



Investigation of evolutionary effects on the relative frequency of sexual reproduction in freshwater diatoms

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Abstract

There are substantial ecological and evolutionary costs to sexual reproduction and there is only a narrow range of conditions that favors the evolution of high rates of sexual reproduction in species that are capable of both sexual and asexual reproduction. Considering the evolutionary costs and benefits of sexual reproduction, it was hypothesized that the frequency of sexual reproduction relative to asexual reproduction in diatoms would be greater for attached pennates and lower for planktonic centrics. This was investigated by comparison of the size-class distributions of the attached pennate, *Rhopalodia gibba* (Ehrenberg) Müller 1895 and the planktonic centric *Cyclotella meneghiniana* Kützing 1844 collected from the littoral zone of Lake Phalen in St. Paul Minnesota, USA. Numerical combination of binomial functions was used to infer the number of significant occurrences of sexual reproduction contributing to the size-class distribution of each population, with the frequency of sexual reproduction relative to asexual reproduction inferred from the number of binomial curves contributing to the distribution. The size-class profile of *C. meneghiniana* was unimodal, dominated by a single peak at 12 µm, with additional contributing curves with peaks at 15 µm and 18 µm. The size-class profile of *R. gibba* was bimodal, dominated by peaks at 46 µm and 60 µm, with additional contributing curves with peaks at 36 µm, 73 µm, and 84 µm. The results of this investigation were robust with respect to difference between the species in sample size and number of size-classes, although the analysis method is sensitive to differences in the number of size-classes. The results supported the hypothesis, and demonstrate the principle that size-class analysis of diatom populations can be used to investigate evolutionary hypotheses about differences among taxa in the relative frequency of sexual and asexual reproduction.

Introduction

There are substantial ecological and evolutionary costs to sexual reproduction (Williams 1975, Maynard Smith 1978, Futuyma 2009), and recent work in theoretical evolutionary biology indicates that there is only a narrow range of conditions that favors high rates of sexual reproduction in species that are capable of both sexual and asexual reproduction (Otto & Lenormand 2002, Agrawal 2006).

Diatoms are an ideal organism for the investigation of the evolutionary factors that determine the relative frequency of sexual and asexual reproduction because diatom species are capable of both sexual and asexual reproduction (Drebes 1977, Round *et al.* 1990, Mann 1993), different taxa are reported to engage in the two modes of reproduction at different frequencies (Mann 1988, 1993, Edlund & Stoermer 1997), and reproduction in diatoms can be studied with a variety of methods including laboratory culture studies (Chepurnov *et al.* 2004, Amato *et al.* 2005), molecular genetics (e.g. Rynearson & Armbrust 2005, Beszteri *et al.* 2007, Alverson 2008) and field observation (Jewson 1992a, 1992b, D'Alelio *et al.* 2010). Furthermore, because of the siliceous frustule and its changes during the life cycle, it is possible to study the population ecology of diatom populations over thousands of generations using paleolimnological methods (e.g. Nipkow 1927, Card 1997). In this paper we present a novel method for testing evolutionary hypotheses using size-class analysis of diatom populations.

The costs and benefits of sexual reproduction for diatoms include some factors that are common to all eukaryotes, some that are peculiar to algae and unicellular organisms, and some that are unique to diatoms.

Costs generally accepted to be widespread or universal include the loss of good genotypes in populations for which natural selection has favoured particular combinations of alleles and a decrease in the number of offspring produced per reproductive event (Williams 1975, Maynard Smith 1978, Futuyma 2009). For diatoms and other algae there are also the costs of lost reproductive opportunity and increased risk of death during the time-consuming processes of gametogenesis, dispersal, zygote formation and development (Lewis 1983, Brawley & Johnson 1992, Edlund & Stoermer 1997).

Benefits that are generally accepted to be universal include the expression and subsequent purging of deleterious recessive alleles (Hurst & Peck 1996, Futuyma 2009), and the creation of new genotypic and haplotypic associations, although the latter may be beneficial only when the population is subject to directional selection or significant genetic drift (Hurst & Peck 1996, Otto & Lenormand 2002, Futuyma 2009). For diatoms in particular the benefits of sexual reproduction may also include effects that result directly from increased cell size, benefits that have been suggested to include a decreased herbivory rate, a change in nutrient uptake rates, and a change in sinking rates of planktonic cells (Drebes 1977, Edlund & Stoermer 1997). Also, because sexual reproduction for diatoms is associated with the abandonment of the parental cell wall and formation of a new cell wall *de novo*, there may be benefits from the shedding of ectoparasites and the restoration of a genetically determined cell wall pattern that is free from teratological features (Edlund & Stoermer 1997).

Of the many potential costs and benefits, there are two that are likely to vary both substantially and systematically across broad taxonomic groups of diatoms: the cost due to the increased risk and lost opportunity during reproduction and the benefit due to the restoration of genetic diversity that has been lost to genetic drift.

For algae, the failure of gametes to successfully meet and form a zygote can be a substantial cost of sexual reproduction (Brawley & Johnson 1992) and this cost is likely to be higher for taxa that are oogamous, planktonic, and occur in relatively low population densities. For diatoms, this suggests that the evolutionary cost of sexual reproduction will be higher for planktonic centric diatoms than for epiphytic pennates.

Genetic drift reduces genetic diversity more quickly in populations that have smaller effective population sizes (Futuyma 2009). Studies of the dispersal of algal gametes have found that dispersal is generally limited to a few meters, causing very fine-scale genetic structure within populations (Williams & Di Fiori 1996, Van der Strate *et al.* 2002). Sessile algal populations are likely to have smaller effective population sizes than motile or planktonic populations and to have correspondingly greater loss of genetic diversity by genetic drift. For diatoms, this suggests that the evolutionary benefit of sexual reproduction will be higher for epiphytic diatoms than for planktonic diatoms.

Considering the cost and the benefit that are likely to vary substantially and systematically among diatoms, we hypothesized that the net benefit and therefore the frequency of sexual reproduction relative to asexual reproduction will be higher for attached pennates and lower for planktonic centrics.

Methods

Epiphytic samples on coontail (*Myriophyllum spicatum* L.) were collected from a depth of approximately one meter in the littoral zone of Lake Phalen, in St. Paul, Minnesota. Samples were prepared by soaking in cold hydrogen peroxide and were mounted in Zrax mounting medium (W.P. Dailey, Philadelphia, Pennsylvania). Two abundant and distinctive species were chosen for analysis, an attached pennate, *Rhopalodia gibba* (Ehrenberg) Müller (1895: 65) and a planktonic centric, *Cyclotella meneghiniana* Kützing (1844: 50). Identifications were made using Krammer and Lange-Bertalot (1999–2000). Over one thousand valves of *R. gibba* and over four hundred valves of *C. meneghiniana* were measured to a precision of 0.1 µm at 1000x magnification with an Olympus BX microscope and SPOT optical analysis system. The *C. meneghiniana* data

were binned into 1 μm size classes and the *R. gibba* data binned into 2 μm size classes and size-class histograms for each species constructed using Microsoft Excel.

The occurrence of sexual reproductive events was inferred by visual analysis of the size-class histograms, following the methods of Mann (1988) and Jewson (1992a, 1992b), and by numerical fitting of binomial curves to the profiles. In this procedure it is assumed that reproduction is sufficiently well described by the MacDonald-Pfizer rule that each significant event of sexual reproduction results in a size-class distribution described by the binomial expansion. The binomial expansion is a discrete function in which the number of valves n in any size-class i at time t is described by the function: $n_i = t! / ((t-i)! \cdot i!)$. This function is referred to hereafter as the binomial curve. According to the MacDonald-Pfizer rule and its major variations (e.g. Jewson 1992b), sexual reproduction produces large cells that, through cell division (asexual reproduction), create a lineage of cells having a unimodal distribution whose mean and modal size decrease, and variance and range increase, over time. A population that is composed of significant numbers of individuals descended from more than one separate events of sexual reproduction is multimodal, composed of the addition of binomial curves each of which reflects the size-class distribution of a lineage resulting from a separate reproductive event. Thus, the size-class distribution of a population can be described as the linear combination of binomial functions using the equation $\sum z_e \cdot n_{it}$, where z_e is the proportional contribution to the total population of each lineage 'e' and 't' is proportional to the number of generations (cell divisions) that have occurred since the formation of the initial cell.

The data were first smoothed with a three step weighted averaging function ($n_i = 0.25n_{i-1} + 0.5n_i + 0.25n_{i+1}$) to facilitate the deconstruction of the size-class histogram into its constituent binomial curves. A binomial curve was then fit to the largest peak in the data, with the modal size (i) of the binomial curve equal to the size of the largest mode in the data, and the values of parameters (t and z) determined numerically to minimize the residuals. Then, a binomial curve was fit to the largest peak of the residuals, using the same method. A third binomial curve was fit by the same method to the largest peak of the remaining residuals. At each step, the correlation between the distribution of predicted (modelled) and observed (un-smoothed data) valve sizes was checked, and the procedure continued until the correlation was greater than 0.95.

To test for possible confounding effects of the difference in sample size, ten sub-samples of 400 valves each were taken at random from the *Rhopalodia gibba* data set and the curve-fitting procedure applied. To test for possible confounding effects of the difference in the number of size-classes, the *R. gibba* data were also divided into a smaller number of wider size-classes (twelve; 4 μm broad) and the curve-fitting procedure repeated.

Results

Cyclotella meneghiniana

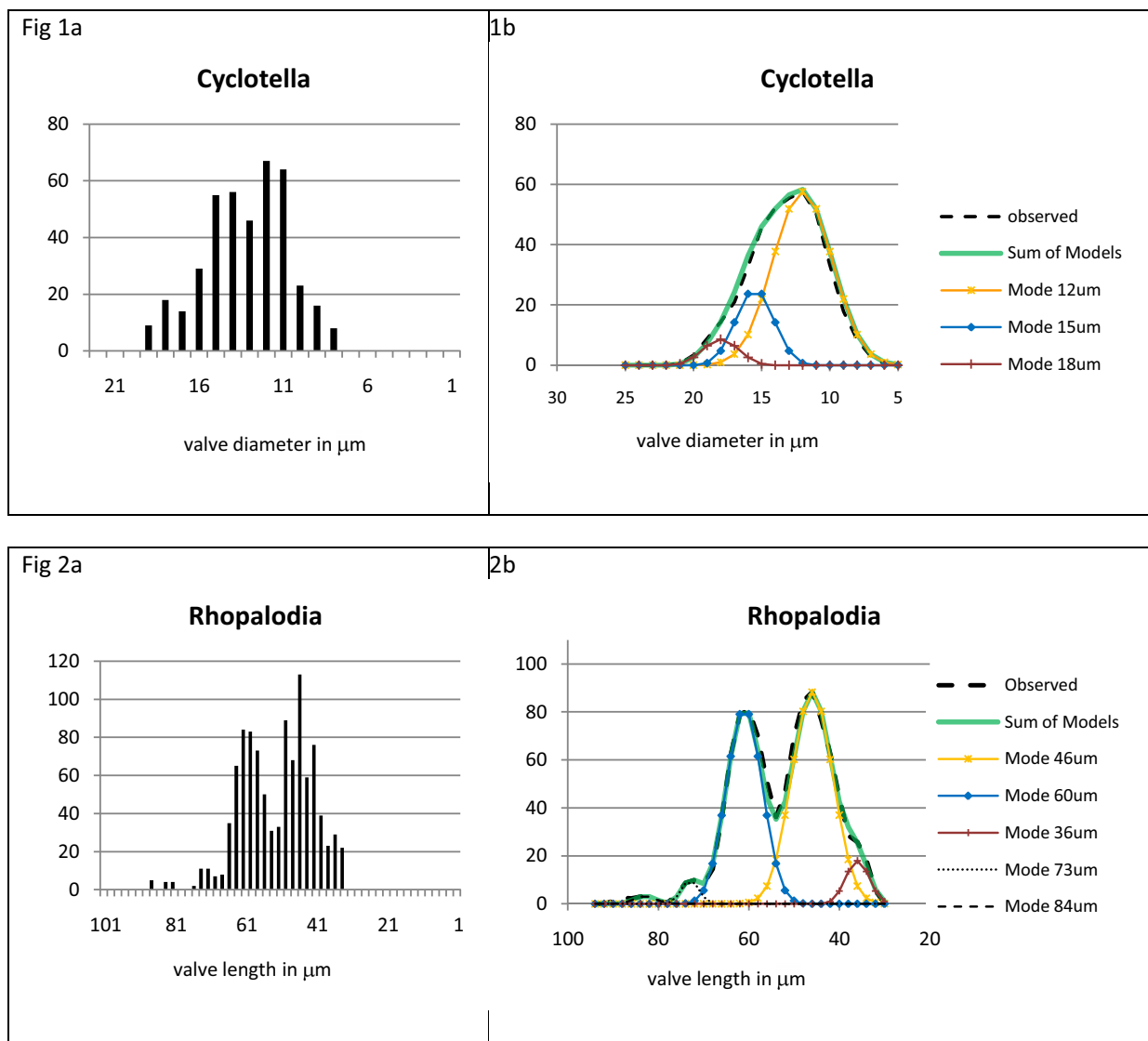
Four hundred and five valves of *Cyclotella meneghiniana*, the planktonic centric taxon, were measured. These valves had an average diameter of 13.2 μm , median diameter of 12.5 μm , and range of 7–19 μm . Valves occurred in twelve 1 μm size-classes. The smoothed size-class profile of the population was unimodal, dominated by a single peak at 12 μm (Fig. 1a). Fitting of binomial curves to the data revealed that it was composed of three binomial curves, the largest with a mode of 12 μm , the second largest with a mode of 15 μm , and the smallest with a mode of 18 μm (Fig. 1b). The first curve accounted for 73% of the valves, the second curve accounted for another 20%, and the third curve accounted for another 6% of the valves. The number of valves in each size-class predicted by the model was highly correlated to the number of valves actually observed in each size-class ($R^2 > 0.95$).

Rhopalodia gibba

One thousand and twenty four valves of *Rhopalodia gibba*, the attached pennate taxon, were measured. These valves had an average length of 52.6 μm , a median length of 48.5 μm and a range of 34–88 μm . Valves

occurred in twenty-five 2 µm size-classes. The smoothed size-class profile of the population was bimodal, with dominant peaks at 46 and 60 µm (Fig. 2a). Fitting of binomial curves to the data revealed that it was composed of at least 5 binomial curves, with modes of 36, 46, 60, 73, and 84 µm. A majority (over 90%) of the valves were accounted for by 46 µm and 60 µm modes (Fig. 2b). The first curve accounted for 50% of the valves, the second curve accounted for another 41%, and the next 3 curves accounted for another 6%, 2% and 1% of the valves, respectively. The number of valves in each size-class predicted by the model based on combination of binomial curves fit to the smoothed data was very highly correlated to the number of valves observed in each size-class ($R^2 > 0.95$).

In eight of the ten 400-valve subsets, the same five binomial curves were identified by the curve-fitting results. Curves with peaks at 46 µm, 60–62 µm and 72–74 µm were present in all ten subsets, the curve with a peak at 82–84 µm was present in nine of the ten subsets, and the curve with a peak at 36 µm was present in eight of the ten subsets.



FIGURES 1, 2: Size distribution and modelled size classes in *Cyclotella meneghiniana* and *Rhopalodia gibba*. Fig. 1a: Observed size class distribution of *Cyclotella meneghiniana* valves. Fig. 1b: Modelled size class distribution based on combination of binomial curves fit to smoothed data. Fig. 2a: Observed size class distribution of *Rhopalodia gibba* valves. Fig. 2b: Modelled size class distribution based on combination of binomial curves fit to smoothed data.

When the data in the 400-valve subsets were divided into a smaller number of size-classes, fifteen size-classes each 4 μm in breadth, the same five curves emerged in a majority of cases, but with greater variability in the modal valve-length of each curve and greater variability in the number of valves accounted for by each curve. The five curves that were consistently detected included a curve with a peak between 44 μm and 46 μm that accounted for 40–48% of the valves, a curve with a peak at 60 μm that accounted for 39–48% of the valves, a curve with a peak between 32 and 35 μm that accounted for 5–12% of the valves, a curve with a peak at 72 μm that accounted for 4–5% of the valves, and a curve with a peak between 84 and 88 μm that accounted for 0–7% of the valves.

Discussion

These results demonstrate the principle that size class analysis of diatom populations can be used to investigate evolutionary and ecological hypotheses about differences among taxa in the relative frequency of sexual and asexual reproduction.

These results support the hypothesis that sexual reproduction is more frequent relative to asexual reproduction in diatom taxa for which the net evolutionary benefit is higher. The analysis follows from work of Mann (1988) and Jewson (1992) and assumes that the predominant cause of size restoration in diatoms is sexual reproduction, while acknowledging that size-restoration by asexual or apomictic reproduction has been described in *Cyclotella meneghiniana* (Rao 1970) and can occur in other diatom taxa as well (Chepurnov *et al.* 2004). With this caveat, this conclusion is supported by reports that have described sexual reproduction as being most commonly observed in attached species and epiphytic communities (e.g. Mann 1988, Edlund & Stoermer 1997). In contrast, sexual reproduction is not unknown for planktonic and centric diatoms, but is most commonly reported from paleolimnological and long-term water sampling studies (e.g. Nipkow 1927, Mann 1988, Jewson 1992a, 1992b, Crawford 1995, Card 1997). This result agrees with the prediction that the relative frequency of sexual reproduction in freshwater diatoms is greater in epiphytic species than in planktonic species.

In order to distinguish the evolutionary influences on the relative frequency of sexual and asexual reproduction, it would be useful to compare multiple species of each group, centric and pennate, planktonic and attached, within the same community. To distinguish between the two hypothetically causal factors it would be useful to compare species with varying degrees of attachment, such as a comparison of firmly attached species within an epiphytic community to motile and meroplanktonic species. We look forward to the insights that will be provided by the use of molecular methods, such as those suggested by Alverson (2006) for the measurement of effective population size. Repeated sampling of a population over time would enable the inference of an absolute time scale for reproductive events (cf. Jewson 1992a, 1992b, D'Allelio 2010), in place of the relative time-scale used in this study.

The novel method presented in this paper for interpreting diatom size-class profiles by numerical combination of binomial curves demonstrates that a size-class profile that appears to be uni-modal or bi-modal can be the product of the linear combination of a larger number of binomial curves. This introduces a new approach for the numerical analysis of diatom size-class data.

The results of this study were robust with respect to differences in sample size and number of size-classes used in the analysis, although the ability of this method to detect the contributing binomial curves is greater for populations that exhibit a greater number of size-classes.

The binomial curves used in this study result from the classical MacDonald-Pfizer theory of cell-size distribution in diatoms, in which the rate of cell division and cell-size reduction are constant with cell-size. This method could be adapted to work with the more mathematically complex patterns that are to be expected in natural populations. For example, it is known that the initial cells of a species can occur in a range of sizes (Edlund & Bixby 2000) and that cells of different sizes may divide at different rates or produce offspring cells with varying degrees of size reduction (Rao & Desikachary 1970, Amato 2005). These topics invite further investigation.

It is hoped that this study will stimulate greater consideration of evolution among diatomists and greater awareness of diatoms among evolutionary biologists. This report suggests several promising directions for future work in this rapidly developing inter-disciplinary field.

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