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Density dependent expression of a diatom retrotransposon

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ABSTRACT

Retrotransposons are mobile genetic elements that encode for their own replication. Many studies have linked their expression to stress caused by environmental factors. Genome sequencing and EST libraries of the coastal diatom *Phaeodactylum tricornutum* indicate that this organism has an active copia-like retrotransposon (*Blackbeard*), which is variably expressed under different culture conditions. In this study, we induce physiologic stress in *P. tricornutum* and measure *Blackbeard* expression over time. However, we find the dominant pattern of *Blackbeard* expression is related to cell culture density, not short-term physiologic stress. Density dependent expression of a retrotransposon in a diatom provides significant insight into the biogeography of diatom genome mutation. We suggest the shallow coastal ocean, where diatom densities are high, may be the geographic locus for generating genomic diversity in diatom lineages.

1. Introduction

Retrotransposons are abundant in eukaryotes, often comprising a large fraction of the genome (Lynch and Conery, 2003). They are capable of autonomous proliferation within a genome through the activity of self-encoded reverse transcriptase, but also are capable of self-deletion through illegitimate recombination (Devos et al., 2002). In addition to encoding reverse transcriptase, many retrotransposons encode group specific antigen, protease, polymerase, enveloping, and endonuclease domains, making the basic structure of a retrotransposon reminiscent of a modern retrovirus (Kazazian, 2004). The activity of retrotransposons can be destructive by interrupting or rearranging crucial sequences, but also potentially beneficial by introducing novel combination of sequences (McClintock, 1984). Because of this activity, these elements are thought to be major drivers of genome evolution by increasing the bulk rate of genome mutation (Kazazian, 2004).

The net impact of retrotransposons on genomic structure depends on transcriptional activity, efficiency of re-insertion, the relative fitness of the insertion, and the effective population size, many of which are unknown. However, there are now many examples of retrotransposons becoming active during environmental stress, most of which have been demonstrated in eukaryotic plant lineages (Grandbastien, 1998). For example, a transcriptionally active retrotransposon was found in experimental tissue cultures of the rice *Oryza sativa* (Hirochika et al., 1996). This study showed that the stress of tissue culture increased the copy number of the Tos17 LTR retrotransposon by at least a factor of 10 over a 16-month period. A more direct study of the OARE-1 a Ty1-copia LTR retrotransposon in oat (*Avenia sativa*) showed that these retrotransposons were also activated by the stress of UV light exposure, and by the addition of jasmonic and salicylic acid and plant wounding (Kimura et al., 2001). In addition, activation of the Tnt1A retrotransposon in tobacco is also induced in response to wounding (Grandbastien, 1998). The natural distribution of the closely related BARE-1 LTR retrotransposon in natural environments also suggests they are active in natural populations. It has also been shown that there was a sharp change in the distribution patterns of the BARE-1 element in wild barley (*Hordeum spontaneum*) in response to microclimate habitats (Kalendar et al., 2000). Populations of barley on adjacent north-facing and south-facing slopes of a canyon had large differences in the copy number of BARE-1. The copy number increase was correlated to the harsher, more stressful environment associated with inhabiting the bright and arid south-facing slope.

In the coastal marine diatom *P. tricornutum* (strain CCMP2561), sequencing, EST libraries and batch culture experiments indicate that a copia-like retrotransposon *Blackbeard* (Accession number AM931159) is transcriptionally active when kept in exponential grown phase in semi-continuous batch culture under low nitrogen conditions (Mamus et al., 2009). This suggests that genome restructuring through the activities of retrotransposons is not a rare occurrence in diatoms, but could be an active response to local environments that increases the rate of genome mutation.

The shallow coastal oceans of the Mid-Atlantic Bight are characterized by large nutrient pulses can occur on day time scales as a result of meteorologically driven upwelling, internal waves, and river plumes, resulting in highly episodic nitrogen pulses to the coastal ocean (Glenn et al., 2004; Malone et al., 1983). Natural diatom populations in a coastal environment experience rapid changes in

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conditions leading to "boom and bust" growth patterns, where diatom biomass increases rapidly over a short period of time achieving very high biomass densities, then rapidly crashes when nutrients are depleted (Moline et al., 2008). In this study, we expose batch cultures of *P. tricornutum* (CCMP630) to rapid changes in nitrogen concentration and measure the transcription of *Blackbeard*, physiologic state and cell densities. We find that *Blackbeard* does exhibit significantly higher transcription in low nitrogen conditions as in work with strain CCMP2561 (Mamus et al., 2009); however, we find that the major predictor of *Blackbeard* expression is biomass density, and not physiologic stress induced by nitrogen limitation. *P. tricornutum* (CCMP630) was isolated in the Mid-Atlantic region. Analysis of historic chlorophyll biomass concentrations in the Mid-Atlantic clearly shows that biomass density is a strongly related to physical geography. Therefore, we suggest that shallow continental shelves like the Mid-Atlantic are likely locations for diatom genome mutation and evolution.

2. Materials and methods

2.1. Culture conditions and treatments

Batch cultures of *P. tricornutum* (CCMP630) were tracked for a period of 14 days (enumerated from 0 to 13) and exposed to various nutrient regimes (Table 1). Our experimental design treats the population as a unit (i.e. it is assumed that each cell in the population responds similarly to the average of the population). All cultures were grown in a 14–10 light–dark cycle at 200 μmol photons m^{-2} s^{-1} and moderately bubbled. On day 0, one 4 L culture was inoculated and grown in F/2 media (Guillard and Ryther, 1962); starting concentration of 800 μM nitrate for a few days to provide enough biomass for the separate nitrogen treatments. On day 3, the biomass was equally split into four cultures: one control culture and three cultures with different nitrogen treatments. Approximately ¼ of the biomass from the initial culture was centrifuged (10,000×*g*, 4 °C, 10 min) and re-suspended in new respective growth media. The control cells were resuspended back into 3 L of F/2 media and the three treatment cultures were placed into 3 L F/2-N media (F/2 media with zero nitrogen added). Before the transfer of these cultures, they were washed with F/2-N media twice to remove residual nitrogen. On day 9, all four cultures were again centrifuged and re-suspended into 2 L fresh media for nitrogen recovery. The control culture and treatment 1 were placed back into nitrogen replete F/2 media. Treatments 2 and 3 were placed into F/2 with either 75 μM nitrate or 75 μM ammonia, respectively. Cells were harvested from each of these conditions approximately twice per day via stepwise centrifugation. First, 250 ml of culture was centrifuged (10,000×*g*, 4 °C, 10 min) and most of the supernatant was decanted. The concentrated cell suspension was then transferred to 2 ml microfuge tubes and centrifuged for 1 min at 10,000 rpm. The supernatant was removed and the pellets were flash frozen in liquid nitrogen before being stored at −80 °C.

2.2. Cell counts

Cell density was measured in triplicate using a Coulter Multisizer II fitted with a 70 μm glass orifice. Cells were diluted between 100 and 1000 times into 0.4 μm filtered seawater and were counted immediately. We counted 500 μl of the diluted samples and coincidence levels were less than 4%.

Table 1
Nitrogen concentrations added to the F/2 growth media.

Days	0–3 (μM)	3–9 (μM)	9–13 (μM)
Control	800	800	800
Treatment 1		0	800
Treatment 2		0	75
Treatment 3		0	75[a]

[a] Nitrogen was added back in the form of ammonia.

2.3. Photosynthetic physiology

The photosynthetic physiological response to nitrogen treatments was measured using a Satlantic *F*luorescence *I*nduction and *Re*laxation (FIRe) fluorometer (Gorbunov and Falkowski, 2004). The ratio of variable chlorophyll fluorescence to the maximum chlorophyll fluorescence (F_v/F_m) is the quantum yield of photosystem II, and thus is an indicator of light energy conversion by a photosynthetic organism. Photosystem II is the primary energy gateway for many photosynthetic organisms and is nitrogen rich, therefore F_v/F_m has been used as a general "health" index of photosynthetic organisms (Kolber et al., 1998). The FIRe instrument automatically adjusts its gain settings for each sample. Therefore to compute trends in F_m/cell, we corrected the F_m signal for the instruments individual variable gain calibration. We used F_m/cell as a proxy for how well the nitrogen additions were incorporated as intracellular pigment.

2.4. Nucleic acid extraction and QPCR

Total RNA was extracted from the frozen pellets using Tri Reagent® according to manufacturer's recommended protocol (Ambion). The RNA was subsequently treated with the Turbo DNA-free™ kit, using the most stringent DNAse treatment recommended by the manufacturer (Ambion). DNAse treated RNA was then reverse transcribed into first-strand cDNA with the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen) using oligo-dT primers. RNA transcription was measured using the Brilliant® SYBR® Green QPCR Core Reagent Kit and the Stratagene MX3000P QPCR instrument (Stratagene).

Genomic DNA (gDNA) was extracted from the frozen pellets using a standard phenol-chloroform extraction (Sambrook and Russell, 2001). The gDNA was subsequently treated with RNAse to remove RNA. Copy numbers of *Blackbeard* were estimated using RNAse treated gDNA with the Brilliant® SYBR® Green QPCR Core Reagent Kit and the Stratagene MX3000P QPCR instrument (Stratagene).

2.5. House keeping genes and primer optimization

The transcription of *Blackbeard* was quantified relative to the transcription of two housekeeping genes, Histone and the TATA box binding protein (*TATA*). The *Histone 4* protein is integral to DNA organization and the *TATA* box binding protein is a basal transcription factor. The *Histone 4* gene has been used as a comparative housekeeping gene in *P. tricornutum* (CCMP2561) experiments. For our study, two genes were chosen to normalize transcription of the *Blackbeard* to avoid bias interpretation stemming from a single normalizing gene (Thellin et al., 1999).

Blackbeard primer sequences were 5'-GTGTTCTTGCTGCAAATGGA 3' (forward) and 5'-ATTCATCGGGGTCACCAATA-3' (reverse). They were designed to amplify a 174 bp region of the *Blackbeard* reverse transcriptase domain. The primers used for *Histone 4* were 5'-AGGTCCTTCGCGACAATATC-3' (forward) and 5'-ACGGAATCACGAATGACGTT-3' (reverse) and amplified a 150 bp fragment. The primers used for *TATA* were 5'-CGGAATGCGCGTATACCAGT-3' (forward) and 5'-ACCGGAGTCAAGAGCACACAC-3' (reverse) and amplified a 180 bp fragment. Amplicons were cloned into plasmids using the TOPO TA Cloning® Kit, which were subsequently linearized by restriction digest with the *Pst1* enzyme. Linearized plasmid DNA was serially diluted from 10× to $1e^{-7}$X and served as pure target for PCR optimization (Table 2) and efficiency calculations. *Blackbeard*, *Histone 4* and *TATA* efficiency of amplification were 99%, 95%, and 95%, respectively.

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Table 2
Optimized primer conditions.

Gene	Forward: reverse primer ratio	Individual primer concentration (μM)
Blackbeard	1:1	800
Histone	1:1	800
TATA	1:1	300

2.6. Chlorophyll biomass analysis

Ocean color data from the MODIS-Aqua satellite mission were downloaded from NASA's Ocean Color website (http://oceancolor.gsfc.nasa.gov/). Chlorophyll data from July 2002 to March 2009 were processed using the SeaWiFS Data Analysis System with the standard atmospheric flags and chlorophyll algorithm (SeaDAS 5.3.0). The boundaries of the satellite imagery were 35° N to 46° N and −77° W to −63° W. Data were downloaded as level-2 standard-suite products at 1 km resolution. Chlorophyll values have log-normal spatial and temporal distribution (Campbell, 1995). Therefore, we first log transformed the spatial chlorophyll values before computing a chlorophyll climatology. One-minute (geographic) resolution bathymetry data was downloaded from the National Geophysical Data Center (http://www.ngdc.noaa.gov/mgg/bathymetry/relief.html) and interpolated to the 1 km MODIS-Aqua grid for comparison with average chlorophyll.

3. Results

3.1. Cell density and photosynthetic physiology

Cell density generally increased throughout the experiment after the inoculation and dilution events (Fig. 1). The first day of exposure to F/2-N media, cells continued to increased in number with high specific growth rates (>1). However, after two days of exposure to F/2-N media, cells reduced their specific growth rate to near zero. F_v/F_m in the control culture was >0.45 throughout the experiment, indicating that the control remained healthy throughout the experiment (Fig. 1A). When the three treated cultures were exposed to F/2-N media, F_v/F_m dropped rapidly for all cultures, stabilizing at a value of ~0.23, indicating that these cultures were physiologically stressed (Fig. 1B–D). In addition, F_m/cell decreased relative to the control indicating that the amount of chlorophyll per cell was decreasing during nitrogen stress. This decrease could have been due to simple dilution of pigments within the population through cell division, or internal harvesting of nitrogen rich chlorophyll by the cells in low nitrogen conditions. In either case, less nitrogen rich pigment is being devoted to light harvesting. For all three treatment cultures, the addition of nitrogen back into the system (Day 9) resulted in a dramatic increase in F_v/F_m within 10 h, approaching control F_v/F_m levels in less than a day. In treatment 1, F_v/F_m remained high after nitrogen addition and F_m/cell increased indicating that the nitrogen addition resulted in a downstream increase of chlorophyll. In treatments 2 and 3, where only 75 μm nitrate or 75 μm ammonia were added back to each respective culture, F_v/F_m increased for a short time, but then dropped quickly as the nitrogen was used by the cells. While cell number increased after the low-level nutrient addition, F_m/cell did not show a large increase indicating that the nitrogen addition was not being used to manufacture chlorophyll.

3.2. Relative transcription of Blackbeard

The change in *Blackbeard* transcript abundance normalized to *Histone* transcription (*Blackbeard*/*Histone*) or the *TATA* box binding protein transcription (*Blackbeard*/*TATA*) increased throughout the experiment for all treatments (Fig. 2). Transcript abundance of the *Blackbeard* element was 3–7 fold higher at the end of the stress time period than at the beginning of the experiment. Transcription levels remained high at subsequent time points, when nitrogen was re-introduced to Treatments 1–3, indicating nitrogen stress is not the primary driver of *Blackbeard* transcription. Also, the different amounts and types of N additions post starvation did not appear to influence *Blackbeard* transcription.

Model II regressions of the fold change in *Blackbeard*/*Histone* and *Blackbeard*/*TATA* to fold change in cell number indicated that *Blackbeard* transcription was significantly positively correlated to fold change in cell density ($p<0.01$ for both normalizer genes) (Fig. 3). The slopes of these two regressions were not significantly different ($p<0.01$), indicating that this result is not sensitive to *Histone* or *TATA* normalization. Notably,

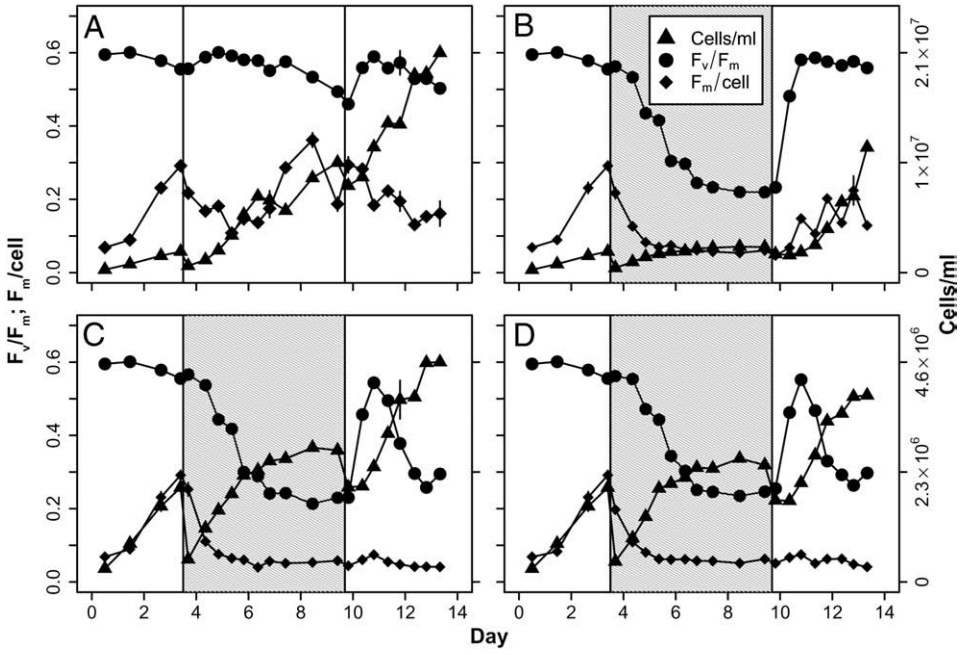

Fig. 1. Cell density, F_v/F_m, and F_m/cell for the control (A) and for treatment 1, 2, 3 (B, C, D respectively – Table 1). Vertical lines indicate time points where cultures were resuspended in new growth media. Grey areas indicate where cultures were exposed to a zero nitrogen condition. Error bars are the second standard deviation from triplicate measurements. Note that the scales for cells/ml are different for panels C and D.

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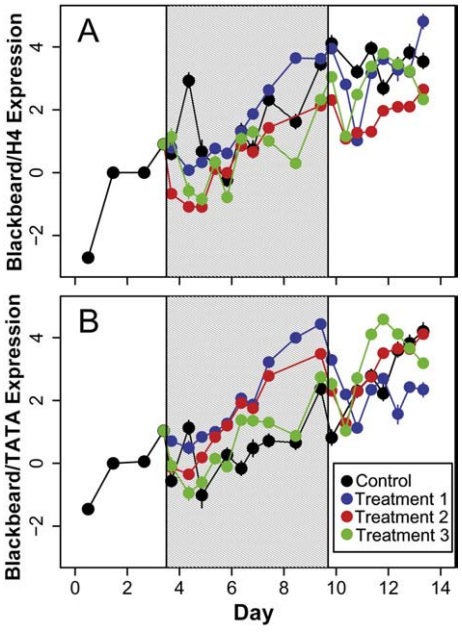

Fig. 2. Time course of *Blackbeard* expression relative to Histone (A) and TATA (B). Areas between the vertical lines indicate when Treatments 1–3 were exposed to zero N conditions. Expression at the end of the experiment is significantly higher than at the beginning of the experiment. Error bars are the second standard deviation from triplicate measurements.

the experimental conditions (i.e. Treatments 1–4) did not overlay each other. The control condition was below the best-fit line while the three treatment conditions were above it; indicating that for equivalent cell densities, cultures exposed to low nitrogen conditions had a higher transcription level of *Blackbeard* compared to the N replete control culture. Hence, nitrogen starvation is a secondary driver of *Blackbeard* transcription. A similar analysis between specific growth rate and *Blackbeard* transcription found no significant relationship for any of the treatments.

In order to specifically assess the role of nitrogen stress on *Blackbeard* expression, we removed the density dependent effect on *Blackbeard* transcription by normalizing its fold transcript abundance to fold increase in cell concentration (cells/ml) and subtracting the control from the three treatment conditions. This analysis revealed significantly higher *Blackbeard* transcription during nitrogen stress only after cells had spent 2–6 days under nitrogen starvation conditions after statistically removing the density dependent effect on *Blackbeard* transcription (Fig. 4). A large excursion in *Blackbeard* transcript abundance relative to the control was observed after the initial dilution, but the second dilution did not show the same effect. It is unknown what caused this large excursion. It is possible that slight differences in media could cause this, but this seems unlikely because all of the media we used in this experiment was made in the same batch. Addition of nitrogen (Day 9) decreased transcript abundance of *Blackbeard* relative to the control and initiated a general down regulation of *Blackbeard* through the end of the time series, which converged with the transcript level in the control.

3.3. Analysis of Blackbeard genome copy number

Blackbeard encodes the ability to reverse transcribe and re-insert itself into the genome, thus increasing copy number in the genome. As in previous work with *Blackbeard* (Mamus et al., 2009), increased copy number of *Blackbeard* relative to copy number of *Histone* or *TATA* in gDNA was not detected by QPCR methods in any of the treatments (data not shown).

3.4. Analysis of chlorophyll concentrations distributions in Mid-Atlantic

Water depth is a strong predictor of mean chlorophyll concentrations on $\log_{10}$ scales in the Mid-Atlantic Bight ($r^2 = 0.83$, $p < 0.01$, Fig. 5). The 5% and 95% percentiles show that chlorophyll values are

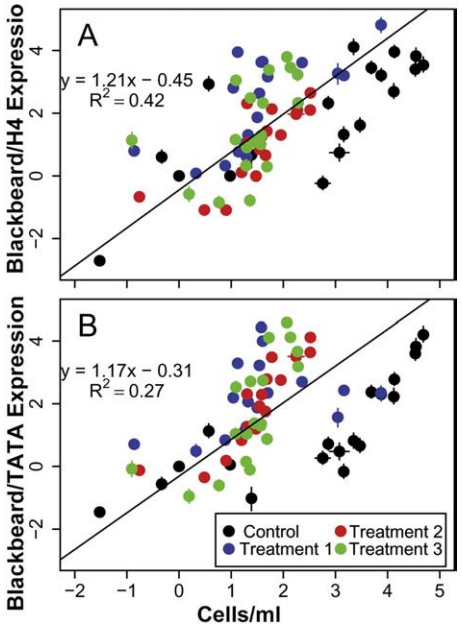

Fig. 3. Model II regression between fold change in *Blackbeard* expression relative to Histone (A) and TATA (B) and fold change in cell density (black, control; blue, Treatment 1; red, Treatment 2; green, Treatment 3). Regression lines were significant at the $p < 0.01$ level and were not significantly different from each other between panel A and panel B. Error bars are the second standard deviation from triplicate measurements.

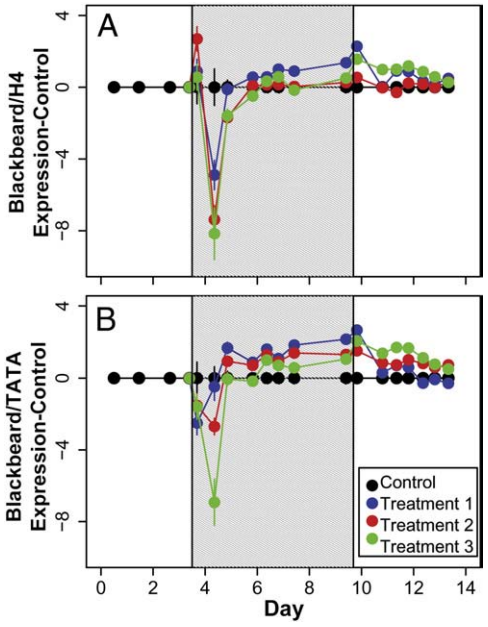

Fig. 4. Fold change in *Blackbeard* expression relative to Histone (A) and TATA (B) normalized to fold change in cell density to remove the effect of cell density (color code is same as Fig. 3). Expression of the control subtracted out for plotting purposes and is indicated by black line. This analysis indicates that culture dilution followed by N stress initially down regulates *Blackbeard* transcription but is then followed by increased expression of *Blackbeard* relative to the control. Nitrogen addition decreases *Blackbeard* expression such that it converges on the expression level observed in the control. Error bars are the second standard deviation from triplicate measurements.

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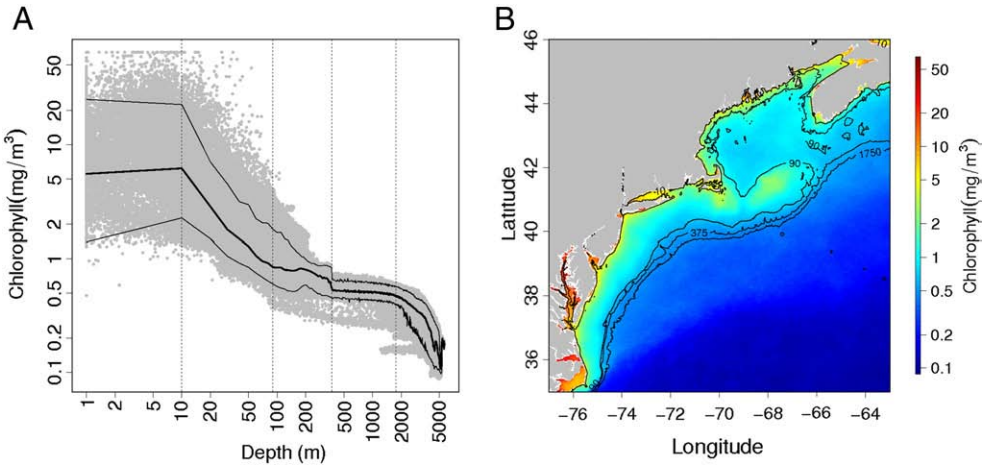

Fig. 5. The depth dependence of average chlorophyll concentration in the Mid-Atlantic region (A). The dark line is the mean of the point cloud, while the thin lines represent the five and ninety-five percentile. Vertical lines highlight changes in the bathymetry-chlorophyll relationship. Average chlorophyll concentration spatial patterns (B) match closely to bathymetric contours at 10, 90, 375 and 1750 m.

more variable in shallow depths. The relationship between depth and mean chlorophyll is stepwise, with at least five discernable changes in the depth-chlorophyll relationship at approximately 10 m, 90 m, 375 m and 1750 m.

4. Discussion

In eukaryotes, a vast majority of total DNA content is probably comprised of retrotransposons and other mobile elements (Lynch and Conery, 2003). Retrotransposons have been traditionally described as genetic parasites because they encode for their own reproduction (Doolittle and Sapienza, 1980; Orgel and Crick, 1980). This activity makes them a potentially harmful mutagenic force within a genome. Why purifying selection has not eliminated these deleterious parasites is a standing question. There are three general solutions to this problem. The first is that purifying selection is weak and unable to rid the cell of deleterious parasitic elements (Lynch and Conery, 2003). The second is that mobile elements and the cost of the extra DNA needed to incorporate them into a genome are generally not deleterious but are neutral (Oliver et al., 2007). The third is that these mobile elements are at some level used by the cell and are therefore preserved (Goodier and Kazazian, 2008; Kidwell and Lisch, 1997). These general explanations are not necessarily mutually exclusive, but dependent on the level of selection and time frame of interest. For example, an individual might experience hard negative selection if an environmentally induced retrotransposon re-inserts into a single copy of a necessary gene. However, if the net frequency of re-insertion of a retrotransposon into the genome is very rare, it may be considered nearly neutral for a whole species on long, evolutionary time scales.

In this study, we focus on the time scale of diatom "boom and bust" patterns (days to weeks) at the population level of a coastal diatom. At this time scale, there are expectations about the effect of retrotransposon transcription on the population based on the three general solutions to the presence of retrotransposons in eukaryotes. We found consistent and significant density and nitrogen starvation driven patterns of *Blackbeard* transcript abundance on these time scales, indicating that it is unlikely that transcription of this retrotransposon is best described as a random neutral phenomenon on short time scales, but rather is environmentally induced. If *Blackbeard* were a deleterious element on the day to week time scale, we would expect that cell physiology would be negatively correlated with *Blackbeard* transcription. We found no significant correlation between growth rate or cell physiology as estimated by F_v/F_m and *Blackbeard* transcript abundance. Furthermore, the higher expression of *Blackbeard* after nitrogen starvation (Fig. 2) did not inhibit physiological recovery of the cultures (Fig. 1). These findings suggest that *Blackbeard* transcription is not deleterious on the day to week time scales. However, it should be noted that a lack of evidence for deleterious effects is not by itself strong evidence for positive selection of *Blackbeard*. For positive selection to be demonstrated, *Blackbeard* needs to be linked to some other nominal cell function or have some net fitness benefit. Clear correlation to normal cell function would suggest that retrotransposons such as *Blackbeard* are in some cases employed by the cell (Kidwell and Lisch, 1997). For example, retrotransposon sequences are often found in promoter regions of genes (Takeda et al., 1999).

In this study, we found that exposing *P. tricornutum* (CCMP630) to nitrogen related stress resulted in up to a two-fold increase in the *Blackbeard* compared to the nitrogen replete control culture (Fig. 4). This indicates that stress related nitrogen starvation up-regulates *Blackbeard* transcription, and is similar to the results found in semi-continuous batch cultures with strain CCMP2561 (Mamus et al., 2009). Also, *Blackbeard* was down regulated when either nitrate or ammonia was reapplied to starved cultures. We observed that while the addition of nitrogen reduced *Blackbeard* expression indicating that nitrogen may have been a stressor to the cells, variable fluorescence, which is a known indicator of physiological nutrient stress in phytoplankton cultures, was not related to *Blackbeard* expression. For example, we still observed relatively high expression of *Blackbeard* in the control culture even though F_v/F_m indicated that the cells were not physiologically stressed.

Our results indicate that the dominant *Blackbeard* induction pattern is related to cell density rather than nitrate concentration or physiological stress as measured by F_v/F_m. However, there did not appear to be a threshold density for *Blackbeard* expression. This may indicate that Blackbeard is always expressed at some low level at all cell densities. Density-dependent expression of a gene has been traditionally linked to cell signaling and quorum sensing (Bassler, 1999; Fuqua et al., 1994). Recent reports have shown how diatom-derived unsaturated aldehydes can regulate intercellular signalling, stress surveillance, and defence against grazers (Ianora et al., 2004; Vardi et al., 2006). There is also evidence that retrotransposon transcription is also triggered by NO, a common diatom signaling molecule (Vardi et al., 2008). These observations are in line with other suggestions that retrotransposons are regulated by cell signaling pathways (Labudova and Lubec, 1998). While it seems that the density dependent transcription of *Blackbeard* could be related to normal cell signaling, could also be related to light levels within the culture population. The interaction of light with water naturally generates a negative feedback relationship between light and phytoplankton population density. Due to light attenuation, light is

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a natural anti-correlate of cell density for all phytoplankton populations in exponential growth, either in batch culture or in the environment.

What is critical here is that this density-dependent expression pattern, irrespective of its proximate cause, points to contemporary ocean environments where increased activity of retrotransposons like *Blackbeard* in diatoms is expected. Diatoms like *P. tricornutum* are far more abundant in nutrient rich, turbulent coastal environments than in the open ocean. This is because the growth rates and nutrient kinetics of diatoms favor these types of environments (Tozzi et al., 2004). In the Mid-Atlantic, the near shore coastal environment is turbulent due to interactions of synoptic wind patterns with bathymetry and continental boundaries (Gong et al., 2010; Malone, 1976; Oliver et al., 2004) and are nutrient rich due to coastal upwelling (Glenn et al., 2004) and coastally trapped river plumes (Chant et al., 2008). These physical interactions predict that the physical geography of coastal environments is predictive of dense populations of diatoms (Fig. 5), and by extension, density dependent retrotransposon activity.

The importance of shallow turbulent areas is also evident in the radiation of diatoms in the late Cretaceous. At that time coastal oceans became more turbulent, and nutrients were delivered to the surface ocean in episodic pulses, thus favoring the diatom "boom and bust" growth strategy (Katz et al., 2004). This potentially explains why diatom genome size evolves at a faster rate than other eukaryotic groups (Oliver et al., 2007), and similar to the rate for Angiosperms which are noted for high retrotransposon activity. Therefore, if diatom retrotransposon activity is linked to population density, and the ecological success of diatoms in the modern ocean is driven by their "boom and bust" growth model, it is not surprising that their genome size is evolving rapidly. This assumes, however that retrotransposons in diatoms generally behave like *Blackbeard* and re-insert themselves in the genome. As with the *P. tricornutum* CCMP2561 (Mamus et al., 2009), we were unable to detect re-insertion in our experiments on weekly time scales. It is curious that a cell would allow potentially mutagenic elements such as *Blackbeard* in nominal cell function under what would seem "normal" environmental conditions that are prevalent in the modern coastal ocean. Although our experiments potentially explains some of the patterns we observe in diatom genome evolution, further study of retrotransposons in diatoms is needed to establish the relative fitness of retrotransposon activity in natural diatom populations. This may possibly be accomplished through over expression techniques in diatoms (Vardi et al., 2008).

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