Climatically driven macroevolutionary patterns in the size of marine diatoms over the Cenozoic

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Numerous taxonomic groups exhibit an evolutionary trajectory in cell or body size. The size structure of marine phytoplankton communities strongly affects food web structure and organic carbon export into the ocean interior, yet macroevolutionary patterns in the size structure of phytoplankton communities have not been previously investigated. We constructed a database of the size of the silica frustule of the dominant fossilized marine planktonic diatom species over the Cenozoic. We found that the minimum and maximum sizes of the diatom frustule have expanded in concert with increasing species diversity. In contrast, the mean area of the diatom frustule is highly correlated with oceanic temperature gradients inferred from the $\delta^{18}O$ of foraminiferal calcite, consistent with the hypothesis that climatically induced changes in oceanic mixing have altered nutrient availability in the euphotic zone and driven macroevolutionary shifts in the size of marine pelagic diatoms through the Cenozoic.

biological pump | carbon cycle | cell size | climate change | phytoplankton

Diatoms are a group of eukaryotic oxygenic photoautotrophs characterized by an opaline silica frustule that can be preserved in the fossil record. The oldest unequivocal fossil diatoms are found in the middle Cretaceous (1, 2), but molecular clock estimates indicate they may have originated as early as 165–240 million years ago (3, 4). The fossil record indicates that diatom diversity and evolutionary tempo (5, 6) has increased over most of the Cenozoic. This trend, in conjunction with the decreasing diversity of fossil calcareous nannoplankton and dinoflagellate cysts, has been cited as evidence for an increase in the relative importance of diatoms to the burial of organic carbon in marine sediments through the Cenozoic (6, 7). In the contemporary ocean, diatoms account for a large proportion of oceanic primary and export production (8).

The size structure of the phytoplankton community is correlated with food web dynamics and export production (9). Small cells are much more likely to be rapidly recycled within the upper ocean in a microbial "loop" (10), whereas communities dominated by large cells tend to be associated with increasing efficiency of trophic transfer to metazoans (e.g., fish) and export of photosynthetically fixed carbon into the deep sea (11). The export of carbon, commonly referred to as the biological pump, contributes to the ocean's capacity to act as a sink for atmospheric carbon dioxide (12). Changes in nutrient availability are often associated with shifts in the size structure of phytoplankton communities and the magnitude and efficiency of the biological pump. Typically, phytoplankton communities are dominated by small phytoplankton cells under oligotrophic conditions, such as the oceanic gyres, whereas larger phytoplankton cells are more abundant along continental margins and in upwelling zones, where nutrient concentrations tend to be higher and more variable (13).

Evolutionary shifts in the size of phytoplankton cells would have had a profound influence on oceanic food web dynamics (9), carbon cycling, and the interpretation of $\delta^{13}C$ of organic carbon over the Cenozoic (14). Several studies on a few single, morphologically defined species of marine diatoms have documented size shifts in response to temperature and upwelling zones over hundreds of thousands to several millions of years (15–17). These observations suggest that environmental factors could drive evolutionary change in phytoplankton cell size, but macroevolutionary changes in the average size of the cells within phytoplankton communities have not been investigated over the Cenozoic.

Variations in the concentration of atmospheric CO_2 and other greenhouse gases can alter nutrient concentrations in the upper ocean through two basic mechanisms. First, greenhouse forcing of planetary temperature influences the equator-to-pole (horizontal) and surface-to-deep (vertical) temperature gradients in the ocean. These two thermal gradients determine the energy required to mix nutrients from the ocean interior to the euphotic zone. Second, the concentration of CO_2 and surface temperatures influence the rate of rock weathering, which affects the flux of nutrients from cratons to the ocean (18). Resource availability in the euphotic zone affects phytoplankton community size structure, which in turn can alter the biological pump and the rate of burial of organic matter along continental margins. Thus, climate-induced changes in nutrient availability have the potential to result in climatic feedbacks through ecological and evolutionary shifts in phytoplankton community size structure and the efficiency of the biological pump.

Macroevolutionary change in body size has been documented in unicellular foraminifera (19, 20) and a variety of aquatic and terrestrial metazoans (21, 22). A combination of size bias in origination or extinction, physiologically imposed boundaries on minimum and maximum sizes, and active selection pressures can result in complex temporal patterns in the evolution of body size. In conjunction with species radiation, passive evolutionary mechanisms tend to result in increases in the maximum and minimum sizes with no change in the mean body size of the group (23, 24). Bias toward the survival of small species after mass extinction events and a physiological boundary on minimum body size often result in increases in the maximum and mean sizes within a taxonomic group, referred to as Cope's rule (23, 25, 26). Active selection pressures, such as trended changes in resource availability or predation pressure, can result in shifts in the size of taxonomic groups toward a particular size with a contraction in the size range (26). Different selection pressures may act on individuals of different size, resulting in a large variety of size distributions.

Here we present a record of macroevolutionary change in the size structure of marine phytoplankton communities from a compilation of the frustule size of planktonic diatoms over the Cenozoic. By using this data set, we evaluate the role of climate change on macroevolutionary changes in the size of diatoms over the last 65 million years.

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Materials and Methods

Diatom Frustule Size Database. A Cenozoic database of marine diatom frustule size and associated geographic and stratigraphic information was compiled from a combination of measurements of light and scanning electron micrographs and floral descriptions from a variety of literature sources, with an emphasis on reports from the Deep Sea Drilling Project and Ocean Drilling Program. Diatoms are primarily represented in the fossil record by their silica frustules. In living diatoms, the frustule is composed of an epitheca that overlays the hypotheca and is held together by concentric girdle bands. Although measurements of frustule volume are preferred because cell volume and mass are correlated to physiological rates and ecological patterns (27), the theca are often separated during preservation or preparation of samples, making the construction of a database of frustule volumes impractical. To compare the size of species with different basic morphologies, the size for each diatom species was estimated as the median (consistently the best metric of central tendency) of the largest observable projected area (valve or girdle view) of the measured theca. The average size of the frustule of the diatoms making up an assemblage was estimated as the community mean of the median size of the extant species within a particular time interval or geographic region without regard to relative abundance. This analysis provides low temporal resolution but large sample sizes that enable a broad comparison of the average frustule size within the diatom community between ocean basins (Atlantic and Pacific) and broad latitudinal bands.

Construction of a High-Resolution Temporal Record of Frustule Size of the Diatom Community. To construct a high-resolution temporal record of change in the frustule size of the dominant diatom community through the Cenozoic, the median size of species was matched to the superior stratigraphic ranges of the dominant marine diatom species provided by the Neptune database (28). Diatom species found only as fragments of the theca, for example *Rhizosolenia* and *Neobrunia* spp., have been excluded from the database. Neptune is a global compilation of micropalaeontological data, including 389 species of diatoms, from 165 drill holes from Legs 1–135 of the Deep Sea Drilling Project and Ocean Drilling Program, adjusted to a uniform taxonomic system correcting for synonyms and using a common time scale. Neptune represents the largest, most comprehensive database of the dominant marine fossil diatoms currently available, providing unprecedented spatial and temporal coverage.

The minimum and maximum size of the diatoms was estimated from the species within the community with the most extreme frustule area for each 1-million-year time interval. These estimates were then used to calculate the percentage rate of change in frustule area per 1 million years. Because the change in minimum and maximum size depends on the origination of rare large or small species and is static over many millions of years, an average rate of change was estimated for 0–65 million years ago. The extreme size ratio was estimated as the maximum area divided by the minimum area. To reduce the effect of a few extremely small or large species, which are hard to sample accurately, the average frustule size of the diatom community was determined from the 90% trimmed mean of the species areas for centric species (radially symmetric), pennate species (bilaterally symmetric), and all species present, in each 1-million-year time interval. A list of the species from the Neptune database with basic size information is provided as Table 2, which is published as supporting information on the PNAS web site.

The mean size of the diatom frustule was compared with paleoenvironmental indicators: global sea level (29), percentage of flooded continental area (30), phosphorus accumulation rates (milligram of phosphorus per cm^2 per every thousand years) (31), $\delta^{18}O$ (‰) of benthic deep-water foraminifera (an indicator of deep sea temperature) (32), difference in $\delta^{18}O$ between tropical planktonic and benthic foraminifera (an indicator of oceanic thermal gradients) (32), and $\delta^{13}C$ (‰) of organic matter in marine sediment (14). Data sets that were not temporally commensurate were interpolated every 5 million years or used the lowest resolution available.

Testing the Significance of Temporal Changes in Average Size of Frustule in the Diatom Community. Permutation tests were used to ensure that changes in the variance in mean size due to the temporally varying sample size did not create a false impression of temporal changes in the size distribution. Our statistic of temporal variation was the sum over time of the squared differences between the time-resolved and time-independent size histograms. We generated 999 samples from two different null distributions, the first by permuting the species present in each 5-million-year interval and the second by permuting sizes among species and computing the temporally resolved size distributions. In both cases, the sample sizes in each time interval was set by species diversity.

Results and Discussion

Macroevolutionary Change in the Size of the Diatom Frustule over the Cenozoic. Over the Cenozoic, the minimum size of the frustule decreased and the maximum size increased, resulting in an ≈1,000-fold increase in the ratio of frustule sizes (maximum area/minimum area) that broadly corresponds to increases in species diversity (Fig. 1 and Fig. 5, which is published as supporting information on the PNAS web site). In contrast, there is a 2.5-fold decrease in mean frustule size over the Cenozoic (Fig. 1). Permutation tests considering the effect of temporally changing sample size confirm that the changes in mean size are statistically significant ($P < 0.05$). The secular change in the mean size of the frustule within the centric and pennate communities is qualitatively identical, but, because the average size of pennates is generally smaller than centrics, the increase in the relative diversity of pennate diatoms over the last 35 million years results in an additional decrease in the overall mean area of the diatom frustule over this time period. Parallel temporal changes in the mean area of the frustule of centric and pennate species suggest that changes in measured frustule area may correspond to changes in frustule and cell volume and changes in aspect ratio.

The largest changes in diatom species diversity, size range, and average area of diatom frustule broadly correspond with the deposition of large chert deposits (33–35) and shifts in ocean circulation associated with changes in climate (36–38). The mean size of the diatom frustule is high through the early Cenozoic, with a peak in early Eocene, and a large decrease in the Miocene. The temporal change in the mean size of the diatom frustule is consistent between communities from the Atlantic and Pacific oceans (Fig. 2*A*). Differences in the average frustule size of communities from the Atlantic and Pacific oceans are largest in the Eocene, decline in the Oligocene, and essentially vanish in the Neogene, consistent with shifts in oceanic circulation (36, 37). Although latitudinal differences in the mean size of the diatom frustule are relatively minor, the average frustule size is smaller in the subpolar and polar latitudes where the differences in size between Atlantic and Pacific communities are largest (Fig. 2*B*). Tropical and subtropical foraminiferal communities (>150 μm) also exhibit a marked change in body size over the last 20 million years (19), indicating that common environmental factors, such as a change in oceanic circulation and changes in nutrient availability, may be driving the macroevolutionary change in the size of planktonic organisms over the Cenozoic.

The fossil assemblage is a small and often biased sample of the species extant in the water column. There is no evidence to

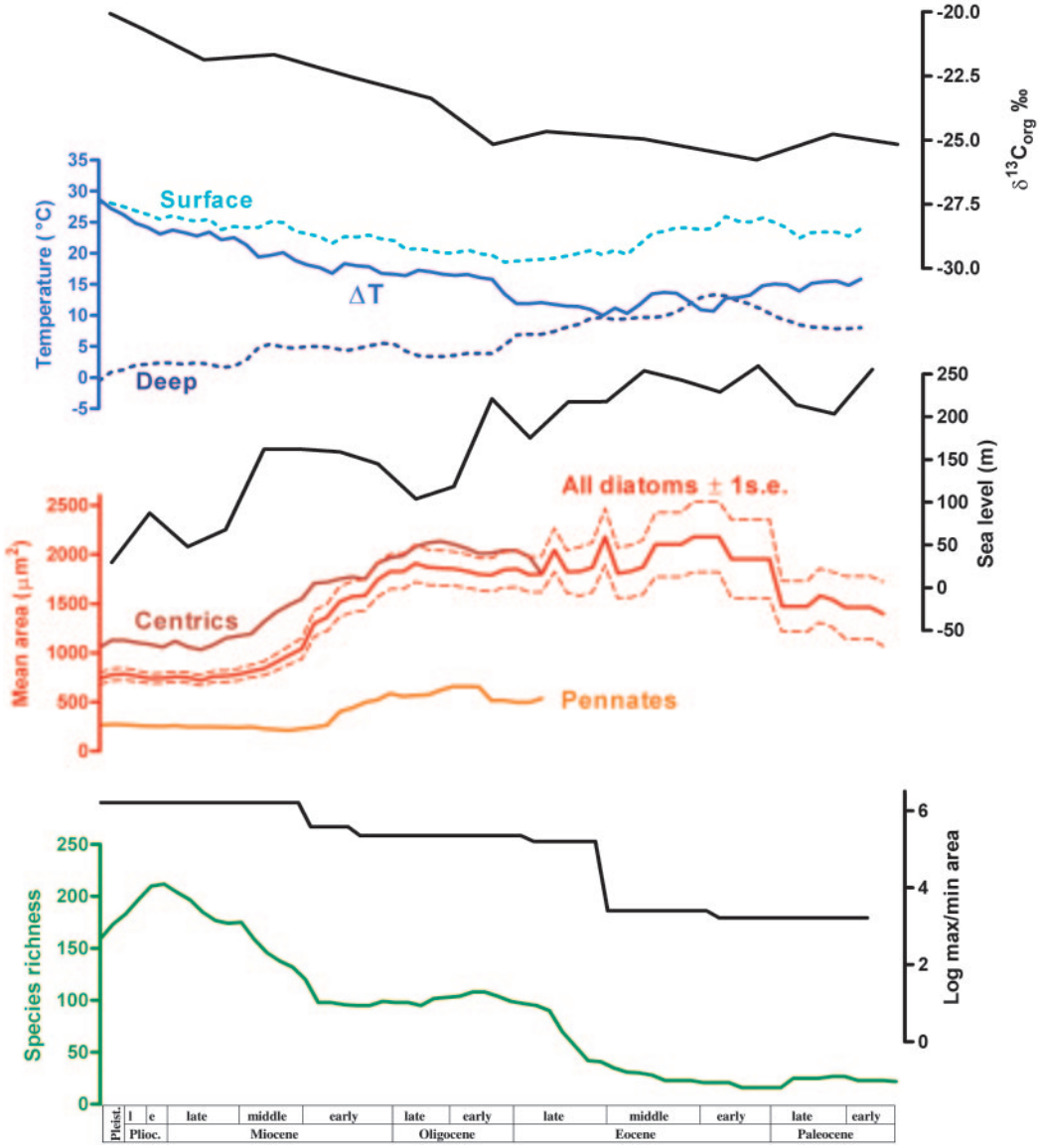

Fig. 1. Macroevolutionary changes in the size structure of the diatom community using the Neptune database associated with paleoenvironmental indicators. Shown from bottom to top are diatom species richness (28); log maximum/minimum frustule area; the 1-million-year, 90% trimmed running mean of median area (μm^2) of pennate species, all diatom species ($\pm$1 SE), and centric species; sea level (m) (59); surface, surface–deep (ΔT), and deep oceanic temperature (°C) determined from tropical Pacific planktonic and benthic $\delta^{18}O$ of foraminifera (32); and the $\delta^{13}C$ of organic carbon (‰) (14).

suggest a strong secular pattern in the preservation of frustules of large or small size. If small or large frustules are less likely to be preserved over time, the minimum or maximum frustule size would be correlated with changes in the mean. In contrast, we find that neither the maximum or minimum frustule size is correlated with the mean. The decrease in the minimum size, increase in the maximum size, and statistically significant decrease in the mean size indicate that size-dependent dissolution is unlikely to have been a primary factor in the observed temporal changes in the size structure of the diatom community over the Cenozoic. Furthermore, examination of extant diatoms indicate that frustule thickness and cell volume are not strongly correlated (39). Therefore, we suggest that the macroevolutionary patterns in the size of marine diatoms in the fossil record are the result of a combination of the physiological, ecological, and microevolutionary factors that affect diatom size.

Diatom Size: Physiological Constraints and Ecological Strategies. Smaller phytoplankton cells have a relatively larger surface area/volume ratio than larger cells, faster growth rates, and an inherently superior ability to harvest light and nutrients under equilibrium conditions (40, 41). The multiple physiological advantages of small size suggest that there should be an inevitable evolutionary trend toward smaller phytoplankton taxa over time. The smallest eukaryotic phytoplankton cells, $\approx$0.8 μm in equivalent spherical diameter, come from one of the most ancient phytoplankton classes, the Prasinophyceae (42). It has been hypothesized that the lower bound on the diameter of single-celled photosynthetic organisms of $\approx$0.5 μm is set by the cellular requirements associated with the maintenance of a functional complement of DNA and photosynthetic machinery (43). The smallest diatoms are 2–4 μm in equivalent spherical diameter. The average rate of decrease in the minimum area of the diatom frustule over the Cenozoic is 4% per million years, but the minimum area has changed little over the last 15 million years of the Cenozoic, suggesting that the diatoms may be approaching their minimum possible cell size. The unique properties of the silica frustule may contribute to their relatively large minimum size. The frustule is constructed from multiple layers of aggregated and polymerized silica spheres that are $\approx$30–40 nm in

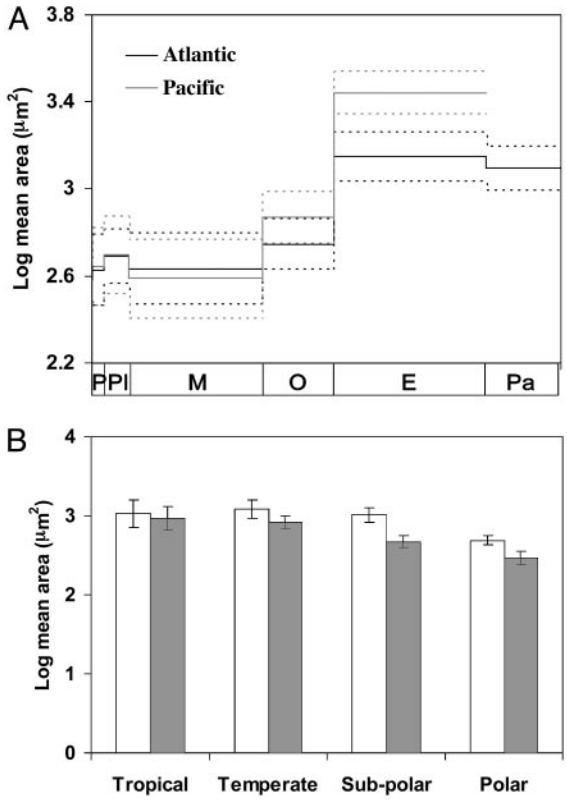

Fig. 2. Log mean frustule size of the diatom community ($\mu m^2 \pm 2$ SE, dotted lines) as a function of epoch and the Atlantic (black, $n = 413$) and Pacific (gray, $n = 235$) ocean basins (*A*) and of ocean basin and latitudinal sites: tropical (0–20°, $n = 64$), temperate (21–45°, $n = 241$), subpolar (46–59°, $n = 419$), and polar (60–90°, $n = 421$) (*B*).

length, which contributes to the size of the diatom frustule and the typical estimated cell size (44). To asexually reproduce, each daughter cell develops within the parental cell wall, inside the theca, often resulting in ≈2-fold decrease in the size of individuals over several generations. Sexual reproduction is cued to a percent decrease in size, resulting in a restoration of the original size (45), indicating no obvious causal relationship between reproductive strategies and evolutionary changes in the size of diatoms over geological time.

Very large changes in maximum body size are generally associated with the origination of fundamentally new taxonomic groups (46). For example, the average cell diameter of fossil phytoplankton assemblages increased from 5 to 13 μm with the establishment of eukaryotic communities, ≈1,500 million years ago (47). Appearing relatively late in the Phanerozoic, diatoms contain species with among the largest cells of all of the autotrophic marine phytoplankton groups; they reach maximum linear dimensions of ≈1–3 mm in the case of *Thalassiothrix longissima* and *Ethmodiscus rex*. Their upper size limit is physically constrained by diffusion; cells with diameters much larger than 1 mm can become seriously nutrient-limited because of a decreased diffusive flux as the surface area/volume ratio decreases and nutrient requirements increase (40). Some of the larger extant diatoms have physiological or ecological strategies that compensate for the decrease in nutrient flux from diffusion. For example, several *Ethmosdiscus* and *Rhizosolenia* spp. have the ability to migrate downward to take up nutrients and then return to the nutrient-depleted surface to photosynthesize (48, 49), whereas other species have nitrogen-fixing symbionts that provide them with an intrinsic source of fixed nitrogen (50, 51). Larger diatoms are often elongated in one dimension, resulting in a greater surface area/volume ratio relative to more compact shapes (52) and have relatively larger nutrient storage vacuoles and lower carbon content on a volume basis (53). Superior storage ability may allow larger diatoms to out-compete smaller diatom species with faster intrinsic growth rates by achieving a slow but steady growth rate in certain pulsed nutrient environments where a small cell can grow rapidly but for only a small proportion of the time (54, 55). These novel strategies may be at the root of the continued evolutionary increase in the upper size of the diatom frustule, which has expanded an average of 7% every million years over the Cenozoic.

Environmental Conditions Actively Select for Macroevolutionary Changes in the Mean Size of the Diatom Frustule. Although changes in the minimum and maximum frustule size of the diatoms correspond with increases in species diversity within physiological boundaries, the macroevolutionary change in the mean size of the diatom frustule is a function of environmental forcing. In the modern ocean, because of size-dependent nutrient requirements and uptake capabilities, small phytoplankton cells often dominate community biomass under low nutrient conditions, whereas larger diatoms tend to be more successful when nutrient availability is high and more temporally and spatially variable (13, 56). Extrapolating this ecophysiological relationship to evolutionary scales implies that the size structure of fossil phytoplankton communities is a biological indicator of the changes in the availability of nutrients in the euphotic zone as a function of climatic change. Qualitatively, the largest changes in the mean size of the diatom frustule occur in the earliest and latest Eocene and early-to-mid Miocene; these size changes correspond with documented shifts in ocean circulation (36, 37) and turnover events in deep sea foraminifera (57) and ostracods (58). The global cooling trend since the early Eocene has led to the expansion of permanent ice on the Antarctic continent, lower bottom water temperatures, an increase in the oceanic surface-to-deep (vertical) temperature gradient (32), an intensified and isolated Antarctic counter current (37), decreases in sea level, and a reduction in flooded continental shelf area (30,

Table 1. Ordinary least squares regression analyses of the mean area of the diatom frustule (μm^2) as a function of paleoclimatic indicators, all adjusted to a uniform timescale (71) and interpolated at 5-million-year intervals

Paleoclimatic indicators	a	b	*R*	Ref.
Sea level, m	-60.0 ± 54.0	$1.1 \times 10^{-1} \pm 3.3 \times 10^{-2}$	0.73	59
Flooded coastal area, %	0.5 ± 3.1	$7.5 \times 10^{-3} \pm 1.9 \times 10^{-3}$	0.78	30
Surface-water temperature, °C	27.5 ± 2.1	$-3.0 \times 10^{-3} \pm 1.3 \times 10^{-3}$	−0.59	32
Deep-water temperature, °C	-2.0 ± 2.5	$5.1 \times 10^{-3} \pm 1.5 \times 10^{-3}$	0.73	32
Temperature gradient, °C	29.5 ± 2.4	$-8.1 \times 10^{-3} \pm 1.5 \times 10^{-3}$	−0.87	32
$\delta^{13}C_{org}$, ‰	-20.7 ± 0.8	$-3.1 \times 10^{-3} \pm 5.6 \times 10^{-4}$	−0.83	14

Shown are the intercept (a) and the slope (b) derived from the ordinary least squares regression. Values for a and b are shown as mean ± SE. All values are significant at $P < 0.01$.

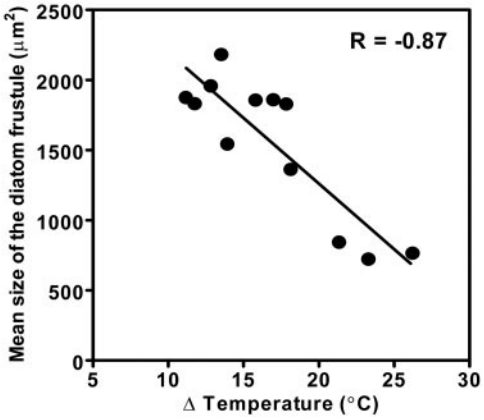

Fig. 3. Mean area of the diatom frustule as a function of the tropical oceanic temperature gradient. The 5-million-year, 90% trimmed running mean of the size of the diatom frustule (μm^2) as a function of the vertical oceanic temperature gradient (ΔT). The temperature gradient is the difference between temperatures determined from planktonic and benthic $\delta^{18}O$ of foraminiferal calcite from tropical Pacific sites (30).

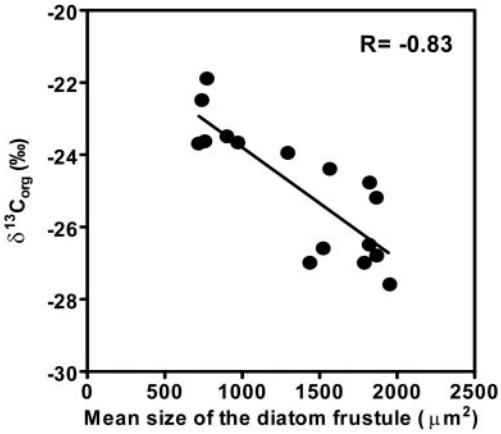

Fig. 4. Cenozoic changes in the $\delta^{13}C$ of organic carbon [$\delta^{13}C_{org}$ (‰)] as a function of the mean area of the diatom frustule (μm^2). The carbon data (14) were interpolated to facilitate comparison.

59). The early Miocene marks the largest change in the average size of the diatom frustule and the beginning of a series of step-like oxygen isotope excursions indicative of large short-term glacial events (36, 38, 60) and increases in biogenic silica deposition from the Caribbean and low-latitude Atlantic to the North Pacific (33).

Quantitatively, the average size of the diatom frustule is significantly correlated with global sea level, percentage of flooded continental area, and oceanic temperature (Table 1). The strongest correlation with the mean size of the diatom frustule is associated with the oceanic temperature gradient as determined from the temperature difference between surface and deep waters in the tropics (Fig. 3). It has been suggested that oceanic temperature is an excellent predictor of planktonic foram diversity because thermocline structure alters the vertical structure of the water column and the available niches (61). For phytoplankton, thermocline structure affects community structure through changes in the availability of light and nutrients for growth in the surface ocean (62, 63). Although the Cenozoic increase in global phosphorus accumulation rates and carbon/phosphorus ratios imply a secular increase in nutrient input to the ocean (31, 64), the phosphorus accumulation rate is relatively weakly correlated ($R = -0.5$, $P = 0.1$) with the mean size of the diatom frustule. We speculate that thermal stratification of the water column and the equator-to-pole temperature gradient moderate the availability of nutrients to phytoplankton in the euphotic zone, accounting for the relatively high correlation between the mean area of the diatom frustule and the oceanic temperature gradient. An increase in the thermal gradient between the equator and the poles acts to increase wind-driven heat transport (65), but the concurrent increase in the average vertical temperature gradient acts to increase the stability of the water column and the energy required to mix nutrients from depth into the euphotic zone. The net effect of these changes in the thermal gradient is to alter the energy required to mix nutrient-poor surface waters with the nutrient-rich waters from below the thermocline (66), perhaps accounting for the large decrease in the average size of the diatom community in the Neogene (Fig. 1). In addition, decreases in sea level and flooded continental areas characterized by shallow, well mixed, high-nutrient upwelling zones may have selected against the larger diatoms that require intense mixing to avoid sinking out of the euphotic zone and against diatoms that form resting stages and require resuspension into the euphotic zone after sinking to the sediment surface (67).

Conclusions and Future Directions. Macroevolutionary change in diatom community size structure provides an additional framework to aid in the interpretation of paleoenvironmental indicators. For example, it has been hypothesized that the Cenozoic increase in the $\delta^{13}C$ of bulk marine organic matter may have resulted from an increase in phytoplankton cell size through time, assuming that primary and export production have remained relatively constant (14). A negative correlation between the mean size of the diatom frustule and the $\delta^{13}C$ of organic carbon (Fig. 4) indicates that alternative hypotheses, such as an increase in export production, a change in the fractionation of carbon due to the increasing dominance of diatoms (68) with C_4 and β-carboxylation pathways (69), or boundary layer effects (70), require further consideration. In addition, the absence of a relationship between the mean size of the diatoms with traditional indicators of nutrient availability in the ocean, such as phosphorus accumulation rates (31), and the high correlation with the vertical temperature gradient in the ocean, suggests nutrient availability in the upper mixed layer has been primarily forced by thermohaline circulation and not chemical weathering and nutrient input from the land into the coastal ocean. The potential of climatically driven changes in the size structure of diatom communities over the Cenozoic to affect and interact with oceanic food web structure and biogeochemical cycles suggests macroevolutionary patterns in the size distribution of plankton can be used to complement geochemical climate proxies to enrich the interpretation of the effects of climatic change on ecosystem structure and function.

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