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Relative stability of ploidy in a marine *Synechococcus* across various growth conditions

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Summary

Marine picocyanobacteria of the genus *Synechococcus* are ubiquitous phototrophs in oceanic systems. Consistent with these organisms occupying vast tracts of the nutrient impoverished ocean, most marine *Synechococcus* so far studied are monoploid, i.e., contain a single chromosome copy. The exception is the oligoploid strain *Synechococcus* sp. WH7803, which on average possesses around 4 chromosome copies. Here, we set out to understand the role of resource availability (through nutrient depletion) and physical stressors (UV, exposure to low and high temperature) in regulating ploidy level in this strain. Using qPCR to assay ploidy status we demonstrate the relative stability of chromosome copy number in *Synechococcus* sp. WH7803. Such robustness in maintaining an oligoploid status even under nutrient and physical stress is indicative of a fundamental role, perhaps facilitating recombination of damaged DNA regions as a result of prolonged exposure to oxidative stress, or allowing added flexibility in gene expression via possessing multiple alleles.

Introduction

Low nutrient concentration across vast tracts of the world's oceans has meant that organisms have to compete for scarce resources (Raven, 1998). In oligotrophic waters, this has led to small cells with high surface area to volume ratios containing a plethora of nutrient transporters to optimize acquisition of these limiting resources. As well as optimising acquisition processes, organisms have also reduced demand, leading to genome streamlining in dominant phototroph [e.g., *Prochlorococcus*, see Partensky and Garczarek (2010)], and heterotroph [e.g., SAR11, see Giovannoni *et al.* (2005)] populations, as well as a decline in %GC content, reducing nitrogen (N) requirements per genome. More specifically in P-deplete waters, a replacement of phosphorus (P) containing lipids for non-P containing lipids occurs, again conserving or recycling previously obtained resources (Van Mooy *et al.*, 2006; Carini *et al.*, 2015; Sebastián *et al.*, 2016). Such resource acquisition or minimization strategies is to some extent in contrast to the observation of the release of extracellular vesicles by such organisms in which hard won resources (proteins, nucleic acids or lipids) are 'lost' to the environment, presumably due to specific communication or horizontal gene transfer functions (Biller *et al.*, 2014).

In cyanobacteria, another potential contradiction to the idea of reducing resource utilization during growth in nutrient poor environments is that many strains of cyanobacteria are known to be oligoploid or polyploid, i.e., contain multiple copies of their chromosome (Griese *et al.*, 2011). Such multiplication of genome copies is in direct contrast to the phenomenon of genome streamlining, where many genes have been lost (Kettler *et al.*, 2007) or potentially outsourced (Morris *et al.*, 2012). High levels of ploidy appear particularly prevalent in freshwater cyanobacteria (Griese *et al.*, 2011), including unicellular species such as *Synechocystis* sp. PCC 6803, the latter strain also demonstrating high variability in chromosome copy number dependent on growth conditions (Zerulla *et al.*, 2016). In contrast, members of the marine *Synechococcus* lineage, which are ubiquitous in oceanic waters (see Scanlan *et al.*, 2009; Scanlan, 2012), are largely monoploid in those few strains characterized (Armbrust *et al.*, 1989; Binder and Chisholm, 1995; Griese *et al.*, 2011), potentially consistent with

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inhabiting this resource limited environment. However, an exception is marine *Synechococcus* sp. WH7803 that possesses between 3 and 10 chromosome copies, dependent on the method of assessment, i.e., flow cytometry or qPCR (Binder and Chisholm, 1995; Griese et al., 2011). Given that *Synechococcus* sp. WH7803 is one of the few genetically tractable marine *Synechococcus* strains (Brahamsha, 1996) we set out here to determine if ploidy status in this strain is regulated by various environmental parameters to ultimately give us a window into understanding the role of ploidy in these organisms.

Results and discussion

Ploidy level in *Synechococcus* sp. WH7803 was determined by qPCR. For more details on the materials and methods, please refer to Appendix S1. DNA extraction for the qPCR assay used mechanical lysis, which was shown to be highly efficient at lysing cellular material compared to chemical lysis (data not shown). The detection limit for the qPCR assay was 10 000 cells (Supporting Information Fig. S1), which was determined by flow cytometric sorting of *Synechococcus* sp. WH7803 cells. Salts in the artificial seawater medium inhibited the qPCR reaction, detecting an order of magnitude less material (Supporting Information Fig. S1). To avoid salt inhibition of the qPCR, different methods were tested for desalting the DNA samples before assay. The most efficient method, i.e., requiring the least amount of time for desalting and without losing DNA, was centrifugation, re-suspension with nuclease-free water and mechanical lysis of cells (Supporting Information Fig. S1). As a control for the potential oligoploid nature of *Synechococcus* sp. WH7803, we also assessed chromosome copy number in a 'sister' strain *Synechococcus* sp. WH7805, which was previously suggested to be monoploid based on flow cytometry analysis (Binder and Chisholm, 1995). Both *Synechococcus* strains were

isolated in the same year (1978) and from the same location (33°45'N, 67°30'W) in the Sargasso Sea at 25 m depth (Waterbury et al., 1986) and are closely related phylogenetically (Urbach et al., 1998; Fuller et al., 2003; Farrant et al., 2016), providing an appropriate comparison of ploidy status. Primer selection for the qPCR assay for *Synechococcus* sp. WH7803 and WH7805 was based both on their specificity to each strain and that the same amplification efficiency for each primer set was obtained (see Supporting Information Fig. S2).

Chromosome copy number was determined using both *Synechococcus* sp. WH7805 cells alone or with an equimolar amount of *Synechococcus* sp. WH7803 cells to assess the reproducibility of the release of nucleic acids by cell lysis. Both experiments gave the same results, with an average of 4.6 ± 2.2 copies for *Synechococcus* sp. WH7803 compared to 1.1 ± 0.3 copies of the *Synechococcus* sp. WH7805 chromosome per cell, confirming the oligoploid nature of *Synechococcus* sp. WH7803 and that *Synechococcus* sp. WH7805 is monoploid. It is important to remember that these copy number values represent a population average with the potential in *Synechococcus* sp. WH7803 at least, that copy number in individual cells is more variable.

Given the higher chromosome copy number in *Synechococcus* sp. WH7803 compared to *Synechococcus* sp. WH7805, this could suggest an increase in cell size in the former strain. We thus assessed cell size by flow cytometry following staining with SYBR Green I in a mix of *Synechococcus* sp. WH7803 and WH7805 cells obtained from the exponential phase of growth. Forward scatter as a proxy for cell size showed cells of both cultures were the same size (Fig. 1A), whereas a shift in green fluorescence was observed for *Synechococcus* sp. WH7803 (Fig. 1B). This indicates that whilst the DNA content was higher in *Synechococcus* sp. WH7803, cell size was the same for both strains.

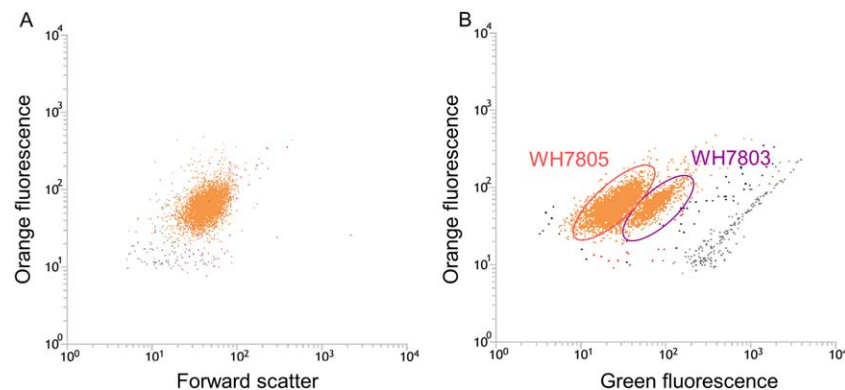


Fig. 1. Flow cytometry plot of a mixed culture of *Synechococcus* sp. WH7805 and *Synechococcus* sp. WH7803 stained with SYBR green I. (A) Orange fluorescence (580 nm), for detection of phycoerythrin, versus forward scatter. (B) Orange fluorescence versus green fluorescence (530 nm), for detection of SYBR green I DNA staining. *Synechococcus* sp. WH7803 and *Synechococcus* sp. WH7805 cells are indicated by purple and red circles respectively.

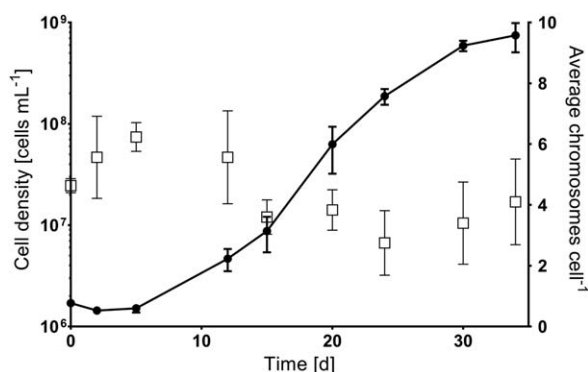


Fig. 2. Assessment of chromosome copy number in *Synechococcus* sp. WH7803 throughout growth. The left y axis depicts cell density (cells mL⁻¹; filled circles) and the right y axis the average chromosome copy number (open squares) of three biological replicates (and three technical replicates). Error bars represent standard deviation.

Determination of chromosome copy number in *Synechococcus* sp. WH7803 through the growth phase

We next determined chromosome copy number in *Synechococcus* sp. WH7803 during the lag phase, mid exponential, late exponential and early stationary phases of growth (Fig. 2). Ploidy level remained relatively stable throughout growth with a mean of 4.8 ± 1.4 copies per cell and no significant difference (t test: $P > 0.05$) between phases, though with a higher value at the end of the lag phase (ca 6 copies per cell) and lower value towards the end of the linear phase (ca. 3 copies per cell). Note though the relatively high coefficient of variation between replicates (CV = 28%), which as mentioned above may be linked to the additional variability in chromosome copy number in individual cells.

The relative stability of chromosome copy number in *Synechococcus* sp. WH7803 during growth is similar to that observed in the oligoploid freshwater cyanobacterium *Synechococcus elongatus* PCC 7942 (Griese *et al.*, 2011), which also shows an increase in ploidy at the end of the lag phase (Watanabe *et al.*, 2015). In contrast, the Archaea *Halobacterium salinarum* and *Halovorax volcanii* and the freshwater cyanobacterium *Synechocystis* sp. PCC 6803 all show higher chromosome copy numbers during exponential growth phase and a subsequent reduction in ploidy level when cells enter stationary phase (Breuert *et al.*, 2006; Griese *et al.*, 2011). Explaining such differences in chromosome copy number across growth also requires knowledge of how chromosomes segregate during cell division. In *Synechococcus elongatus* PCC 7942, chromosomes are spatially ordered along the long axis of the cell in a uniform way, which along with accurate mid-cell septum placement allows for near-optimal segregation of chromosomes to daughter cells (Jain *et al.*, 2012). In contrast, in *Synechocystis* sp. PCC

Table 1. *Synechococcus* sp. WH7803 growth rates and chromosome copy number under nutrient deplete and physical stress conditions.

	μ [d ⁻¹]	Chromosomes cell ^{-1a}	CV %
Control	0.24 ± 0.01	4.8 ± 1.4	28
-P	0.16 ± 0.02	3.7 ± 1.4	36
-N	0.19 ± 0.03	3.7 ± 1.4	36
-Fe	0.14 ± 0.03	3.0 ± 1.1	36
UV	nd	5.1 ± 1.7	33
High temperature	nd	4.7 ± 1.8	37
Low temperature	nd	4.2 ± 1.9	46

nd, not determined.

a. Average chromosomes cell⁻¹ across all time points.

CV %, Coefficient of variation expressed in percentage.

6803, segregation occurs late during the cell cycle potentially in a random and passive way (Schneider *et al.*, 2007) similar to that found in a filamentous cyanobacterium (Hu *et al.*, 2007). However, no information is currently available for marine *Synechococcus* strains.

How does variation in environmental conditions affect *Synechococcus* sp. WH7803 chromosome copy number?

Synechococcus sp. WH7803 grown under -N, -P or -Fe conditions showed a relatively stable chromosome copy number (Table 1) despite either a reduced growth rate (-P conditions) or growth arrest (-Fe, -N) (Bergkessel *et al.*, 2016). Specifically, the copy number per cell decreased marginally by 1.2 ± 0.5 chromosome copies per cell under -N and -P conditions compared to the control (t test: $P < 0.05$), but more so under Fe starvation (1.9 ± 0.4 fewer chromosomes per cell; t test: $P < 0.0001$) (Table 1, Fig. 3). The marginal decrease in chromosome copy number under P-deplete conditions in *Synechococcus* sp. WH7803 contrasts with that observed in the archaeon *H. volcanii* and the cyanobacterium *Synechocystis* sp. PCC 6803, which decrease chromosome copy number substantially under such conditions, from 20 under normal growth conditions to 2 under P-deplete growth [*H. volcanii*, Zerulla *et al.* (2014)] or from 50 (P-rich) to 1 (P-starved) for *Synechocystis* sp. PCC 6803 (Zerulla *et al.*, 2016).

Physical stressors (high temperature, low temperature and UV exposure) also had little effect on the ploidy status of *Synechococcus* sp. WH7803 with no significant difference compared to unstressed control cultures (t test: $P > 0.05$; Table 1).

Conclusions

Synechococcus sp. WH7803 appears unusual amongst those marine *Synechococcus* strains assessed for ploidy

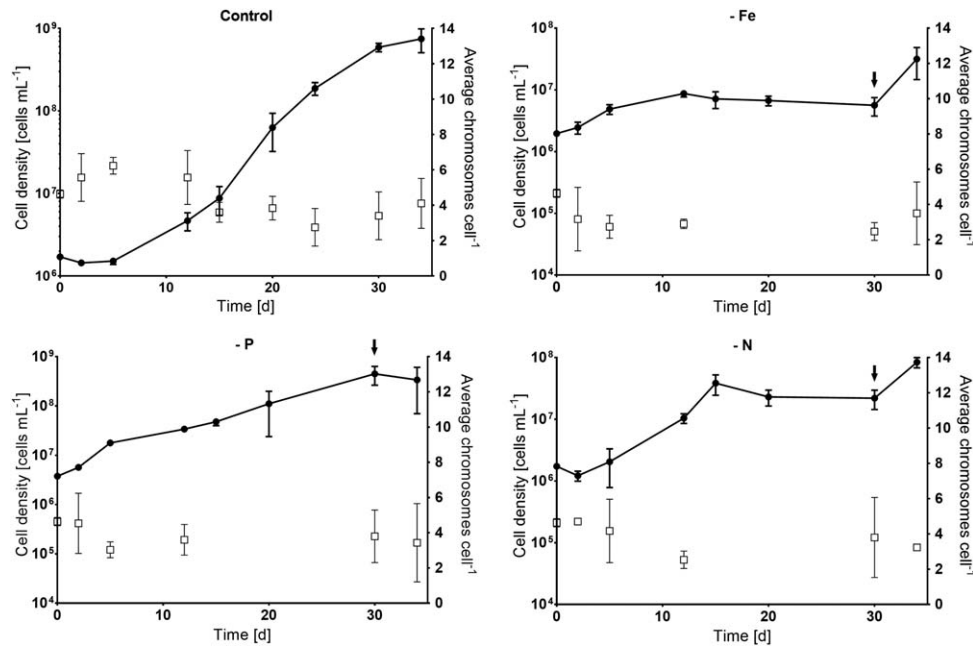


Fig. 3. Assessment of chromosome copy number during growth of *Synechococcus* sp. WH7803 under standard (control) and nutrient deplete (-Fe, -P and -N) conditions. Growth curve (left y-axis; filled circles) and the average chromosome copy number (right y-axis; open squares) of three biological replicates (and three technical replicates) during growth. After 30 days of growth in nutrient deplete medium, nutrient levels were restored to those in ASW medium (black arrows) and growth monitored for a further 4 days. Chromosome copy number was determined at all time-points except time 12 h and 15 h in nutrient-deplete experiments. Error bars represent standard deviation. Note that for clarity, the left y-axis scale varies between conditions.

status in containing on average around 4 chromosome copies per cell. As shown here, such a ploidy status appears relatively invariant irrespective of environmental conditions and whilst it is unknown whether polyploidy only arose following isolation of this organism into culture, ploidy level indeed appears stable across time given similar indications of ploidy in the same strain in the mid-90's (Binder and Chisholm, 1995). In contrast, most marine *Synechococcus* so far examined appear to be monoploid, including *Synechococcus* sp. WH7805, a strain closely related to WH7803 and despite the growth of these strains in a nutrient-rich medium over several decades. Such a monoploid nature is consistent with the idea of reducing resource demand for organisms inhabiting nutrient-poor conditions but does not explain the polyploid character of the *Synechococcus* sp. WH7803 strain. Perhaps a polyploid species like WH7803 possesses gene/DNA sequence redundancy that could facilitate recombination of DNA damaged regions as result of oxidative stress resulting, e.g., from prolonged exposure to high irradiance or UV radiation, using unmodified chromosomes as a template. Alternatively, ploidy may allow added flexibility in gene expression via possessing multiple alleles, a phenomenon recently observed in a diploid diatom (Mock *et al.*, 2017), providing an advantage over monoploid organisms to adapt to different environments and threats (Makarova *et al.*, 2001;

Soppa, 2013). Certainly, further work is required to assess whether the WH7803 strain is unusual in this respect or whether polyploidy is more prevalent in natural marine *Synechococcus* populations than currently thought.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Assessing the detection limit and the effect of salt on qPCR quantification of chromosome copy number in *Synechococcus* sp. WH7803 and WH7805. 10^0 – 10^7 sorted *Synechococcus* sp. WH7803 cells were used for qPCR with ASW medium (black circles) or washed with nuclease free water (white circles) to remove excess salt. The detection limit was determined to be 10^4 cells.

Fig. S2. Standard curves constructed with *Synechococcus* sp. WH7803 and WH7805 genomic DNA. The efficiency is calculated as R^2 .