because they are disconnected from neighboring elements at the outer margin, and they rarely extend beyond the proximal shield diameter. The inner tube cycle extends into the central area (CA) with irregularly shaped elements (similar to E. huxleyi Type R; Young et al., 2003), which display variable degree of calcification, maximum thickness at the inner cycle and irregularly cover the CA, which has not been described for Type R (cf. Young et al., 2003; but see a recent illustration of a heavily calcified Type R in Beaufort et al., 2011). Type T also differs from Type R in that its distal shield elements are not as thick to appear merged together (but see rarely encountered 'combination' coccospheres that challenge this distinction; Plate 1e).

Type T coccoliths from offshore Namibia do not have the same smooth, over-calcified appearance of morphotypes observed in the upwelling region offshore Chile (Beaufort *et al.*, 2008; 2011), the heavily calcified forms that have been observed in the Bay of Biscaye under summer conditions (Beaufort & Heussner, 2001) or Type A "overcalcified" *cf*. Young *et al.* 2003. Instead, the presence of a gap between the distal shield elements and inner tube

element (Plate 1d,e) is a very consistent feature in the sampled populations. Young (1994) argued that such features are due to secondary dissolution of the central tube, and part of a spectrum from lightly etched to 'collapsed' coccoliths. No data on *in situ* pH were available for this study (see also discussion in Henderiks *et al.*, 2012), but it is interesting to note that the heavily calcified *E. hux-leyi* morphotypes discovered in low-pH upwelling waters offshore Chile did not show such supposedly 'etched' features (Beaufort *et al.*, 2008; 2011).

We also note that, despite obvious differences in coccolith size and coccosphere diameter (compare Plate 1g), the CA of Type T is very similar to the CA of Reticulofenestra parvula var. tecticentrum (Okada & McIntyre 1977) Jordan & Young, 1990, an overcalcified form of Reticulofenestra in which the inner tube cycle closes the central area, without a central collar between the CA and distal shield elements.

Based on its irregular grill structure in the CA, its heavily calcified, but short and incomplete distal shield elements, as well as size ($<4\mu$ m), we conclude that *E. huxleyi* Type T is a transitional form on a morphological spectrum between Type A and Type R (compare classification in Hagino *et al.* (2011); their Table 1). Unfortunately, no cells were isolated and kept in culture, so we have not been able to test the phenotypic stability of this form. However, the morphological features of Type T were highly consistent within and between the sampled populations, while being distinctly different from the other *E. huxleyi* morphotypes that were encountered further offshore and later in the plankton succession (Plate 1a-c).

4. Concluding remarks

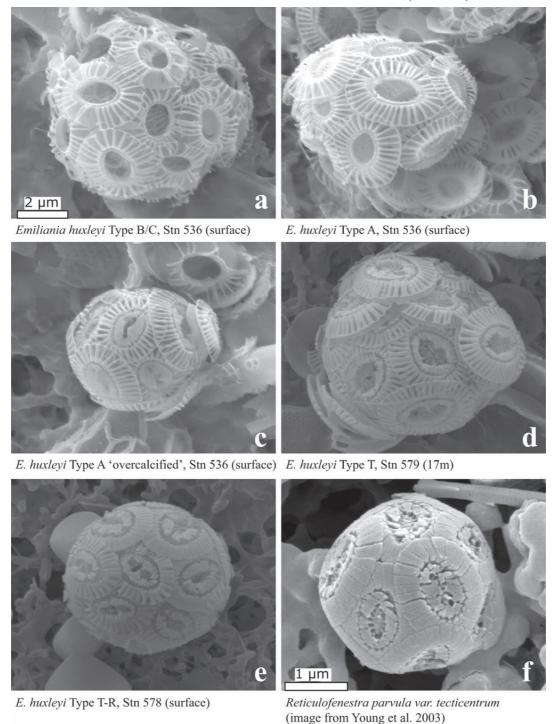
Over the past two decades, our research community has collected extensive evidence for distinct morphotypes, ecotypes and genotypes of *E. huxleyi*, in the quest to understand why this cosmopolitan taxon has a competitive edge over most other coccolithophores. Even if the relationship morphotype - ecotype - genotype is not necessarily a straightforward one (e.g. Langer *et al.*, 2009; Hagino *et al.*, 2011), it is very important to differentiate ("split") between morphotypes in natural samples, test strains for morphological stability/plasticity in the laboratory, and further develop a classification scheme that includes transitional forms between already established morphotypes.

References

Alheit, J. 2000. METEOR-Berichte 06-5, Cruise 48, Leg 5, Walvis Bay - Walvis Bay, October 13- October 30, 2000, www.dfg-ozean.de/fileadmin/DFG/Berichte/Report_M48_5KS_.pdf.

Plate 1

Scanning electron micrographs of *Emiliania huxleyi* morphotypes encountered offshore Namibia (cruise 48/5 of the R/V *Meteor* in October 2000). Scale bars: $(a-e) = 2 \mu m$, $(f) = 1 \mu m$.



- a. Type B/C coccosphere with double layer of coccoliths. Station 536 (surface).
- b. Type A coccosphere, rarely encountered in our samples. Station 536 (surface).
- c. 'over-calcified' Type A, nearly entirely covered central area, found to co-exist with Type B/C. Station 536 (surface).
- d. Type T coccosphere with double layering of coccoliths. Station 579 (chlorophyll maximum).
- e. Type T-R coccosphere, transitional form, merged distal shield elements (Type R-like) on some coccoliths, others as described for Type T, with short and thick I-elements. None of the distal elements connected by central collar. Station 578 (surface).
- f. Reticulofenestra parvula var. tecticentrum, as illustrated in Young et al., 2003 (image credits M. Geisen & J. Young). Inner tube cycle closing the CA irregularly, distal shield elements not connected by central collar. However, note scale bar = 1 μm; coccoliths and coccosphere diameter of this specimen are significantly smaller than the illustrated E. huxleyi morphotypes.

- Beaufort, L., Probert, I., de Garidel-Thoron, T., Bendif, E.M., Ruiz-Pino, D., Metzl, N., Goyet, C., Buchet, N., Coupel, P., Grelaud, M., Rost, B., Rickaby, R.E.M. & de Vargas, C. 2011. Sensitivity of coccolithophores to carbonate chemistry and ocean acidification. *Nature*, 476: 80-83.
- Beaufort, L., Couapel, M., Buchet, N., Claustre, H. & Goyet, C. 2008. Calcite production by coccolithophores in the south east Pacific Ocean. *Biogeosciences*, 5: 1101-1117.
- Beaufort, L. & Heussner, S. 2001. Seasonal dynamics of calcareous nannoplankton on a West European continental margin: the Bay of Biscay. *Marine Micropale-ontology*, **43**: 27-55.
- Brand, L. 1994. Physiological ecology of marine coccolithophores. *In*: A. Winter & W. G. Siesser (Eds). *Coccolithophores*, University Press, Cambridge: 39-49.
- Cook, S.S., Whittock, L., Wright, S.W. & Hallegraeff, G.M. 2011. Photosynthetic pigment and genetic differences between two Southern Ocean morphotypes of *Emiliania huxleyi* (Haptophyta). *Journal of Phycology*, **47**: xxx-xxx. (available online 9 May 2011).
- Cubillos, J.C., Wright, S.W., Nash, G., de Salas, M.F., Griffiths, B., Tilbrook, B., Poisson, A. & Hallegraeff, G.M. 2007. Calcification morphotypes of the coccolithophorid *Emiliania huxleyi* in the Southern Ocean: changes in 2001 to 2006 compared to historical data. *Marine Ecology Progress Series*, 348: 47-54.
- Hagino, K., Bendif, E.M., Young, J.R., Kogame, K.,
 Probert, I., Takano, Y., Horiguchi, T., de Vargas, C.
 & Okada, H. 2011. New evidence for morphological and genetic variation in the cosmopolitan coccolithophore *Emiliania huxleyi* (Prymnesiophyceae) from the cox1b-atp4 genes. Journal of Phycology, 47: 1164-1176
- Hagino, K. & Okada, H. 2006. Intra- and infra-specific morphological variation in selected coccolithophore species in the equatorial and subequatorial Pacific Ocean. *Marine Micropaleontology*, **58**: 184-206.
- Hagino, K., Okada, H. & Matsuoka, H. 2005. Coccolithophore assemblages and morphotypes of *Emiliania huxleyi* in the boundary zone between the cold Oyashio and warm Kuroshio currents off the coast of Japan. *Marine Micropaleontology*, **55**: 19-47.
- Henderiks, J., Winter, A., Elbrächter, M., Feistel, R., van der Plas, A., Nausch, G. & Barlow, R. 2012. Environmental controls on *Emiliania huxleyi* morphotypes in the Benguela coastal upwelling system (SE Atlantic). *Marine Ecology Progress Series*, **448**: 51-66.
- Iglesias-Rodríguez, M. D., Schofield, O., Batley, J., Medlin, L. K. & Hayes, P. K. 2006. Intraspecific genetic diversity in the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae): The use of microsatellite analysis in marine phytoplankton population studies. *Journal of Phycology*, **42**: 526-536.
- Langer, G., Nehrke, G., Probert, I., Ly, J. & Ziveri, P. 2009. Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry. *Biogeosciences*, **6**: 2637-2646.

- Medlin, L. K., Barker, G. L. A., Campbell, L., Green, J. C., Hayes, P. K., Marie, D., Wrieden, S. & Vaulot, D. 1996. Genetic characterisation of *Emiliania huxleyi* (Haptophyta). *Journal of Marine Systems*, **9**: 13-31.
- Paasche, E. 2002. A review of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions. *Phycologia*, **40**(6): 503-529.
- Schroeder, D. C., Biggi, G. F., Hall, M., Davy, J., Martínez Martínez, J., Richardson, A. J., Malin, G. & Wilson, W. H. 2005. A genetic marker to separate *Emiliania huxleyi* (Prymnesiophyceae) morphotypes. *Journal of Phycology*, 41: 874-879.
- Young, J., Geisen, M., Cros, L., Kleijne, A., Sprengel, C., Probert, I. & Ostergaard, J. 2003. A guide to extant coccolithophore taxonomy. *Journal of Nannoplankton Research*, *Special Issue*, 1: 125 pp.
- Young, J. & Westbroek, P. 1991. Genotypic variation in the coccolithophorid species *Emiliania huxleyi*. *Marine Micropaleontology*, **18**: 5-23.
- Young, J. R. 1994. Variation in *Emiliania huxleyi* coccolith morphology in samples from the Norwegian EHUX experiment, 1992. *Sarsia*, **79**: 417-425.