that closely mimics the observed pattern results when $\alpha = 2.95$ and $\beta = 0.02727$ [see (*11*)].
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Covariance Patterns of Foraminiferal $\delta^{18}O$: An Evaluation of Pliocene Ice Volume Changes Near 3.2 Million Years Ago

Abstract. *Oxygen isotope data for a Pliocene interval from 3.6 to 2.8 million years ago show a mean increase (0.5 per mil) of benthic $\delta^{18}O$ at about 3.2 million years ago, whereas planktic $\delta^{18}O$ does not increase. This lack of covariance indicates that the event at 3.2 million years did not result in a permanent increase in the ice budget of either the Northern or the Southern Hemisphere.*

The history of continental ice sheets and their effect on the composition and climates of the ocean is a pervasive theme in paleoceanography. For example, within the Neogene, much of the evidence for the magnitude and timing of the initiation of major ice sheets in the Northern Hemisphere has come from analysis of the stratigraphy of ice-rafted deep-sea sediments (*1, 2*) and increased $\delta^{18}O$ of benthic foraminifera (*3, 4*). Although the first major glaciation in the Northern Hemisphere has traditionally been placed in the Pliocene (*5*), some investigators have suggested that ice sheets were present as early as the late Miocene (*6*). In this report I examine the oxygen isotope evidence for major glaciation at 3.2 million years ago (Ma) a date often cited for the initiation of Northern Hemisphere glaciation (*1–5*).

Prior to the availability of Deep Sea Drilling Project (DSDP) hydraulic piston cores, the highest quality oxygen isotopic record relevant to the question of Pliocene glaciation was that of core V28-179 from the equatorial Pacific (*7*). At approximately 3.2 Ma, this record contains a steplike increase of about 0.4 per mil in the average $\delta^{18}O$ of benthic foraminifera. This baseline shift separates an interval of low variability and relatively low isotopic values, called the "preglacial" Pliocene, from an interval of higher and variable isotopic values (the "glacial" Pliocene) (*7*). The increased $\delta^{18}O$ values were interpreted to indicate increased $\delta^{18}O$ of the ocean due to the presumed initiation of Northern Hemisphere ice volume. With one apparent exception (*2*), this pattern of benthic $\delta^{18}O$ is widely observed in all oceans (*3, 4*). A subsequent increase in the mean $\delta^{18}O$ at about 2.4 Ma has recently been correlated with the onset of major ice rafting on Rockall Bank in the North Atlantic (*2, 7*). This 2.4-Ma isotopic event is also clearly recorded, although with lower amplitude, in a detailed planktic isotopic record from the equatorial Pacific (DSDP site 572) (*8*).

If these mean $\delta^{18}O$ increases (*9*) do represent a greater average volume of continental ice sheets, the isotopic composition of the entire ocean should be increased. Therefore, the change in mean $\delta^{18}O$ observed in benthic records should also be observed in the average $\delta^{18}O$ of surface-dwelling planktic foraminifera. At the 2.4-Ma event, the planktic and benthic $\delta^{18}O$ records do covary. However, preliminary data suggest that planktic and benthic $\delta^{18}O$ data do not covary across the 3.2-Ma event. I examine here simple models of foraminiferal isotopic variations and new data to identify the pattern of covariation between the planktic and benthic $\delta^{18}O$ data before and after 3.2 Ma.

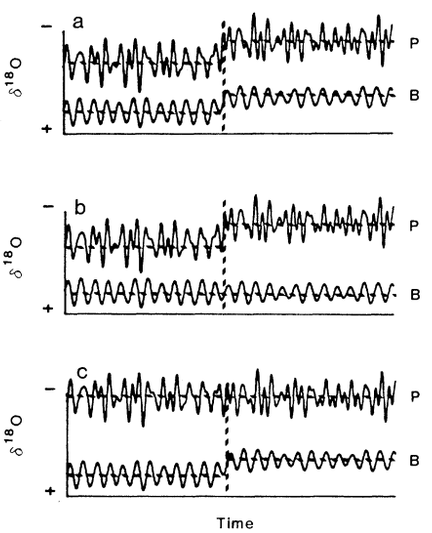

Fig. 1. Schematic records of planktic (P) and benthic (B) foraminiferal $\delta^{18}O$. A steplike change in mean $\delta^{18}O$ is imposed on the normal variability, which is simulated by frequency components that are observed in paleoceanographic data. The low-latitude planktic record is simulated by a combination of 0.023-Ma (precession) and 0.041-Ma (tilt) cycles. The benthic record is simulated by the 0.041-Ma tilt cycle, which is predominant at high latitudes. Comparison of the P and B records shows the patterns expected from an increase in ice volume (a), a decrease in sea-surface temperature (b), and a decrease in bottom-water temperature (c). The pattern of covariation of P and B records allows one to distinguish between ice volume changes and other processes.

Conceptual models for the expected covariation between low-latitude benthic and planktic $\delta^{18}O$ records are shown in Fig. 1 for three plausible changes in the ocean-climate system. An increase in oceanic $\delta^{18}O$, due to increased ice volume, would increase both records equally, yielding covariation (Fig. 1a). A decrease in local sea-surface temperature would increase planktic $\delta^{18}O$ but would not affect the benthic $\delta^{18}O$ record (Fig. 1b). A decrease in bottom-water temperature would increase the benthic $\delta^{18}O$ but would not affect the planktic record (Fig. 1c). The $\delta^{18}O$ effect of these various processes may be estimated at about 0.23 per mil per degree Celsius (*10*), 0.11 per mil per 10 m sea level equivalent (*11*), and 0.11 to 0.29 per mil of salinity (*12*). Hence, the relative amplitude of these processes can be considered in the context of the three conceptual patterns of planktic-benthic $\delta^{18}O$ covariation.

To define the degree and pattern of benthic-planktic $\delta^{18}O$ covariance in the "preglacial" to "glacial" Pliocene, I compared isotopic time series over the interval from 3.6 to 2.8 Ma. This 0.8-million-year interval includes a significant length of record before and after the 3.2-Ma event so that reliable statistics can be calculated to characterize changes in the mean $\delta^{18}O$. Figure 2 compares a representative benthic record (core V28-179) with planktic records from DSDP site 502 (Caribbean Sea), DSDP site 572 (equatorial Pacific), DSDP site 573 (equatorial Pacific), V28-179 (equatorial Pacific), and V20-163 (Indian Ocean) (*13*). All isotopic data were generated in the Benedum Stable Isotope

Laboratory at Brown University according to published procedures (*14*). The planktic foraminifera *Globigerinoides sacculifera-triloba* (300 to 355 μm) was analyzed in cores DSDP 502, DSDP 572, and V20-163. *Globorotalia tumida* (355 to 500 μm) was analyzed in samples V28-179 and in DSDP 573 (*15*). I constructed a time series of the isotope data, using paleomagnetic data (four sites), biostratigraphy, and carbonate stratigraphy (*16*).

An examination of Fig. 2 reveals that the planktic $\delta^{18}O$ records do not exhibit the steplike increase in mean value at 3.2 Ma that the benthic record does. Although the planktic records do show a brief interval (from about 3.3 to 3.15 Ma) of increased $\delta^{18}O$ of as much as 0.5 per mil (the average increase is only about 0.2 per mil), the values do not remain high (Fig. 2). In fact, the intervals from 3.6 to 3.25 Ma and 3.15 to 2.8 Ma have almost exactly the same mean values in most cases (Fig. 2). Similar results, but with lower temporal resolution, have been reported from the South Atlantic at about 30°S (*4*). Hence, in the interval subsequent to 3.2 Ma, the pattern of $\delta^{18}O$ increase is not the same for records reflecting the surface and deep ocean.

Two simple hypotheses may be proposed to explain the apparent paradox between the benthic and planktic data. First, the benthic $\delta^{18}O$ reflects a mean, although variable, increase in ice volume (*7*). This hypothesis requires that the surface waters either permanently warmed about 2°C or become less saline by several parts per mil in order to compensate for the increased $\delta^{18}O$ of the ocean (0.5 per mil). Second, the benthic $\delta^{18}O$ record reflects colder bottom waters (also about 2°C) to account for the observed $\delta^{18}O$ increase. This hypothesis requires that the colder bottom waters must become a permanent feature of the bottom circulation and that the mean ice volume was not increased in the interval subsequent to the 3.2-Ma event. Here I note that the records do covary over the short interval from 3.25 to 3.15 Ma, which suggests that a brief ice growth phase did occur but that the mean increase did not persist.

Unfortunately, independent records of ice volume changes, such as radiometrically dated tills and the terrestrial glacial geology of the Arctic and Antarctic, do not have the resolution and age control to permit one to choose between these hypotheses (*6, 17*). Related studies of plankton biogeography (*18*) and circum-Antarctic sediments (*19*) document a mid-Pliocene cooling of the high latitudes and intensified bottom currents during the mid-Pliocene. Although these data support the cooling of high-latitude surface waters and hence the production of colder bottom waters, they may or may not reflect an increase in ice volume. Thus we return to the paradox of the lack of covariation of the benthic and planktic $\delta^{18}O$ records subsequent to about 3.2 Ma.

The choice between these two hypothesis has important implications for understanding the heat budget of the ocean. Although a combination of these two hypotheses is possible, one is still left with the prospect that either the bottom waters cooled or the low-latitude surface ocean warmed. The widespread distribution of the planktic data (*13*) requires that any low-latitude warming must be global in extent rather than local or regional. I interpret the lack of planktic-benthic $\delta^{18}O$ covariation to indicate that the mean ice volume did not increase subsequent to 3.2 Ma. Rather, the increased benthic isotopic values represent new or increased production of cold bottom waters in the high latitudes. This hypothesis is consistent with available isotopic data and modern patterns of deep circulation. Cooling of high-latitude surface waters and production of cooler deep water seems more plausible than widespread warming of the low-latitude surface ocean, which is required by the "increased ice volume" hypothesis. These data do not disprove the existence of Northern Hemisphere ice sheets prior to 3.2 Ma, but they do indicate that increased ice volume does not adequately account for the isotopic data in the interval from 3.2 to 2.4 Ma.

This study demonstrates the need to combine planktic and benthic $\delta^{18}O$ data in the interpretation of Cenozoic oceans. Each data set provides a separate set of constraints. The high covariance displayed by planktic and benthic $\delta^{18}O$ data in the late Pleistocene is not necessarily a characteristic of the Cenozoic ocean. Hence, the decision on how to partition the effects of temperature and ice volume on $\delta^{18}O$ composition must be evaluated at each interval with both planktic and benthic data at an appropriate resolution.

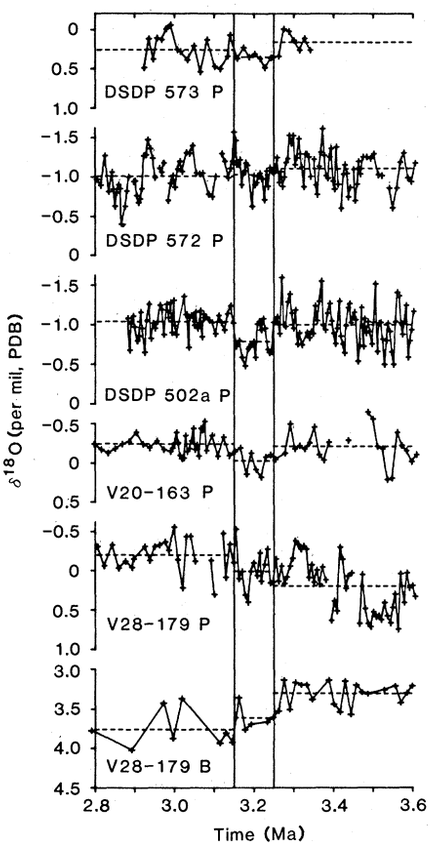

Fig. 2. Time series of $\delta^{18}O$ of benthic (B) and planktic (P) foraminifera over the Pliocene interval from 3.6 to 2.8 Ma. Data and age models are discussed in the text and elsewhere (*9–11*). The mean values for the intervals from 3.6 to 3.25 Ma and from 3.15 to 2.8 Ma are shown by dashed lines and are, respectively, 3.30 and 3.76 per mil (V28-179 B); 0.19 and −0.20 per mil (V28-179 P); −0.21 and −0.24 per mil (V20-163); −1.00 and −1.04 per mil (DSDP 502a); −1.11 and −1.01 per mil (DSDP 572); and 0.16 and 0.26 per mil (DSDP 573).

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9. Note that I refer to the mean $\delta^{18}O$ of intervals rather than to individual data or ranges. Significant natural variability, at a variety of frequency bands, occurs around the mean $\delta^{18}O$ value. Hence, unless sampling density is adequate to define these variations, reference to single values can be misleading.
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13. Locations and water depths of cores in Fig. 2 are as follows: for V28-179, 4°37′N, 139°36′W, 4502 m; for DSDP 502, 11°30′N, 79°23′W, 3051 m; for DSDP 572, 1°26′N, 113°51′W, 3893 m; for DSDP 573, 0°30′N, 133°19′W, 4301 m; and for V20-163, 17°12′S, 88°41′E, 2706 m.
14. Laboratory procedures are given in W. L. Prell and W. B. Curry, *Oceanol. Acta* **4**, 93 (1981). All data are referred to Peedee belemnite (PDB) by the standard δ notation [H. Craig, *Geochim. Cosmochim. Acta* **12**, 133 (1957)]. Calibration to PDB is through three intermediate laboratory standards, which are within 0.2 per mil. Analytical precision (weighted average $\frac{1}{2}\Delta\delta^{18}O$) is based on the number of duplicate analyses for

each core and is 0.10 per mil for $\delta^{18}O$ and 0.10 per mil for $\delta^{13}C$.

15. *Globorotalia tumida* was analyzed for $\delta^{18}O$ in samples V28-179 and DSDP 573 because carbonate dissolution has eliminated shallow-dwelling species such as *G. sacculifer*. C. G. Adelseck and T. F. Anderson [*Geology* **6**, 388 (1978)] showed that $\delta^{18}O$ stratigraphy based on *G. tumida* correlates well with other late Pleistocene isotope stratigraphy.
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Shiga-Like Toxin–Converting Phages from *Escherichia coli* Strains That Cause Hemorrhagic Colitis or Infantile Diarrhea

Abstract. Escherichia coli *K-12 acquired the ability to produce a high titer of Shiga-like toxin after lysogenization by either of two different bacteriophages isolated from a highly toxinogenic* Escherichia coli *0157:H7 strain that causes hemorrhagic colitis. One of these phages and another Shiga-like toxin–converting phage from an* Escherichia coli *026 isolate associated with infantile diarrhea were closely related in terms of morphology, virion polypeptides, DNA restriction fragments, lysogenic immunity, and heat stability, although a difference in host range was noted. These phages are currently the best-characterized representatives from a broader family of Shiga-like toxin–converting phages.*

Scotland *et al.* (*1*) described a toxin-converting bacteriophage released spontaneously from a plasmid-negative *Escherichia coli* K-12 strain that had become toxinogenic after cocultivation with *E. coli* 026 strain H-19. Smith *et al.* (*2*) isolated two different converting phages, H-19A and H-19B, that were released spontaneously from *E. coli* H-19. They also isolated converting phages from several other *E. coli* isolates. *Escherichia coli* H-19, which caused an outbreak of infantile diarrhea in Great Britain more than 15 years ago, is noninvasive and does not produce heat-labile or heat-stable enterotoxin (*3*). It makes large amounts of a cytotoxin that appears to be the same as the *Shigella dysenteriae* 1–like (Shiga-like) toxin (*4*, *5*). Shiga-like toxin is produced in various amounts by some strains of *E. coli* and is defined by its ability to be neutralized by antibodies against purified Shiga toxin (*6*). Shiga-like toxin is cytotoxic for Vero cells and was originally called Vero cell cytotoxin (*1*, *2*, *4*, *5*).

We report that *E. coli* 0157:H7 strain 933, which causes hemorrhagic diarrhea and produces large amounts of Shiga-like toxin, also harbors two different toxin-converting phages designated 933J and 933W. We present morphological, biochemical, and genetic evidence that converting phage 933J from strain 933 is closely related to phage H-19A and provide data indicating that a family of Shiga-like toxin–converting phages exists in *E. coli* strains in nature.

Excherichia coli of the serotype 0157:H7 was recently reported as being a causative agent of hemorrhagic colitis in the United States (*7*) and Canada (*8*) and has been isolated from feces of patients with the hemolytic-uremic syndrome (*9*). It has been suggested that these geographically diverse isolates represent a single, widely dispersed clone of virulent *E. coli* (*10*). Riley *et al.* (*11*) characterized several *E. coli* 0157:H7 strains that were obtained from hamburger and from diarrheal stools of patients during investigation of two food-borne outbreaks of hemorrhagic colitis in the United States. They found that none of the *E. coli* 0157:H7 isolates were enteroinvasive and that none elaborated *E. coli* heat-labile toxin (LT) or *E. coli* heat-stable toxin (ST). The virulence of *E. coli* 0157:H7 for animals was demonstrated by Farmer *et al.* (*12*), who showed that infant rabbits developed diarrhea when fed *E. coli* 0157:H7. The possibility that a toxin other than LT or ST might be involved in the pathogenesis of *E. coli* 0157:H7 diarrheal disease was suggested by Johnson *et al.* (*13*). They reported that Canadian isolates of *E. coli* 0157:H7 made a cytotoxin, which O'Brien *et al.* (*5*) subsequently found could be completely neutralized by antitoxin to purified Shiga toxin. The level of Shiga-like toxin made by three 0157:H7 isolates examined by O'Brien *et al.* was equivalent to that of *Shigella dysenteriae* 1, or 10^5 to 10^6 50 percent cytotoxic doses (CD_{50}) per milliliter of culture supernatant and $\geq 10^6$ CD_{50} per milligram of protein in lysates of bacteria. In a separate study, O'Brien *et al.* (*14*) purified Shiga-like toxin from one of the *E. coli* 0157:H7 strains (designated 933) to homogeneity by the same procedure previously used to purify toxin from *E. coli* 026 strain H30 (*15*). The pure toxins from *E. coli* 0157:H7 strain 933, *E. coli* 026 strain H30, and *Shigella dysenteriae* 1 strain 60R had the same subunit structure and the same biological activities (*14*).

We developed methods to induce phages from *E. coli* strains H-19 and 933 and to optimize conditions for performing plaque assays for the phages. Bacterial cultures (5 ml) were grown to an optical density of 0.5 (wavelength, 600 nm) in LB broth (*16*) prepared with half the usual amount of NaCl and supplemented with 10 m*M* $CaCl_2$ and 0.001 percent thiamine. Cells were then harvested by centrifugation, resuspended in 5-ml samples of 10 m*M* $CaCl_2$, and irradiated (40 J/m^2) in 100-mm glass petri dishes. The irradiated bacteria were diluted tenfold in modified LB broth and then incubated for 5 hours at 37°C in foil-

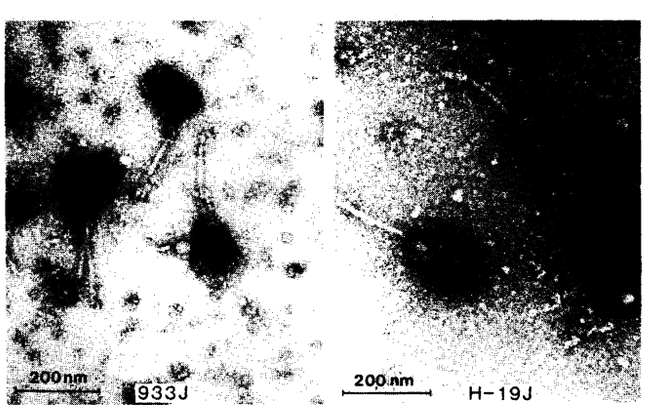

Fig. 1. Electron micrographs of plaque-purified H-19J and 933J phages negatively stained with uranyl acetate (*p*H 4.5).