

## Macroevolutionary trends in silicoflagellate skeletal morphology: the costs and benefits of silicification

Helena M. van Tol, Andrew J. Irwin, and Zoe V. Finkel

**Abstract.**—The silicoflagellates are a class of enigmatic chrysophytes characterized by netlike skeletons composed of opaline silica. Other major groups of siliceous plankton—the diatoms and radiolarians—exhibit evidence of decreasing size or silicification over the Cenozoic. We investigated trends in the silicoflagellate fossil record by constructing a species-level database of diversity and morphological metrics. This new database reveals a proliferation of silicoflagellate species with spined skeletons along with an increase in the mean number of spines per species over the Cenozoic. Although there is little change in skeleton size or silicification among species with spines, those without spines are larger than species with spines and exhibit a decrease in size toward the present. Increased grazing pressure combined with declining surface silicate availability may have shifted the costs and benefits of silicification, causing divergent responses in skeletal morphology between these different morphological lineages of silicoflagellates over time. We postulate that diminishing Cenozoic surface silicic acid availability may have predisposed large spineless silicoflagellate species to extinction, whereas increased grazing pressure may have contributed to the extinction of all remaining spineless species within the edible size range of grazers.

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### Introduction

Each year,  $5.5 \pm 1.6$  Tmol of silicon is delivered to the upper ocean through surface runoff, groundwater flow, continental weathering, and aeolian dust deposition while  $0.6 \pm 0.4$  Tmol dissolves into the deep ocean through seafloor weathering and hydrothermal activity. Therefore, it may seem surprising that broad expanses of the upper ocean are characterized by silicic acid concentrations of less than  $2 \mu\text{M}$ , whereas silicic acid concentrations in the deep ocean can reach up to  $180 \mu\text{M}$  (Tréguer et al. 1995). The settling of precipitated biogenic silica out of the surface ocean can account for this discrepancy. Although ocean silicon precipitates only abiotically when silicic acid concentrations are higher than  $1000 \mu\text{M}$ , biologically mediated reactions by diatoms and other siliceous plankton can precipitate opal at lower environmental concentrations of silicic acid (Hurd 1973; Ragueneau et al. 2000). In diatoms (and perhaps other siliceous plankton) these biochemical transformations are made possible by the activity of silicon transporters which augment intracellular

silicic acid concentrations to several hundred  $\mu\text{M}$  (Martin-Jézéquel et al. 2000). The organic matrix chemistry of siliceous phytoplankton may also act as a catalyst that is optimized to lower the kinetic energy barrier of silica nucleation (Wallace et al. 2009). Because the density of opaline silica is approximately 2.5 times the density of seawater, heavily silicified phytoplankton sink out of the photic zone faster than unsilicified plankton, creating a flux of silicon and carbon to the deep ocean (Berner et al. 1983; Reynolds 1984; Dugdale and Wilkerson 1998). The contribution of heavy diatom frustules to atmospheric carbon sequestration is quantitatively important in regions where silicic acid wells up from the ocean depths or where silica-rich dust is deposited (Harrison 2000; Yool and Tyrrell 2003; Allen et al. 2005).

Over the last 600 Myr, surface silicic acid availability is thought to have decreased alongside the appearance of organisms with the ability to use silicon (Maliva et al. 1989). The disappearance of siliceous sponges from neritic shelf environments but not from deeper, more silica-rich water during the

Mesozoic may be evidence of early depletion of surface silicic acid (Maliva et al. 1989; Maldonado et al. 1999). Furthermore, these sponges demonstrate the phenotypically plastic capability of lightening their skeletons under lower silicic acid concentrations and secreting heavier structures such as isochelae and desmas only under higher silicic acid concentrations (Maldonado et al. 1999). A decline in radiolarian test weight over the Cenozoic era coupled with rising diatom diversity is indicative of increased competition between these two groups, possibly for increasingly scarce silicic acid (Harper and Knoll 1975; Lazarus et al. 2009). Declining diatom frustule size over the Cenozoic in addition to evidence that diatoms produce lighter frustules at lower silicic acid concentrations provides additional support for the hypothesized decline in silicate availability over the last 65 Myr (Finkel et al. 2005, 2010a; Finkel and Kotrc 2010). In the contemporary regime, the diatoms are major carbon and silicon flux contributors to the deep ocean. Radiolarians and siliceous sponges may be quantitatively important in some local regions, whereas silicoflagellates probably have a minor impact on biogeochemical cycling in the modern ocean (Tréguer et al. 1995; Maldonado et al. 2005, 2010). However, the relative contribution of silicoflagellates may have been much higher during their acme in the Miocene Epoch as well as during the early Tertiary Period when silicoflagellate species richness may have been higher than diatoms (see below) (Lipps 1970; Bukry 1981).

If surface silicic acid concentrations have decreased over the Cenozoic (Harper and Knoll 1975; Lazarus et al. 2009; Finkel and Kotrc 2010), then concurrent decreases in silicification are expected in the fossil record of all siliceous plankton including silicoflagellates. Silicoflagellates are members of the family *Dictyochaceae*, a group of understudied chrysophytes with siliceous skeletons. *Dictyocha speculum* SSU rDNA and *rbcL* sequence data have indicated that silicoflagellates are a sister taxon to the pedinellids and *Rhizochromulina* heterokonts, but in their pigment composition they bear closer resemblance to some haptophytes (Daugbjerg and Henriksen

2001). Silicoflagellate taxonomy is traditionally defined by paleontologists on the basis of skeleton morphology. Although laboratory and field studies on modern species indicate that this trait is phenotypically plastic, it is the only method of identifying the majority of extinct species (Van Valkenburg and Norris 1970; Thomas et al. 1980; Boney 1981). The silicoflagellate skeleton is composed of a tubular network of fused structural opaline silica rods associated with traces of aluminum, chlorine, calcium, sodium, and magnesium (Lipps 1970). The silicoflagellate cell is exposed to the environment; unlike diatom frustules, silicoflagellate skeletons do not provide any protection from smaller pathogens (Hamm and Smetacek 2007). Silicoflagellate skeletons are relatively common and well-preserved in the Cenozoic fossil record and extend into the Mesozoic (Bukry 1981; McCartney 1987; Perch-Nielsen 1985; McCartney and Wise 1990; McCartney et al. 1990, 2010; Desikachary and Prema 1996). In comparison to Cenozoic forms, silicoflagellate fossils from the Albian exhibit higher levels of morphological diversity and asymmetry (McCartney et al. 1990; McCartney et al. 2010). It has been suggested that silicoflagellates may maintain a genetic memory of ancient morphotypes that may be exhibited under stressful conditions, and that the phenotypic plasticity of the skeleton may be useful for the reconstruction of past environmental conditions (Bukry 1981; Guex 2006).

We expect that micro- and macro-evolutionary trends in silicoflagellate skeleton morphology may provide insight to the changing global selection pressures on this group and on silicification over geologic time. If the surrounding environment became increasingly undersaturated in silicic acid, then the costs of silicification should correspondingly increase for all groups of siliceous phytoplankton. Consequently, a decrease in silicoflagellate skeleton size and silicification may provide additional evidence for declining silicic acid availability over the Cenozoic. However, it is important to recall that size and silicification are also under selection by food web interactions, such as predation, and are constrained by the physics of the

pelagic marine environment (Knoll 2003; Finkel and Kotrc 2010; Finkel et al. 2010b). To test these hypotheses, we constructed a database of silicoflagellate morphological metrics, using the first and last appearance data of species that existed since the Cretaceous but with a focus on the Cenozoic, to explore (1) changes in morphology over time, (2) morphological changes that resulted in modified silicate use, and (3) correlations with the diversity and size of diatoms.

### Materials and Methods

**Morphological Database Construction.**—To create the morphological database including first and last appearance data for 248 species, we used all silicoflagellates with known temporal ranges recognized by Desikachary and Prema (1996) as well as any remaining species recognized by Perch-Nielsen (1985); these are the only available global compilations of silicoflagellate ranges. Adhering to the classification system of one authority reduced the effect of synonyms. Epochs were converted into absolute ages using the Geological Society of America's 2009 geologic time scale. Morphological metrics were collected from SEM and light microscope images of silicoflagellate skeletons in the literature, primarily from Desikachary and Prema (1996), Perch-Nielsen (1985), and Deep Sea Drilling Project (DSDP) reports (original data available on request). The maximum skeleton diameter (from opposing spine tips or from opposing basal ring edges in the absence of spines), the maximum basal ring width (a measure of the diameter of the skeletal rods that make up the skeleton), the two-dimensional surface area covered by the complete skeleton (an estimate of skeletal surface area), and a two-dimensional estimate of the outer skeleton contour were used to derive an upper approximation of maximum cell surface area (Fig. 1). A silicification index (2D skeleton surface area  $\div$  2D estimated cell surface area) was used as a proxy of silica use and silicification. Spine number enumerates the larger spines protruding from the basal ring, not the microspines (or pikes) that tend to be oriented inward. We assume that these larger spines could have a role in deterring

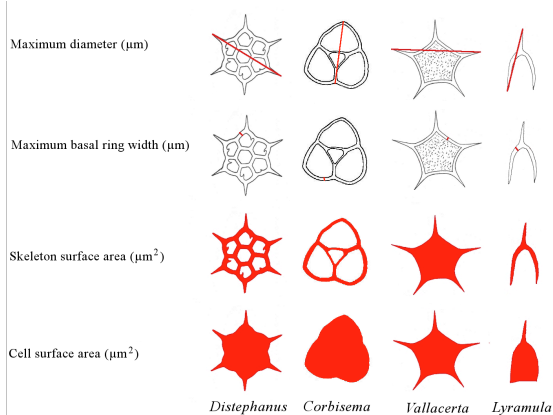


FIGURE 1. Morphological measurements taken from silicoflagellate species included in the database. The base images in this figure were modified from Loeblich et al. (1968) and Lipps (1970).

grazers or altering sinking velocity. Spine length was calculated as the difference between maximum skeleton diameter and basal ring diameter divided by two. Spine length was then normalized by the basal ring diameter to account for the fact that larger cells probably support longer spines. Cell size and skeleton surface area may be underestimated, particularly in more three-dimensional species. *ImageJ* version 1.43u (NIH, USA) and a drawing tablet were used to collect the morphometric data. For 70% of the species in the database we measured three or more images. Using the drawing tablet and *ImageJ*, we repeated test measurements of some images to estimate the level of precision achieved. The percentage of variation within these test measurements never exceeded 12% and was typically less than 2%.

**Statistical Analyses.**—We calculated silicoflagellate species richness for each sub-epoch over the Cenozoic, using first and last appearance data from the morphological database and statistical computing software R, v. 2.11.1 (R Development Core Team 2010). Mean morphological metrics were calculated at sub-epoch resolution and plotted for the Cenozoic; we limit interpretations to the Cenozoic because of gaps in sampling and lower data quality in the Cretaceous. For morphological analyses we separated the silicoflagellates into two groups, those with and without spines. Calculations of mean

number of spines were based only on species with spines—omitting species without spines—to determine whether the trend in spine number is independent of the extinction rates of spined relative to spineless species. To compare the mean skeleton area of silicoflagellates with the size of diatoms, we obtained median frustule area data from Finkel et al. (2005). We then calculated diatom frustule area for each sub-epoch to match the silicoflagellate data resolution and plotted both metrics over the Cenozoic era.

Bootstrapping was used to estimate 95% confidence intervals from 1000 subsamples for the mean and median of each morphological metric for the epochs divided into their early, middle, and late subdivisions. We split the data into five intervals of geologic time to compare mean morphometrics from the Paleocene, Eocene, Oligocene, Miocene, and the last 5.3 Myr (Pliocene through to the present). The program PASW Statistics 18 was used to calculate and compare mean morphometrics for spined species and species without spines. Independent *t*-tests were performed for each time interval to determine whether the two groupings were significantly different from each other at a significance level of 0.05. A one-way ANOVA was also performed on spine count data for species with spines. The spine count data were square root transformed to improve normality, silicification index data were arcsine square root transformed, and the remaining morphometric data were log-transformed. These transformations corrected for positively skewed distributions, although Kolmogorov-Smirnov and Shapiro-Wilk tests continued to detect a significant difference between these data and normality. The data set almost never failed to meet the homogeneity of variance assumption using Levene's test. Standard error of the mean was used to indicate the level of variability throughout the text.

## Results

The compiled silicoflagellate species richness data set (Fig. 2) from the combined Desikachary and Prema (1996) and Perch-Nielsen (1985) compilation, ranging from the Albian to the present, comprises 248 species.

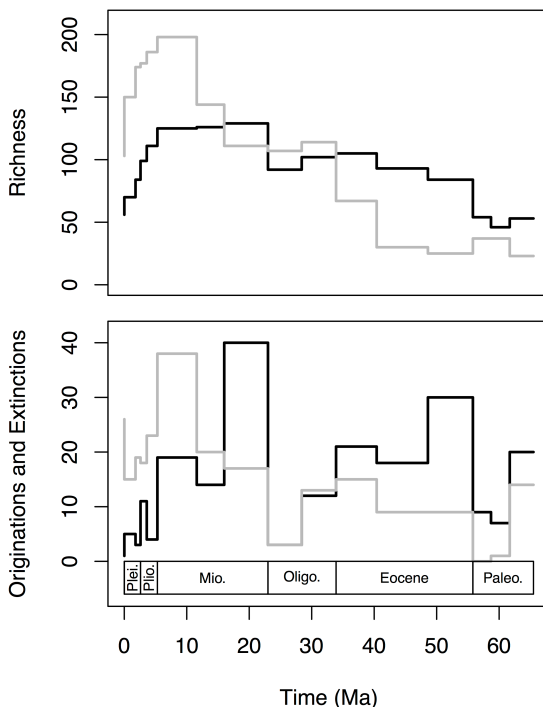


FIGURE 2. Silicoflagellate (black line) and diatom (gray line, Finkel et al. 2005) species richness (top panel). Silicoflagellate species origination (black line) and extinction rates (gray line) are shown in the bottom panel. Silicoflagellate data calculated from the combined Desikachary and Prema (1996) and Perch-Nielsen (1985) compilation.

The change in species richness over time confirms trends observed in previous estimates including a general increase in richness from the Cretaceous to the Miocene and the decrease in richness from the Miocene to the present (Lipps 1970; Bukry 1981). At the Eocene/Oligocene boundary, diatom species richness approaches silicoflagellate species richness. From the Oligocene/Miocene boundary to the present, extinction rates are high, eclipsing origination rates and thus resulting in declining silicoflagellate richness toward the present. There is little silicoflagellate species turnover in the Cretaceous relative to the Cenozoic, likely due to obvious gaps in sampling. For this reason, we focus on the last 65 Myr of the record for all analyses. Note that these richness data come from first and last occurrence data and are not corrected for changes in sampling effort over time. Differences in species concepts across workers, especially if this varies over the Cenozoic, and any errors in compilation will affect

patterns in species richness over time. In all subsequent analyses we focus on morphological trends over time, which are much less likely to be influenced by these potential biases than estimates of species diversity.

*Changes in the Species Richness of Morphologically Distinct Groups over Time.*—The three most abundant genera in the database are *Dictyocha*, *Distephanus*, and *Corbisema*. *Dictyocha* encompasses a group of diamond-shaped species with four spines and no apical ring. *Distephanus* is a genus of hexagon-shaped species that typically have six spines and an apical ring (Fig. 1) (Desikachary and Prema 1996). *Dictyocha*, *Distephanus*, and *Octactis* are the only genera that still exist in the modern ocean, though some taxonomists combine these under the genus *Dictyocha* (Van Valkenburg and Norris 1970; Ling and Takahashi 1985). *Corbisema* includes all three-sided species with rods inside the basal ring and includes species with and without spines. *Mesocena* (*Bachmannoecena*) and *Naviculopsis* are the other major genera that include species without spines.

Species with spines dominate the database (87% of all species) and their relative proportion increases over the Cenozoic (Fig. 3A). The mean number of spines per species (omitting species without spines) increased significantly over the Cenozoic from an average of about three to five spines (Fig. 3B). There is an increase in the percent abundances of genera with four to eight spines (*Dictyocha*, *Octactis*, and *Distephanus*) alongside the disappearance of groups with fewer than three spines (*Corbisema*, *Lyrarmula*, and *Naviculopsis*). The median remains close to four throughout the Cenozoic primarily because *Dictyocha* (mostly species with four spines) are often the most common members of the family *Dictyochaceae*. The increasing proportion of species with spines toward the present (Fig. 3A) may be attributed to the increased origination rates of spined relative to spineless species combined with the gradual extinction of species without spines.

*Changes in Silicoflagellate Size over the Cenozoic.*—*Dictyocha stelliformis*, a species with spherical nodules rather than spines that existed during the mid-Eocene, has the

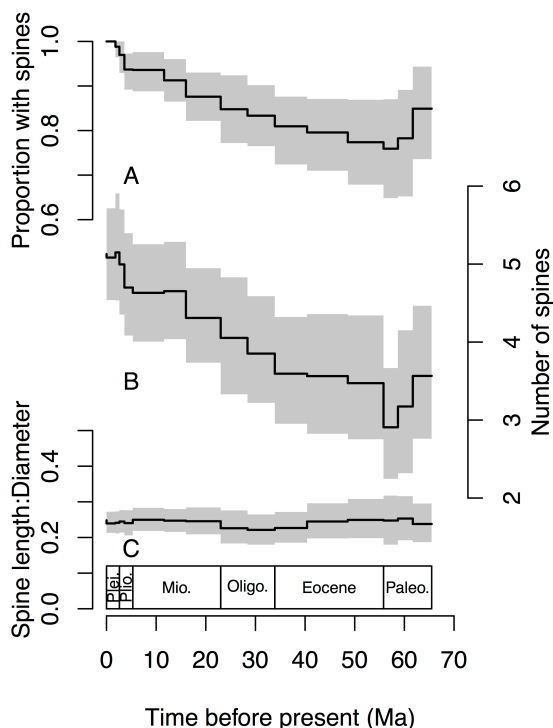


FIGURE 3. Macroevolutionary changes in the proportion of silicoflagellate species with spines (A), the mean number of spines per spined species (B), and spine length normalized by diameter (C) over the Cenozoic era. The black line represents the mean and the gray shaded region represents the 95% confidence interval.

thickest basal ring ( $18 \pm 0.7 \mu\text{m}$ ), as well as the largest skeleton ( $9600 \pm 360 \mu\text{m}^2$ ) and estimated cell surface area ( $17,000 \pm 140 \mu\text{m}^2$ ) in the database, though other members of this genus are typically much smaller. Members of *Crassicornisema*, a genus that originated during the Cretaceous and went extinct before the Miocene, have the largest average basal ring widths ( $7.0 \pm 1.1 \mu\text{m}$ ), skeleton surface areas ( $2000 \pm 610 \mu\text{m}^2$ ), and estimated cell surface areas ( $4700 \pm 1200 \mu\text{m}^2$ ). The smallest silicoflagellates are not confined to any one genus. *Distephanus bukryi*, a four-spined species that existed during the Pleistocene, has the thinnest basal rings ( $1.2 \mu\text{m}$ ) as well as the smallest skeleton ( $61 \mu\text{m}^2$ ) and estimated cell surface areas ( $81 \mu\text{m}^2$ ) in the database.

Silicoflagellate species without spines have, on average, larger basal ring widths than species with spines (Table 1). The mean basal ring width of spineless species decreases

TABLE 1. Summary of key morphometric characters for the species database.

	All species ( <i>n</i> = 248)		Spined species ( <i>n</i> = 215)	Spineless species ( <i>n</i> = 33)
	Min-max	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
Spine number	0–22	4.0 $\pm$ 0.2	4.7 $\pm$ 0.2	
Spine length ( $\mu\text{m}$ )	0–36	9.2 $\pm$ 0.5	11 $\pm$ 0.5	
Skeleton diameter ( $\mu\text{m}$ )	15–210	66 $\pm$ 1.6	65 $\pm$ 1.7	69 $\pm$ 4.5
Maximum basal ring rod width ( $\mu\text{m}$ )	1.2–18	3.5 $\pm$ 0.1	3.4 $\pm$ 0.1	4.2 $\pm$ 0.3
Skeleton surface area ( $\mu\text{m}^2$ )	61–9600	710 $\pm$ 49	680 $\pm$ 52	950 $\pm$ 130
Cell surface area ( $\mu\text{m}^2$ )	81–17,000	1700 $\pm$ 110	1500 $\pm$ 110	2800 $\pm$ 380
Silicification index	0.09–1.00	0.52 $\pm$ 0.01	0.54 $\pm$ 0.01	0.39 $\pm$ 0.03

through time toward the present, from  $4.7 \pm 0.6 \mu\text{m}$  in the Paleocene to  $3.7 \pm 0.4 \mu\text{m}$  by the Pliocene (Table 2). In contrast, there is little change in the mean basal ring width of spined silicoflagellates ( $3.4 \pm 0.1 \mu\text{m}$ ) over the last 65 Myr (Table 1, Fig. 4A). Independent *t*-tests show that the basal ring width of species without spines is significantly larger than the basal ring width of spined species during the Paleocene, Eocene, and Oligocene, but not during the Miocene and Pliocene to present. The skeleton surface area of species without spines is on average 40% larger than the skeleton surface area of spined species (Table 1). The skeleton surface area of spineless species shifts from  $1100 \pm 270$  to  $780 \pm 190 \mu\text{m}^2$ , whereas the skeletons of spined species remain relatively constant over the last 65 Myr ( $680 \pm 52 \mu\text{m}^2$ , Fig. 4B). Silicoflagellates without spines have significantly larger skeletal surface areas than spined silicoflagellates during the Paleocene, Eocene, Oligocene, and Miocene but not from the Pliocene to the present (Table 2).

A similar trend was observed in estimated silicoflagellate cell surface area (Fig. 4C). The mean estimated cell surface area of species

without spines is nearly twice the mean surface area of spined species (Table 1). For spineless species, cell surface area shifts from  $3500 \pm 690$  to  $2100 \pm 470 \mu\text{m}^2$  while the cell surface area of spined species stays near  $1500 \pm 110 \mu\text{m}^2$  over the Cenozoic. The difference between these two subgroups is highest in the Paleocene and becomes nonsignificant from the Pliocene onward (Table 2), perhaps indicating a convergence of cell size toward the present, primarily due to a decrease in the size of species without spines (Fig. 4C).

*Changes in Silicoflagellate Silicification over the Cenozoic.*—The silicification index is defined as the dimensionless ratio of skeleton surface area to estimated cell surface area in silicoflagellates and always lies between 0 and 1. Overall, there is no change in the silicoflagellate silicification index over the last 65 Myr (Fig. 5A); however, species with spines appear to support significantly more Si per cell than spineless species despite their smaller cell size (Table 2). The silicification index is  $0.54 \pm 0.01$  for spined species and  $0.39 \pm 0.03$  for species without spines. In general, species with long spines have higher silicification values than species with shorter

TABLE 2. *p*-values from independent *t*-tests comparing size and silicification metrics for spined and spineless species of silicoflagellates. *n* = sample size of spineless species, spined species.

	Millions of years before present				
	0–5.3 ( <i>n</i> = 7, 124)	5.3–23.0 ( <i>n</i> = 16, 144)	23.0–33.9 ( <i>n</i> = 18, 87)	33.9–55.8 ( <i>n</i> = 26, 97)	55.8–70 ( <i>n</i> = 14, 55)
Skeleton diameter ( $\mu\text{m}$ )	0.527	0.377	0.037	0.618	0.127
Maximum basal ring rod width ( $\mu\text{m}$ )	0.389	0.051	0.009	0.003	0.001
Skeleton surface area ( $\mu\text{m}^2$ )	0.391	0.016	0.001	0.004	<0.0001
Cell surface area ( $\mu\text{m}^2$ )	0.056	<0.0001	<0.0001	<0.0001	<0.0001
Silicification index	0.02	0.001*	<0.0001*	<0.0001	<0.0001*

\*Equality of variances not assumed in these cases.

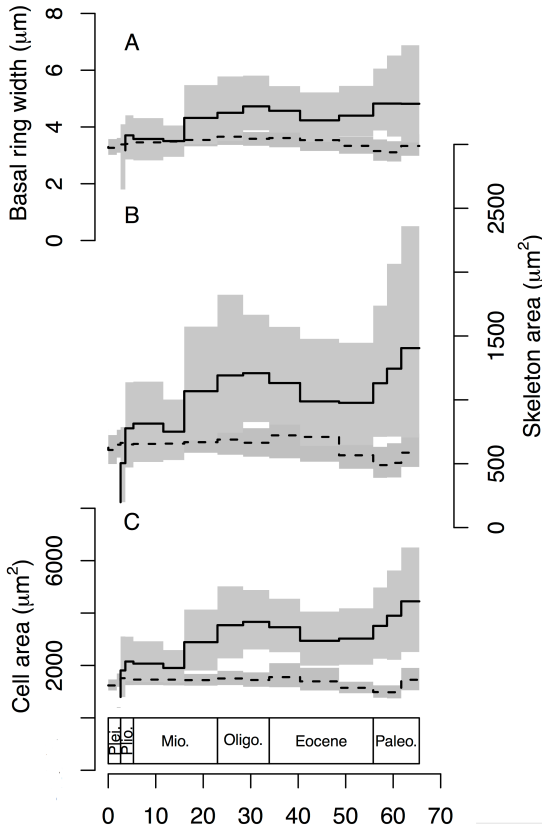


FIGURE 4. Macroevolutionary changes in silicoflagellate skeleton over the Cenozoic era, mean maximum basal ring width in  $\mu\text{m}$  (A), mean skeleton surface area in  $\mu\text{m}^2$  (B), and mean estimated cell surface area in  $\mu\text{m}^2$  (C). The solid black line represents the mean value for the species without spines, the dashed line represents the mean value for species with spines, and the gray shaded region represents the 95% confidence interval.

spines (Fig. 5B). There is little change in diameter-normalized spine length over the Cenozoic (Fig. 3C).

Over the Cenozoic, median diatom frustule area decreases alongside the skeleton surface area of spineless silicoflagellate species (Fig. 6). However, over some shorter periods of time the size of silicoflagellates without spines appears to be negatively correlated with the size of diatoms.

**Discussion**

There are macroevolutionary changes in the silicoflagellate skeleton over the Cenozoic (Figs. 2–4). Within the lineage as a whole, the proportion of species with spines increases from ~78% to 100%, and within species with spines the average number of spines increases from the Paleocene to the present (Fig. 3). There is no significant change in average skeleton size or silicification for silicoflagellate species with spines over the Cenozoic (Fig. 5). In contrast, species without spines until recently tended to be larger than species with spines, and have decreased in size through the Cenozoic (Fig. 4). We interpret these macroevolutionary changes in the morphology of silicoflagellate skeletons in the context of hypothesized costs and benefits of the silicified skeleton in response to changes in the biotic and abiotic environment over time.

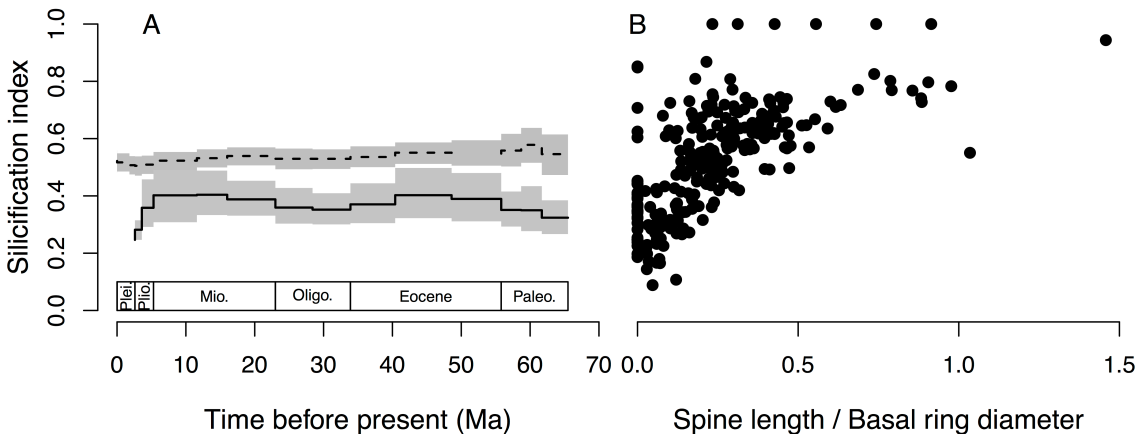


FIGURE 5. Macroevolutionary changes in silicoflagellate silicification (dimensionless), mean silicification for species without spines (black line), species with spines (dashed line), and 95% confidence interval gray shaded region (A) and the relationship between silicification and spine length normalized by basal ring diameter, dimensionless (B).

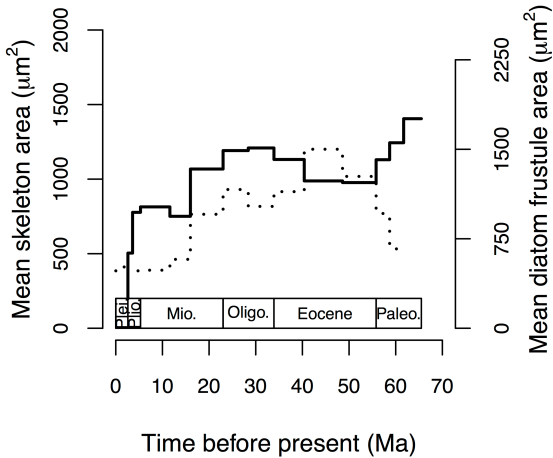


FIGURE 6. Macroevolutionary changes in median diatom frustule size (dotted line) and the mean skeleton surface area of silicoflagellate species without spines (black line) in  $\mu\text{m}^2$ .

Siliceous skeletons are spread across a wide phylogenetic distribution, produced in five out of the eight eukaryotic clades (Knoll 2003; Dove 2010). Likely costs of a siliceous skeleton include the metabolic expense of silicic acid uptake, transport, and precipitation in a low-nutrient environment, resulting in longer generation times (Brzezinski 1992). Certainly, skeleton-bearing silicoflagellates have much lower growth rates and lower maximum cell abundances than naked cells (Fanuko 1989; Jochem and Babenerd 1989; Henriksen et al. 1993). Silicification may increase intracellular density and sinking rates out of the pelagic zone; an effect that may be either detrimental, as it restricts access to light, or beneficial, as it increases access to nutrients and may reduce grazing pressure (Smetacek 1985; Raven and Waite 2004). When accounting for the success of silicification in the pelagic marine environment it is important to recall that defense mechanisms are under high selective pressure in a system intermittently ruled by top-down herbivory and predation (Smetacek 2001; Hamm and Smetacek 2007). Silicification may be expected to increase among siliceous marine phytoplankton in response to increased grazing pressure if it provides a significant evolutionary benefit through grazer deterrence (Sommer 1998; Pondaven et al. 2007), yet silicification may be constrained by availability of silicic acid in the upper ocean

and associated direct and indirect metabolic costs. The actual costs and benefits of biomineralization will vary over geologic time as a function of changes in selective pressures such as predation and the physical chemistry of the surrounding environment, specifically decreased silicic acid availability over the Cenozoic (Harper and Knoll 1975; Knoll 2003; Lazarus et al. 2009; Finkel and Kotrc 2010).

There appear to be two distinct morphological subgroups within the *Dictyochaceae* family. Silicoflagellate species with spines are more heavily silicified (higher silicification index) and are therefore likely to be denser than species without spines (Fig. 5A). Spined silicoflagellate species are characterized by smaller apical ring diameters, smaller basal ring rod widths, smaller skeleton and cell surface areas, and larger cell surface area to volume ratios, assuming cell volume is correlated with the total area of the skeleton (Table 1). Because of their smaller size, species with spines are likely to have lower total nutrient requirements as well as higher nutrient uptake rates per unit volume (Finkel et al. 2010a). The benefits of small size in the spined silicoflagellates will be somewhat muted for Si relative to other nutrients because of their higher levels of silicification. Spines may be a strategy to maintain a relatively small cell size with high surface area to volume ratio and a relatively larger physical size as experienced by grazers (Fig. 1). By contrast, the larger silicoflagellates, which tend to lack spines, will likely have had lower cell surface area to volume ratios, decreasing their competitive advantage under low-nutrient environments relative to spined forms owing to less efficient uptake kinetics and higher nutrient requirements (Cushing 1989; Aksnes and Egge 1991).

Lipps (1970) first postulated that silicoflagellates would sink according to Stokes's law of frictional force, with the broad surface perpendicular to the direction of descent, thereby slowing passive sinking and maximizing chromatophore exposure to light (Reynolds 1984). Because of unknown trade-offs between cell density and silicification, and skeleton and cell volume and shape, it is difficult to assess whether extinct silicoflagellate species



with or without spines had higher sinking rates. According to Stokes's law, sinking rate is linearly related to increases in organism density and the square of the organism's diameter, but is further mediated by the cell or skeleton shape. Spines can decrease sinking velocity relative to a sphere of equivalent radius by increasing form resistance (Sarjeant et al. 1987; Padisák et al. 2003), but because silicoflagellate spines are biomineralized, increasing the density of the cell, they could increase sinking velocity. Quantitative analyses of sinking velocity will require improved estimates of the density of the silicoflagellate skeleton, the density of the cell, and the ratio of cell to skeleton volume ratio.

As the base for the entire marine food web, plankton have developed a myriad of defense mechanisms to deal with a range of predators (Smetacek 2001; Hamm and Smetacek 2007). The two morphologically distinct subgroups of silicoflagellates, species with and without spines, may represent distinct strategies for grazer deterrence. Silicoflagellate species without spines may achieve some level of protection from grazing pressure through their larger size (Chase 1999) and the mechanical resistance of their skeletons, which are constructed of the same high-strength material as diatom frustules (Hamm et al. 2003). The absence of sharp angles, notches, and slits in the silicoflagellate skeleton as well as the presence of symmetrical structural rods may allow stress to be evenly distributed across the skeletal structure. Mechanical resilience may be enhanced through increases in thickness of the basal ring (Hamm and Smetacek 2007), which is proportional to skeleton size (Fig. 4). Therefore, silicoflagellates with large structurally sound skeletons may have fewer potential grazers than smaller naked species, which are susceptible to a wider range of grazers (Perissinotto 1992; Hansen et al. 1994; Hamm and Smetacek 2007).

Spines are common in smaller silicoflagellate species as well as some species of diatoms, radiolarians, dinoflagellates, and planktonic foraminifera. Spines have the potential to injure the digestive tract or feeding apparatus of grazers and would likely

increase grazer handling time (Hamm and Smetacek 2007). Skeleton-bearing silicoflagellate blooms have been associated with fish mortality events, leading to the hypothesis that the silicoflagellate skeleton may cause mucus clogging of fish gills following irritation by spines, as has been documented in response to certain blooms of spined diatoms (Bell 1961; Thomsen and Moestrup 1985; Fanuko 1989; Henriksen et al. 1993; Hargraves and Maranda 2002). Fish and benthic mortalities associated with non-skeleton-bearing (naked cell stages) silicoflagellate blooms have been attributed to anoxia (Jochem and Babenerd 1989; Fanuko 1989). Several workers have suggested that few animals graze upon modern silicoflagellates, yet there is little quantitative evidence that zooplankton grazing rates is inhibited by the presence or abundance of silicoflagellates (Fanuko 1989; Gowen et al. 1999). However, silicoflagellate grazing by salps, echinoderm larvae, and copepods has been documented (see references in Gowen et al. 1999). More work is required to assess predation rates and the preference of grazers for naked or skeleton-bearing silicoflagellates. Unlike diatom frustules, which provide some protection against digestion, the open skeletons of silicoflagellates leave cells more vulnerable to digestive enzymes and pathogens. Silicoflagellates are therefore unlikely to survive gut passage irrespective of any injury to the grazer. Nevertheless, the skeleton could result in a net benefit by increasing grazer handling time, deterring grazing, or injuring silicoflagellate ingestors. Large or spined skeletons may be much more effective against grazers than small skeletons without spines. The extinction of species without spines and increased origination rates of spined species toward the present indicate that only one of these anti-grazing strategies achieved evolutionary success over the Cenozoic (Fig. 3A).

Grazing pressure may have increased with the recovery and diversification of grazers since the end-Cretaceous mass extinction, perhaps stimulating an arms race in the planktonic realm (Dawkins and Krebs 1979), as indicated by the extinction of silicoflagellates without spines and the increasing mean number of

spines per species over the Cenozoic (Fig. 3). Major changes in the acquisition and proportion of basal ring spines since the Paleocene in *Corbisema*, *Naviculopsis*, and *Mesocena* have been used as biostratigraphic guides (Bukry 1981). Similarly, proximochorate and chorate dinoflagellate cysts exhibit a recovery in average crest height and spine length from the end-Cretaceous to Eocene, followed by a general decline until the Pliocene (Sarjeant et al. 1987). If grazing pressure were the sole driver of macroevolutionary change in silicoflagellates, metrics representing skeleton size would be expected to increase, particularly among species that rely on size rather than spines to deter grazers (Pondaven et al. 2007). However, the basal ring width, skeleton surface area, and estimated cell surface area actually decrease from the Paleocene through to the Pleistocene among silicoflagellates without spines (Fig. 4). Mathematical analysis of the apical surface area, length of the skeletal elements (a proxy for total silica use if the various structure all have the same diameter and density), and volume enclosed by the silicoflagellate skeleton (all non-dimensionalized) suggests that *Corbisima*, *Dictyocha*, and *Distephanus* skeletal morphologies are the consequence of a variety of trade-offs between silicic acid use and total surface area (McCartney and Loper 1989, 1992).

Macroevolutionary trends in the fossil records of diatoms, radiolarians, and siliceous sponges have been taken as evidence for decreases in silicic acid availability at the ocean surface over geologic time (Harper and Knoll 1975; Maliva et al. 1989; Maldonado et al. 1999; Finkel et al. 2005; Finkel and Kotrc 2010). The skeleton surface area of spineless silicoflagellate species and the area of the diatom frustule area both decrease over the Cenozoic (Fig. 6). Because the metabolic costs associated with silicic acid uptake, transport, and precipitation are expected to have increased as surface silicic acid became increasingly scarce over the Cenozoic, the decrease in size among silicoflagellate skeletons without spines provides additional support for this hypothesis. By contrast, species with spines appear to remain unaffected by changes in silicic acid concentration (Fig. 3).

Within the spined silicoflagellates, total silica use does not change, but Si appears to be redistributed to increase the number of spines. The recent extinction of silicoflagellates without spines as they approach the smaller size of the silicoflagellates with spines indicates that the benefits provided by siliceous spines exceed the metabolic costs of constructing and maintaining a siliceous skeleton even as silicic acid availability may have become increasingly scarce. Selection toward smaller skeleton sizes may also have been affected by high sinking rates, but the relative costs or benefits of sinking are not currently well understood for silicoflagellates.

Why do silicoflagellate species without spines decrease in size and then go extinct while silicoflagellate species with spines show little change in size or silicification over the Cenozoic? We argue that declining silicic acid availability in the upper sunlit layer of the ocean acted to increase the costs associated with constructing and maintaining a siliceous skeleton, favoring small and more lightly silicified skeletons while grazing pressure favored the persistence of the siliceous skeleton, especially with spines. If the silicoflagellate skeleton, and in particular the spines, provide a benefit against predation then a decline in the availability of silicic acid and/or an increase in predation pressure over the Cenozoic would be expected to favor species with spines and a decrease in the size of the skeleton of species without spines, as observed. The larger size of the silicoflagellate species without spines may have provided them with some refuge against grazing pressure, but as silicic acid became increasingly scarce the cost of larger skeletons would have increased, favoring a reduction in size and further reducing the fitness advantage associated with skeletons without spines.

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