Diatoms favor their younger daughters

Samuel R. Laney,* Robert J. Olson, and Heidi M. Sosik

Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

Abstract

We used a time-lapse imaging approach to examine cell division in the marine centric diatom *Ditylum brightwellii* and observed that daughter cells who inherited their parents' hypothecal frustule half were more likely to divide before their sisters. This is consistent with observations in *Escherichia coli* of a bias between sister cells, where faster growth in one sister is thought to arise from its inheriting parental material with less oxidative damage. We also observed that hypothecal sisters in *D. brightwellii* were more likely to inherit a greater proportion of their parents' cellular material, similar to what has been seen in *E. coli*. We found a statistically significant correlation between the amount of parental material inherited by a hypothecal daughter and its relative division rate, indicating that this extra material inherited by the hypothecal daughter plays a role in its more rapid division. Furthermore, the intercept in this regression was greater than zero, indicating that other factors, such as differences in the quality of inherited material, also play a role. This similarity between two taxonomically distant microbes suggests that favoritism toward one daughter might occur broadly among unicellular organisms that reproduce asexually by binary fission. Such a bias in cell division might be advantageous, given model predictions that show that favoring one daughter at the expense of the other can result in higher population growth rates, increasing the chance that a cell's genotype will survive compared to a model where the daughters divide at equal rates.

Many unicellular organisms reproduce primarily by binary fission, an asexual process in which a parent cell undergoes first mitosis, then cytokinesis, to form two new daughter cells. Mitosis ensures that these sister cells are effectively identical in terms of genotype, but it is likely that they will differ at least slightly in phenotype. Phenotypic differences between two nascent sister cells can arise from stochastic effects during cell division that result in the inexact division of parental material between the two progeny. Non-stochastic mechanisms can also bias the allocation of parental material between two sister cells, sometimes systematically and predictably. The degree to which phenotypic differences occur between sister cells—and whether these differences reflect purely stochastic or instead biased processes—may have important consequences not only to the relative growth and fitness of these two cells as individuals, but also at the population level, depending on how these between-sister asymmetries are manifested.

A well-known example of a systematic, between-sister phenotypic asymmetry is seen in the diatoms, arising from the morphology of their rigid exterior frustules. Diatom frustules are composed of two halves of slightly different diameters, and during cell division one daughter inherits the parent's larger-diameter epithecal half and its sister inherits the smaller-diameter hypothecal half. Each daughter then forms a new hypotheca to fit inside whichever frustule half it inherited (Macdonald 1869; Pfitzer 1869). A consequence of this between-sister morphological asymmetry is that the sister inheriting the epitheca will be the same diameter as its parent and the other will be slightly smaller. Although this difference in diameter is slight, over many generations it will lead to the well-known diminution in cell

diameter in lineages of cells that repeatedly inherit the smaller-diameter hypotheca (Fig. 1). This asymmetry between every pair of diatom sisters structures not only the distribution of size in diatom populations but also the distribution of sex, because sex in diatoms occurs primarily among the smallest cells, those whose ancestors predominantly inherited their parents' hypothecae and who have become smaller than their species-specific size threshold for gametogenesis (Round 1972; Drebes 1977).

That a slight but systematic phenotypic difference between every two diatom sisters, established at the time of their formation, can structure the distributions of size and sex at the population level is both well understood and long accepted (Rao and Desikachary 1970; Crawford 1981; Edlund and Stoermer 1997). Less well understood is how such phenotypic differences between two nascent sister cells affect their metabolism, growth, or fitness as individuals. Historically, inheritance of either the hypotheca or the epitheca has not been expected to confer any significant benefit or penalty on the fitness of the daughter that inherits it because the two frustule halves differ too little in size to introduce any meaningful differential effect. Yet in the prokaryote *Escherichia coli*, a bacterium that forms two daughters that had been assumed to be identical, small but systematic differences in size and division rates have been observed between pairs of sister cells (Stewart et al. 2005). This between-sister phenotypic difference is thought to arise during cell division where one sister is preferentially endowed with parental cellular material that is of higher quality. Individual *E. coli* cells are rod shaped and each cell inherits one of its poles (ends) already formed from its parent. The other pole is completed later as a cell separates from its sister. Because a cell's more newly formed pole contains material that has experienced less cumulative oxidative damage, it is expected that the *E. coli* daughter

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* Corresponding author: slaney@whoi.edu

Time

1st gen

2nd gen

3rd gen

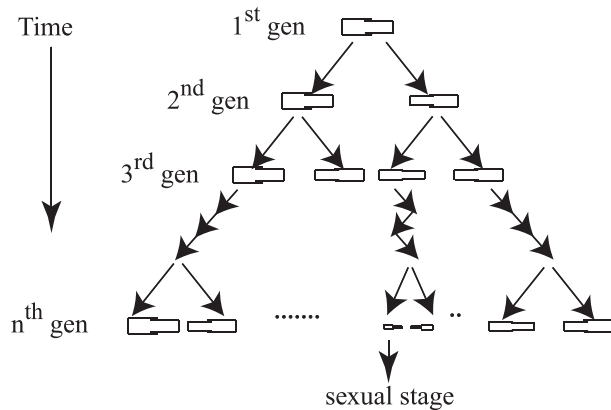nth gen

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sexual stage

Fig. 1. The canonical MacDonald-Pfitzer rule for diatom reproductive ecology. Binary division produces two daughters of near-equal size that inherit either the parent's slightly larger-diameter epitheca or its slightly smaller-diameter hypotheca. Each daughter then forms a new hypotheca to fit inside whichever frustule half it inherited. Cells in lineages that repeatedly inherit the hypotheca (center of diagram) decrease in diameter with each successive generation, eventually becoming too small to divide viably. Small cells can escape this fate by undergoing a sexual stage, producing gametes that fuse to eventually form a new, large cell of nearly maximal diameter.

that inherits this pole will enjoy a relative metabolic advantage over its sister that has to expend metabolic effort repairing or maintaining that "older" pole. The younger sister instead can allocate more of its resources to growth. Although segregation of cellular material by quality during cell division remains putative in *E. coli*, it can be observed clearly in yeasts, where mother cells retain material with greater levels of oxidative damage and thus provide their budding daughter cells with material that requires comparatively less effort to repair (Aguilaniu et al. 2003).

This dichotomy in *E. coli* of a cell inheriting either a metabolically younger or older pole from its parent has a direct analog in diatoms, where the hypothecal half of a diatom parallels the metabolically younger pole of an *E. coli* cell. If the hypothesized mechanism for between-sister biases in *E. coli* also occurs in diatoms, its effects should be detectable as faster growth and more rapid division in hypothecal sister cells. We used time-lapse imagery of cell division in *Ditylum brightwellii*, a centric diatom, to examine this possibility. Despite being taxonomically distant from *E. coli*, this diatom is a morphologically appropriate eukaryotic analog because it is similarly roughly rodlike in shape and divides in half along its minor axis. The question we address is whether the younger hypothecal daughters in *Ditylum* divide more rapidly on average than their epithecal sisters, which would be expected if the asymmetric cell division observed in *E. coli* also occurs in diatoms.

Methods

Cells of the *D. brightwellii* clone CCMP359 were grown in semi-continuous culture in f/2 medium (Anderson 2005) for over 10 generations at 15°C. The cells used in this study

were on the order of 20 μm in width, smaller than the ca. 26-μm upper limit of the sexually inducible size range reported in a study of field isolates of *D. brightwellii* (Koester et al. 2007). This indicates that cells in this study were likely capable of sexual reproduction, but we did not observe any indication of gametogenesis in any of these cells. Light was provided by F30T12/CW/RS cool-white fluorescent bulbs on a 14:10 light:dark (LD) cycle with an intensity of $\sim$ 130 μE m^{-2} s^{-1}. After one final dilution a small volume of culture containing $\sim$ 50 cells was injected into a water-jacketed borosilicate capillary (Vitrotube 4410-100) mounted to a standard microscope slide. The capillary-slide assembly was placed on a motorized stage of a Zeiss Axioskop microscope equipped with an infrared-sensitive digital camera (Sony XC-7500). The water jacket around the capillary-slide assembly was maintained at 15°C and growth light was provided by a F15T8/CW 15-W cool-white lamp above the stage, with an intensity of $\sim$ 100 μE m^{-2} s^{-1} on an LD cycle of 12:12. An infrared long-pass filter (Schott RG-780) was placed in front of the microscope's own incandescent light source to minimize any growth effect from the photosynthetically active wavelengths of that lamp, allowing cells to be imaged with infrared illumination during both the light and dark periods.

The initial location of each cell in the capillary was determined manually, and this information was entered into a custom software program that controlled the microscope's motorized stage. This program moved the stage to the initial location of each cell and captured an image every 5 min over a period of $\approx$ 4 d. The repeated images at all of these locations produced time-lapse sequences of each initial cell and its progeny over 3 to 4 subsequent generations. At 28 of these locations the progeny cells stayed in focus over the entire course of the experiment, and the time-lapse sequences from these locations were used in our subsequent analyses. Custom software was written in MATLAB (The Mathworks) to mark the time of each cell's division manually in these time-lapse sequences. This software also was used to determine the relative allocation of parental cell material between each pair of daughter cells following every cell division, using a manual morphometric approach to measure each sister's cytoplasm length and width immediately after division.

Diatom cell cycles include both light-requiring and light-independent stages, and so for cells grown under an LD cycle the time between a cell's separation from its sister and its own subsequent division can vary depending on its relation to the LD cycle. Absolute time therefore was not an appropriate metric for a cell's generational period, and so we instead used the number of hours of light each cell experienced before it divided (its total light dose [TLD]). This metric reduces the influence on cell division times of the dark periods, during which cell cycle progress may halt. Cells in this study divided primarily during the light period, similar to what has been observed in diatom light cycle phasing experiments (Olson and Chisholm 1983).

In our cell images it was not possible visually to distinguish the hypothecal end of an individual *D. bright-*
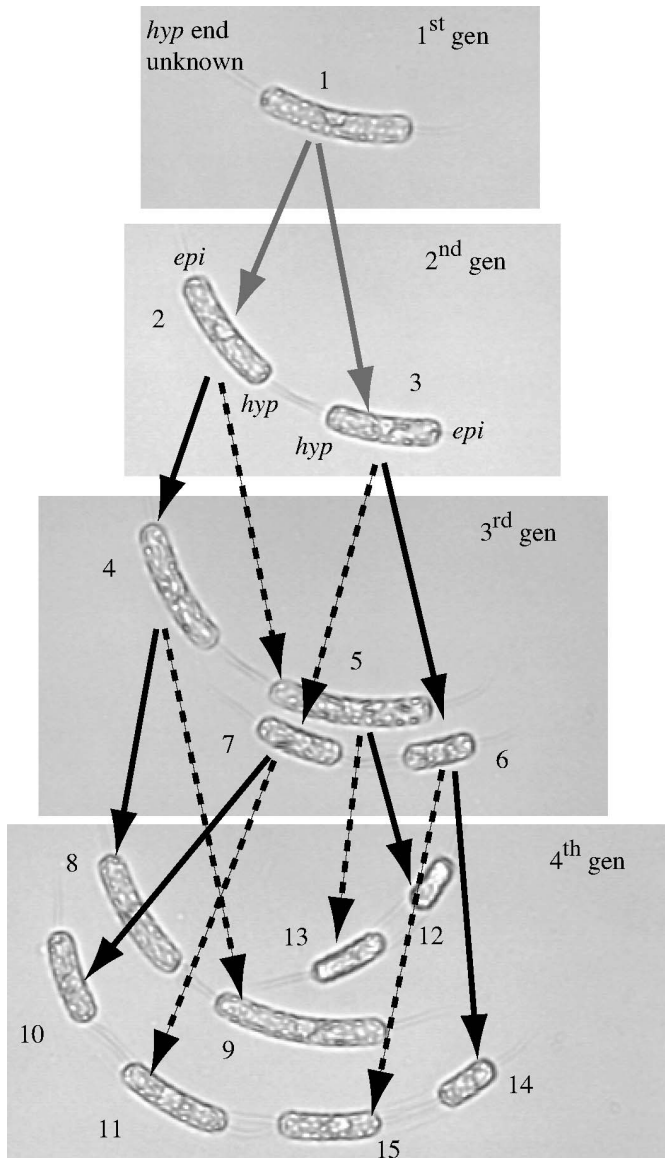
Fig. 2. Images of an individual *Ditylum brightwellii* cell and its progeny over three generations, illustrating how younger daughters were identified. In the first generation (gen) the hypothecal end of the cell cannot be identified but after it divides (grey arrows) the neighboring poles of the new daughter cells indicate their newly formed hypothecae. Inheritance of the hypothecae (*hyp*, dashed arrows) and the epithecae (*epi*, solid arrows) can then be inferred by tracking cell division in intervening images (not shown). In this time-lapse sequence daughters 5, 7, 9, 11, 13, and 15 inherit the more newly formed, metabolically younger hypothecal frustule halves from their parent. Note that because divisions are not synchronous, these images show cells at different stages of growth.

wellii cell from its epithecal end, but it was possible to infer which daughter inherited its parent's hypotheca or epitheca from information in these time-lapse sequences spanning multiple generations. Once a cell was observed to divide, the hypothecae of the two resulting daughter cells could be identified as corresponding to the two new poles formed from the parent's midpoint (Fig. 2). As *D. brightwellii* are

not motile and each cell moved little in the 5 min between images, the inheritance of these frustule halves could be tracked over subsequent generations. Although the initial cell densities in the capillary were very low, after four to five generations the progeny of any one cell overlapped each other at a particular location in the capillary so that the individual cells could no longer be tracked accurately. This meant our ability to examine progeny was limited to 3 to 4 generations.

Sister cells in this experiment often remained touching after dividing from their parent, which precluded the use of automated techniques for tracking individual cell morphometrics over multiple generations. At one location in the capillary, however, the progeny of that initial cell remained physically separated over subsequent generations so that automated methods could be applied. An image-processing cell morphometric approach developed by the authors (Sosik and Olson 2007) was modified to measure changes in the major axis and minor axis lengths of each cell at this particular capillary location for over three generations in these 1105 images. We used these measurements to estimate the biovolume of cells in this lineage by approximating their shape as an equilateral triangular prism, where the minor axis length measured in girdle view was assumed to represent the leg length of the triangle. Changes in biovolume over the course of a cell's lifespan could then be used as a proxy for its individual growth rate with 5-min resolution over three generations.

Results

In this time-lapse study we observed a statistically significant difference in generational period between hypothecal and epithecal daughters of *D. brightwellii*. The cell that inherited its parent's hypotheca tended to divide sooner than its sister, requiring on average 0.68 h less exposure to light to grow and subsequently divide (Fig. 3a, paired right-tailed *t*-test, $t = 1.91$, df $= 80$, $p = 0.03$ for $n = 81$ pairs of sisters, SD $= 2.16$ h). This average 0.68-h difference represents $\approx 4\%$ of the total light dosage that cells in this culture needed to achieve cell division under these conditions. In this analysis we omitted three instances in the difference in TLD between the two sisters that fell far outside the distribution (arrows in Fig. 3a). These outliers reflect cases when one of the sisters divided close to dusk whereas the other sister's division was delayed over the subsequent dark period and occurred after illumination was restored the next day. Removing these three individuals from the above analysis did not alter the results materially. When examining cells in the following generation that shared a common grandparent, an average difference of 0.87 h in light requirement (Fig. 3b, paired right-tailed *t*-test, $t = 1.81$, df $= 23$, $p = 0.042$ for $n = 24$ pairs of sisters, SD $= 2.35$ h) was observed between the metabolically oldest cousin (i.e., the cell out of these four that inherited an epitheca from a parent that also started from an epitheca, e.g., cell 8 in Fig. 2) and that of the metabolically youngest (i.e., its counterpart in the same generation that inherited a hypotheca from the parent that also started from a hypotheca, e.g., cell 13 in Fig. 2). The
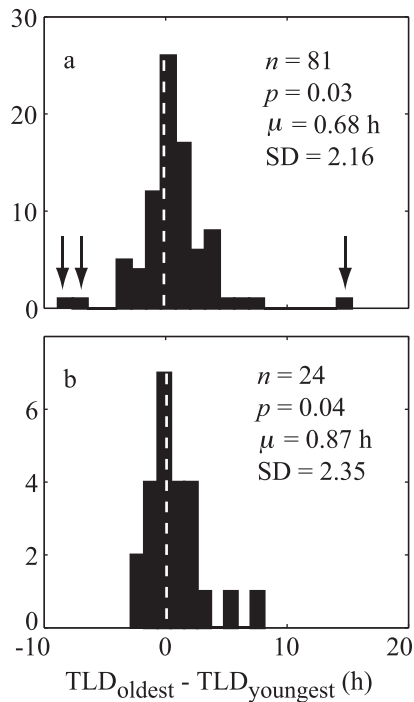
Fig. 3. Distributions of the difference in TLD (h) for division between a metabolically "older" cell (one inheriting the epitheca) and a younger cell (one inheriting the hypotheca), where (a) illustrates the distribution comparing two sister cells and (b) illustrates an identical comparison in the subsequent generation comparing two cells of a common grandparent. In each case the number of cell pairs compared (n), the p value (p), the mean difference in hours (μ), and its standard deviation (SD) are indicated. The vertical dashed line indicates a zero difference in the TLD required in each pair of cells. (a) Arrows indicate three outlier sister pairs referenced in the text.
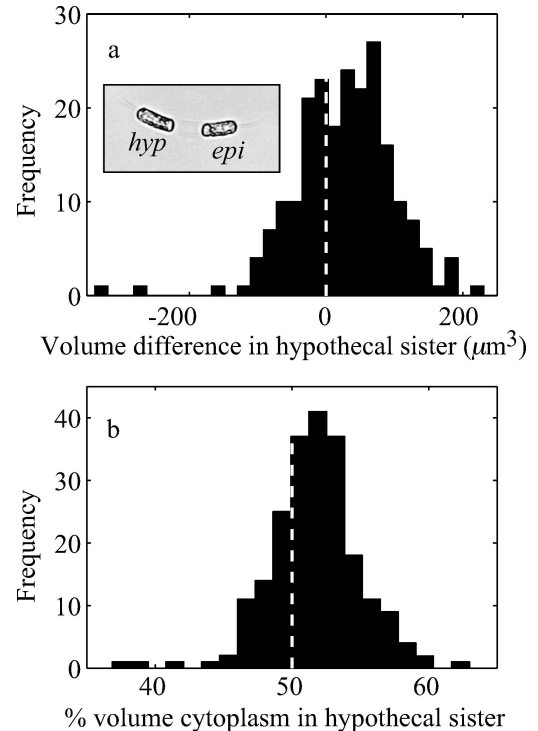
Fig. 4. The bias in parental material allocated to the hypothecal daughter cell (a) in absolute units of cell volume and (b) in terms of the relative percentage of parental material inherited by the two daughters, each measured within 5 min following cell division from the parent. The vertical dashed line in each panel represents no measureable difference between two sister cells.

means of these two distributions were not significantly different statistically (two-sample t-test for means being equal, $t = 0.65$, df $= 103$, $p = 0.74$).

A closer examination of these time-lapse sequences showed that the metabolically younger daughters (i.e., those inheriting the more newly formed hypothecae) also tended to inherit a greater volume of parental material. This might not be expected a priori given that the hypothecal daughter inherits the smaller-diameter frustule half. When we measured the sister cells immediately after division we found that the hypotheca-inheriting daughters received a greater volume of the parent's cell material in 63% of all divisions, considerably more often than would be expected by chance (Fig. 4a). The average difference between hypothecal and epithecal sisters is significantly greater than zero (one-sided t-test, $t = 3.78$, df $= 214$, $p < 0.0001$ for $n = 215$ pairs of sisters, mean $= 26.6$ μm^3, SD 72.9 μm^3). In terms of the relative allocation of parental material between two daughter cells, the hypothecal daughter received more parental cytoplasm in 69% of all divisions, significantly greater than half (Fig. 4b, one-sided t-test, $t = 3.78$, df $= 214$, $p < 0.0001$ for $n = 215$ pairs of sisters, mean $= 51.7\%$, SD 3.32%).

At the one location in the capillary where the progeny cells remained physically separated even after multiple

generations, automated measurement of individual cell biovolumes with 5-min resolution revealed that the sister whose volume increased more rapidly (i.e., the one that grew faster) was the one that (1) initially inherited the greater volume of cytoplasm from the parent, (2) inherited the parent's hypotheca, and (3) also eventually divided sooner (e.g., cell 2 in Fig. 5). Because we were not able to measure biovolume with full temporal resolution in the progeny of any other initial cell in this study, we cannot support this interpretation statistically. We note, however, that this observation is consistent with results in *E. coli* where both faster growth and more rapid division were observed more often in metabolically younger daughters.

We also examined whether the absolute differences in parental material inherited by hypothecal daughters were correlated with their division rates. We expect some spatial heterogeneity in illumination in the capillary and also that the nutrient conditions and cell growth characteristics might have changed during the four generations followed in our study. We avoided these confounding factors by performing a regression analysis on the pairwise, between-sister measurements shown in Figs. 3a, 4a, with the three outliers indicated in Figs. 3a omitted for this analysis. This regression revealed a statistically significant positive correlation between the absolute amount of parental material inherited by a hypothecal daughter and the comparatively fewer number of hours of TLD it needed
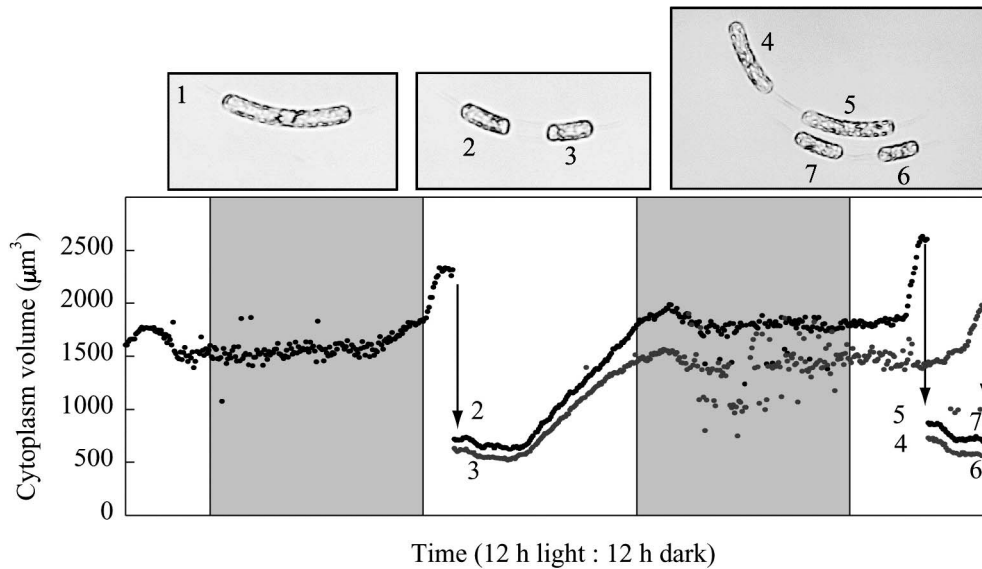
Fig. 5. Changes in cytoplasm volume over time for the first through third generation of cells in Fig. 2. Shaded regions indicate dark periods of the 12 : 12 LD cycle, and arrows indicate when a particular cell divided. The daughter that inherits more cellular material (e.g., cell 2) grows faster and eventually divides sooner than its sister (3). The daughters identified as inheriting their parents' hypotheca (5 and 7) not only inherited larger volumes but also themselves eventually divided sooner. Increased variability in estimated cell volume, seen here during the dark periods, reflects nocturnal changes in cell morphology that degraded the automated morphometric measurements of cell width and length, not actual variability in cell volume per se.

to divide (Fig. 6, Model I linear regression, $\beta_1 = 0.0068$ h μm^{-3}, $n = 81$, $p = 0.09$ for the hypothesis that slope is zero; $\alpha = 0.1$, $r^2 = 0.03$). The y-intercept was also significantly different from zero ($\beta_0 = 0.48$ h, $p = 0.07$ for $\alpha = 0.1$), indicating that in the absence of any volumetric difference between sister cells the hypothecal one would divide sooner on average.

Discussion

The degree to which phenotypic differences occur between two sister cells—and whether these differences
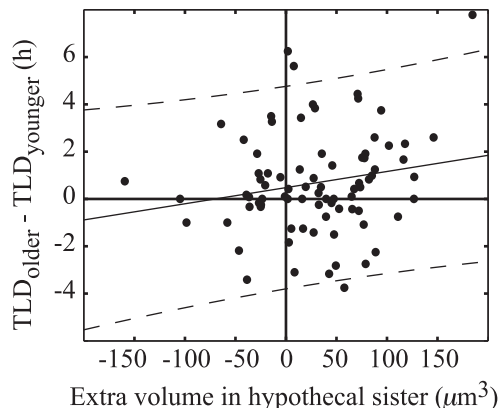

Fig. 6. A Model I linear regression of the difference in volume of parental material inherited by a hypothecal daughter compared to its epithecal sister (abscissa) and the hours sooner that it divided (ordinate), where dashed lines indicate the 95% confidence interval.

are stochastic or biased—is an important aspect of microbial ecology. Phenotypic differences should be expected because of the improbability that a parent cell can divide exactly in half in all respects. A simple example would be a parent cell with an odd number of chloroplasts, which must unavoidably endow one daughter with numerically more of those plastids. It is reasonable to expect that such a gross asymmetry between the two resulting sister cells would result in differences in their individual growth and fitness. Such a phenotypic asymmetry between two sister cells could presumably be stochastic and thus unbiased as to which sister inherited more of its parent's chloroplasts. Yet there are also non-stochastic aspects of cell division that can introduce systematic and biased partitioning of parental material between two daughter cells. These include the frustule morphology of diatoms but also a variety of other phenotypic differences that have only recently been identified (Ackermann et al. 2003; Stewart et al. 2005; Aldridge et al. 2012). The frustule-driven morphological difference between nascent diatom sister cells is both predictable and systematic, with important consequences to both the size structure and distribution of sex within a population. In contrast, the ecological ramifications of these other modes of phenotypic differences between diatom sisters in natural populations remain poorly explored.

Unequal partitioning of material during cell division has now been demonstrated in organisms from three kingdoms (*E. coli* in Bacteria, yeasts in Fungi, and *D. brightwellii* in Protista), suggesting that this phenomenon is widespread in microbial asexual reproduction. To our knowledge, no organism has been shown to have truly symmetric division,

and so this mode of asexual reproduction may be the norm. The similarity between our observations with *D. brightwellii* and prior findings with *E. coli* (Stewart et al. 2005) is striking. Both organisms exhibited a statistically significant difference in the generational periods of two sister cells, and the one likely to grow faster and divide sooner was the one that inherited its parent's metabolically younger half and more of its biomass. It is intriguing that the roughly 4% shorter TLD needed for hypothecal *D. brightwellii* sisters to divide is comparable to the 4% shorter division periods observed in younger daughters in *E. coli*. Based on the prior findings with *E. coli* we expected to see the difference in generation period compound in successive generations, i.e., that the difference between the oldest and youngest granddaughters would be twice that observed between sisters. The average difference in TLD between extreme granddaughters was greater than that for sisters (Fig. 3b) but not significantly so.

Stewart et al. (2005) proposed a mechanism related to the quality of material inherited to explain the disparate division rates of younger and older sisters seen in *E. coli*, but did not discuss the relationship between biomass advantage and decreased division time. Results from this study suggest that this latter factor is probably important in the *Ditylum* strain we examined. Regression analysis of our data suggests that both the relative quality and quantity of inherited parental material contribute to the more rapid division seen in hypothecal daughters; both the intercept and slope of the regression in Fig. 6 were significantly greater than zero at the 10% level. Given potential problems with using TLD as a metric for division time and with determining robust volumes (e.g., of curved cells), we cannot say which has the greater effect. With these time-lapse observations, we are limited to such regression-based inferences, but follow-up studies may consider using microscopy techniques that incorporate metabolic stains in order to provide additional insight into this issue of inherited quality vs. quality. Stains that target reactive oxidative species in unicellular algae (Affenzeller et al. 2009) may help quantify the disparity in quality of material inherited.

A systematic difference in growth rate between two sister cells has intriguing implications for adaptation not only in diatom populations but potentially in other unicellular eukaryotes that also reproduce both sexually and asexually. When the shortening of generational periods is included in the canonical MacDonald-Pfitzer paradigm (Fig. 1), the result is a skewing of generations, with hypothecal lineages reaching smaller sizes sooner (Fig. 7). This effect over multiple generations may represent a previously unconsidered mechanism that affects the "sexual clock" of diatoms, i.e., the interval between sexual events in a population (Lewis 1984). Within-species variations in inducible size ranges and in the rates of reduction in frustule width per division, are two mechanisms thought to have allowed diatoms to adjust their sexual clock on evolutionary time scales, presumably to adapt better to changes in environmental conditions. Asymmetrical division, with faster growth along hypothecal lineages, will effectively shorten the average interval between sexual events in a lineage and
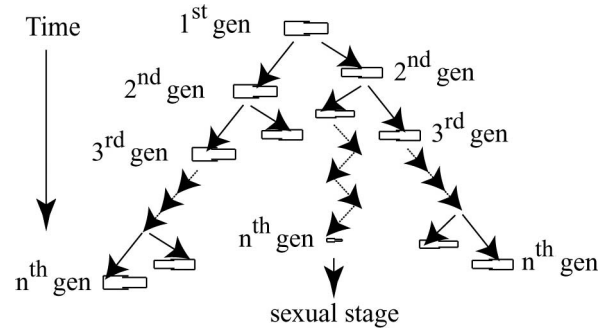

Fig. 7. Modification of the MacDonald-Pfitzer paradigm of Fig. 1 to incorporate the faster growth and division we observed among hypothecal daughters. Cells that repeatedly inherit the hypotheca still decrease in diameter with each generation (center of diagram) but these lineages create terminally small cells sooner than in the standard model. When the wholly epithecal lineage (cells on the outside left) reaches its nth generation, cells in other lineages with hypothecal ancestors will have reached the same generation earlier and may potentially be at a subsequent generation. This particular diagram illustrates the extreme case where the hypothecal daughter grows faster than its sister in every instance, not just typically as was seen in this study.

as such is a potential third mechanism for adjusting the diatom sexual clock. This skewing of the canonical MacDonald-Pfitzer model has further implications for the cells in wholly epithecal lineages and whether they are identical replicates of their parents. A mechanism that favors hypothecal daughters with less damaged material is also one that relegates material with greater damage to the daughter whose progeny are less likely to engage in sex, i.e., these epithecal daughters.

Asymmetric cell division may also play another role in diatom reproductive ecology through its preferential endowment of better parental material to the hypothecal daughter. Age–class matrix models that have explored population-scale effects of symmetric vs. asymmetric binary division predict that favoring one daughter at the expense of the other can result in higher population growth rates (Watve et al. 2006), thus increasing the absolute number of copies of an ancestor cell's genome within a population. Such studies remain hampered, however, by the lack of observational data necessary for parameterizing critical aspects of such comparative models. Laboratory studies like the one described here can provide observational data that is critical for improving these models' realism, including constraining the average difference in generational period and initial biomass between two sister cells, the persistence of these biases over subsequent generations, and the distribution of these disparities within a population. Such models predict that under certain situations asymmetric division in microbes can increase fitness within a population. Our observations of biased division in *D. brightwellii* suggest that these modeled dynamics and their ramifications to fitness may also apply to diatoms.

The considerable genetic diversity seen in natural *Ditylum* populations at the clonal level (Rynearson and Armbrust 2005; Rynearson et al. 2006) provides compelling arguments to repeat these studies on a range of *Ditylum*

isolates beyond the CCMP359 strain we examined here. We have demonstrated bias between sister cells only with a laboratory culture, but there is no reason to not expect this phenomenon to occur also in natural populations. Images of recently divided *Ditylum* sister cells in the northwest Atlantic indicate that sister cells differ in size soon after division from their parents (data not shown). It will be necessary to isolate such cells and examine them in comparable time-lapse studies to determine if biases between sister cells in growth rate and division period play any role in the patterns that have been observed in nature, in the reproductive ecology of this species and of diatoms in general (Koester et al. 2007; D'Alelio et al. 2010).

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