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Seasonal distribution of genetic types of planktonic foraminifer morphospecies in the Santa Barbara Channel and its paleoceanographic implications

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Kate F. Darling,[1,2] Michal Kucera,[3] Christopher M. Wade,[4] Peter von Langen,[5] and Dorothy Pak[5]

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[1] We present data on the temporal distribution of planktonic foraminifer genotypes (small subunit (SSU) ribosomal (r) RNA gene) and morphospecies (sediment traps) collected during 1999 in the Santa Barbara Channel. The sampling was undertaken with special emphasis on paleoceanographically important morphospecies, predominantly *Globigerina bulloides*. We found the same genotype of *G. bulloides* (type IId) in all the changing hydrographic regimes associated with this region throughout the annual cycle with the exception of January, when we recorded the additional presence of the high-latitude *G. bulloides* type IIa. We identified three new genotypes: *Neogloboquadrina dutertrei* type Ic, *N. pachyderma* dextral type II, and *Turborotalita quinqueloba* type IId. Our data suggest that *G. bulloides* type IId and possibly even the new genotypes listed above may be associated specifically with the complex hydrography or other environmental features characteristic of this area. Since *G. bulloides* type IId occurs throughout the year and its peak fluxes are related to different hydrographic regimes, we argue that the physical properties of the water column are not the major factor influencing the distribution and growth of this genotype. In sediment trap samples we found a skewed coiling ratio for *G. bulloides* (most likely representing type IId), which is related neither to sea surface temperature nor to genotypic difference. This study illustrates the necessity to map both the spatial and temporal distribution of the genetic types, especially in areas of paleoceanographic interest, where geochemical and paleontological proxies are being calibrated. *INDEX TERMS:* 3030 Marine Geology and Geophysics: Micropaleontology; 9355 Information Related to Geographic Region: Pacific Ocean; 4855 Oceanography: Biological and Chemical: Plankton; *KEYWORDS:* planktonic foraminifera, genotypes, paleoceanographic proxies, Santa Barbara Channel

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1. Introduction

[2] Many marine groups with similarly high dispersal potential to the planktonic foraminifera have been shown to include sibling species complexes [*Knowlton*, 1993]. Indeed, recent molecular genetic studies show that there is a considerably greater degree of diversity within the planktonic foraminifera than morphological studies have predicted [*Huber et al.*, 1997; *Darling et al.*, 1999, 2000; *de Vargas et al.*, 1999, 2001]. Many morphospecies represent complexes of different and sometimes highly divergent genotypes, some of which are now considered to be cryptic sibling species [*Huber et al.*, 1997]. Planktonic foraminiferal morphospecies therefore do not represent genetically continuous species throughout their entire environmental range. For example, the higher-latitude morphospecies *Globigerina bulloides* and *Turborotalita quinqueloba* represent multiple genotype clusters, which have specific genotypes confined within the warmer waters of their morphospecies ranges [*Darling et al.*, 2000]. This observation has potentially large implications for the use of planktonic foraminifera as paleoceanographic proxies. Stable isotope and geochemical analyses of planktonic foraminiferal shells and census-based transfer function techniques derived from pooled morphospecies must include significant noise, if not error [*Darling et al.*, 2000]. Therefore it is imperative to understand the nature and adaptations of these genotypes and their spatial and temporal distribution in the ocean.

[3] Genetic diversity among planktonic foraminifera has so far been studied only in temporally discrete samples. Yet to be able to assess the biological significance of these genotypes, we need to understand their behavior in the ocean. The oceanic water masses constitute complex dynamic biological systems, which vary spatially and temporally. Sampling such a dynamic environment is fraught

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with difficulty, as plankton blooms build and decay through quite short periods of time.

[4] Here we report on the first attempt to investigate seasonal dynamics of cryptic species of planktonic foraminifera. The Santa Barbara Channel (SBC) (Figure 1) was selected as an ideal candidate for such a study. The Santa Barbara Channel waters experience high seasonality (in 1999, average daily temperatures ranged between 10° and 18°C), resulting from the varying intensities of the cold California Current and the warmer Davidson Current. We have concentrated on paleoceanographically important species and succeeded in obtaining a representative collection of *G. bulloides* sequences from five sampling intervals throughout 1999. We discuss the genetic character of all collected specimens, the spatial and temporal distribution of individual genotypes and morphospecies, and the paleoceanographic significance of our observations.

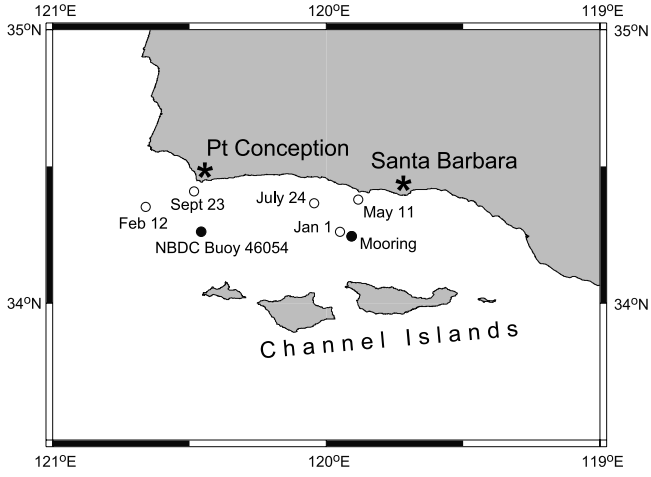

Figure 1. Map of the Santa Barbara Channel showing the exact positions and dates of the five 1999 plankton tow samples used for planktonic foraminiferal genotyping. Also shown are the position of the University of California Santa Barbara mooring where the sediment trap data were collected and the position of the National Data Buoy Center Buoy 46054 from which sea surface temperature records were extracted.

2. Methods

2.1. Oceanographic Setting of Santa Barbara Channel

[5] The SBC extends for ~100 km from its east entrance near Point Hueneme to its west entrance at Point Conception, where the California coastline takes a dramatic bend to the north. The channel is ~50 km wide, and the four Northern Channel Islands delineate its southern boundary (Figure 1). The SBC is a region of oceanic transition characterized by mixing of cold and fresh waters, derived from the California Current and the regional upwelling, with the warmer, saltier waters of the Davidson Current flowing from the Southern California Bight. The large-scale flow is generally equatorward during the spring and poleward summer through winter, although reversals for several days occur [*Hendershott and Winant*, 1996]. Strong northwest winds result in intense upwelling off Point Conception (Figure 1) that occurs year round, although it is predominant during spring and summer. Highly productive waters resultant from the upwelling flow along the SBC southern boundary and reach the Southern California Bight through the eastern SBC entrance [*Hendershott and Winant*, 1996]. Data on average sea surface temperature (SST) (1 m beneath the surface) during 1998 and 1999 were obtained from continuous monitoring by the West Santa Barbara Channel Buoy 46054 (34°16′08″N, 120°26′54″W) (Figure 1), maintained by the National Data Buoy Center (NDBC).

2.2. Planktonic Foraminiferal Collection

2.2.1. Santa Barbara Basin

[6] Living planktonic foraminifera were collected on five occasions in 1999 from sites along the northern coastline and central region of the SBC (Figure 1). To ensure that our collections were representative of the entire water column population of planktonic foraminifera, integrated plankton tows (mesh size 0.063 mm) were taken between the surface and the approximate level of the thermocline (10–50 m). This was determined on board using a temperature depth profiler. Features and accuracy of hand-deployed temperature profiles were verified on several occasions by comparison with simultaneous conductivity-temperature-depth casts.

[7] The plankton sample was diluted with surface seawater and the mixture kept in a cooler at approximately the collection temperature by the addition or removal of blue ice. Specimens of planktonic foraminifera with visible cytoplasm were picked using a stereo microscope and cleaned in filtered seawater. Each specimen was then transferred into a lysis buffer solution on the tip of a picking brush,was crushed, and was incubated at 60°C for 1 hour [*Holzman and Pawlowski*, 1996; *Darling et al.*, 1999].

2.2.2. Coral Sea

[8] To obtain a better perspective on the molecular phylogeny and geographical distribution within the Pacific Ocean of the *Neogloboquadrina* clade, we have sequenced new material from the Great Barrier Reef (GBR) off Queensland, Australia. *N. dutertrei* (d'Orbigny) and *Pulleniatina obliquiloculata* (Parker and Jones) were collected in September 1997, 0.8 nautical miles (1.48 km) due east of Ribbon Reef 10 in the Cairns section of the GBR (14°39′S, 145°42′E). Specimens were collected by drift net (mesh size 0.075 mm) at depths between 5 and 50 m. Specimens were identified and processed onshore at Lizard Island Research Station, located in the Cairns section of the GBR. Taxonomic identification was confirmed using a stereomicroscope, and specimens were crushed in a lysis buffer as described in section 2.2.1.

2.3. Sediment Traps

[9] To document the temporal variation in fluxes of planktonic foraminifer morphospecies in the SBC, we have used sediment trap samples, which were collected between 1998 and June 1999 as a part of the University of California Santa Barbara-Santa Barbara Basin Mooring Project (1995–1999). The mooring was located in the eastern part of the central portion of the basin (34°15.33′N, 119°56.29′W) (Figure 1). A Parflux Mark 7, GW-13, cone-shaped, baffled sediment trap was deployed at 470 m

depth, 50 m above the sediment. The trap was equipped with a 13 cup sample carousel, which was programmed to rotate every 10 to 14 days. A trap failure led to a gap in the time series from May to July 1998.

[10] Sediment trap samples were preserved in 4% borate buffered formalin (pH 8.2) and were refrigerated after collection. Planktonic foraminiferal shells were picked wet from splits of each of the sample cups recovered. Only specimens larger than 0.125 mm were identified by morphospecies and counted. Counts are reported as shell flux (number of shells m^{-2} d^{-1}) and relative abundance. Coiling ratios for *G. bulloides* were determined in all samples from 1999. We calculated 95% confidence limits for all samples with >15 specimens.

2.4. Molecular Methods

2.4.1. DNA Extraction, Amplification, and Sequencing of the Small Subunit Ribosomal RNA Gene

[11] DNA was extracted from the Santa Barbara specimens as described previously by *Darling et al.* [1996, 1999]. We have examined an ~1000 base pair (bp) region at the terminal end of the small subunit (SSU) ribosomal (r) RNA gene. This gene encodes one of the RNA subunits of the ribosome, the site of protein synthesis within the cell. The target fragment was amplified using the polymerase chain reaction (PCR) and sequenced using a direct automated sequencing procedure using fluorescent dyes (Applied Biosystems "Big Dye" cycle sequencing). PCR primers were as described by *Darling et al.* [1996, 1997]. A full ~1000 bp sequence was obtained for each genotype with the exception of Santa Barbara *N. dutertrei* type Ic and *T. quinqueloba* type IId. In these cases, only a 5′ 500 bp fragment was successfully amplified. Subsequent characterization of individual specimens was carried out by sequencing 500 bp duplicates. In each case, the genetic types were identical across the entire sequenced fragment.

2.4.2. Sequence Analysis

[12] The amplified sequences were aligned manually within the Genetic Data Environment package [*Smith et al.*, 1994]. Of the ~1000 bp sequenced, only 506 sites were sufficiently conserved to permit alignment across all foraminiferal taxa. However, when closely related groups are considered alone, additional sites can be recruited into analyses. This enabled us to utilize 729 sites in analyses for *G. bulloides*, 781 sites for *T. quinqueloba*, and 686 sites for the *Neogloboquadrina* clade. Phylogenetic trees were constructed using distance-based (neighbor-joining and Fitch-Margoliash), maximum likelihood, and parsimony methods in the PAUP* package [*Swofford*, 2002]. Distance and maximum likelihood trees were constructed using the general time reversible model with gamma correction to account for unequal rates across sites. This allows undetectable changes in the DNA sequence (due to reverse or multiple mutations) to be taken into account. All construction methods showed similar topology. Neighbor-joining trees are shown in this study, with bootstrap resampling (1000 replicates) [*Felsenstein*, 1985] employed to assign support for particular nodes. The planktonic foraminiferal SSU rDNA sequences presented in this study are deposited in the GenBank database with the accession numbers AY241707, AY241708, AY241709, AY241710, AY241711, AY241712, and AY241713.

3. Results

3.1. Sediment Traps

[13] Planktonic foraminifer fluxes in the Santa Barbara Channel are influenced both by interannual variations in the California Current System related to the El Niño Southern Oscillation (ENSO) effect and by the seasonal cycle of sea surface temperature, thermocline depth, and upwelling intensity. *Kincaid et al.* [2000] studied the export flux of planktonic foraminifera in the SBC between August 1993 and December 1996, with an interruption between May 1995 and March 1996. They observed that the ENSO-related pattern is mainly manifested by decreased total flux in the year 1995, which followed a strong ENSO event, while the seasonal variation in total flux seems to involve a peak export flux occurring consistently in September and a spring peak ranging between March and May. These two main flux peaks largely reflect the dynamics of *G. bulloides*, which is the dominant species for most of the year, except for April–August, when *T. quinqueloba* makes up well over 50% of the total assemblage [*Kincaid et al.*, 2000]. Both *N. pachyderma* dextral (DEX) and *N. dutertrei* showed the highest fluxes and the highest relative abundance during the late summer and autumn months [*Kincaid et al.*, 2000].

[14] Our sediment trap data show a fourfold decrease in average total flux between 1998 and 1999 (Figure 2). This can be explained by the fact that 1998 was a strong ENSO year. Despite the overall decrease in total flux our data show a similar pattern as documented by *Kincaid et al.* [2000]. We observed two seasonal peaks driven by *G. bulloides*, a late spring–summer dominance of *T. quinqueloba*, and the highest fluxes and highest relative abundance of *N. pachyderma* DEX and *N. dutertrei* during the late summer and autumn months.

[15] From the hydrographic point of view it could be argued that the spring peak flux of *G. bulloides* is related to the onset in spring of regional upwelling off Point Conception [*Hendershott and Winant*, 1996]. The upwelling continues until early summer, and during this period, *T. quinqueloba* dominates. In July the SST increases, and a thermocline develops, resulting in higher fluxes of *N. pachyderma* DEX and *N. dutertrei*, which are both considered thermocline species [*Hemleben et al.*, 1989]. Then, in September, another export flux peak of *G. bulloides* occurs following a period with the highest SST and well-established thermocline in late August.

3.2. Genetic Characterization of the Santa Barbara Channel Planktonic Foraminifer Morphospecies

3.2.1. *Globigerina bulloides* Lineage

[16] The evolutionary relationships within the *G. bulloides* lineage are shown in Figure 3. The phylogeny is rooted on the subtropical *G. bulloides* type I genotypes, thus providing a direction of evolution within the type II cluster. These genotypes have been shown to consistently fall basal within the *G. bulloides* cluster in foraminiferal phylogenetic trees [*Darling et al.*, 2000], indicating an earlier divergence than those within the type II cluster.

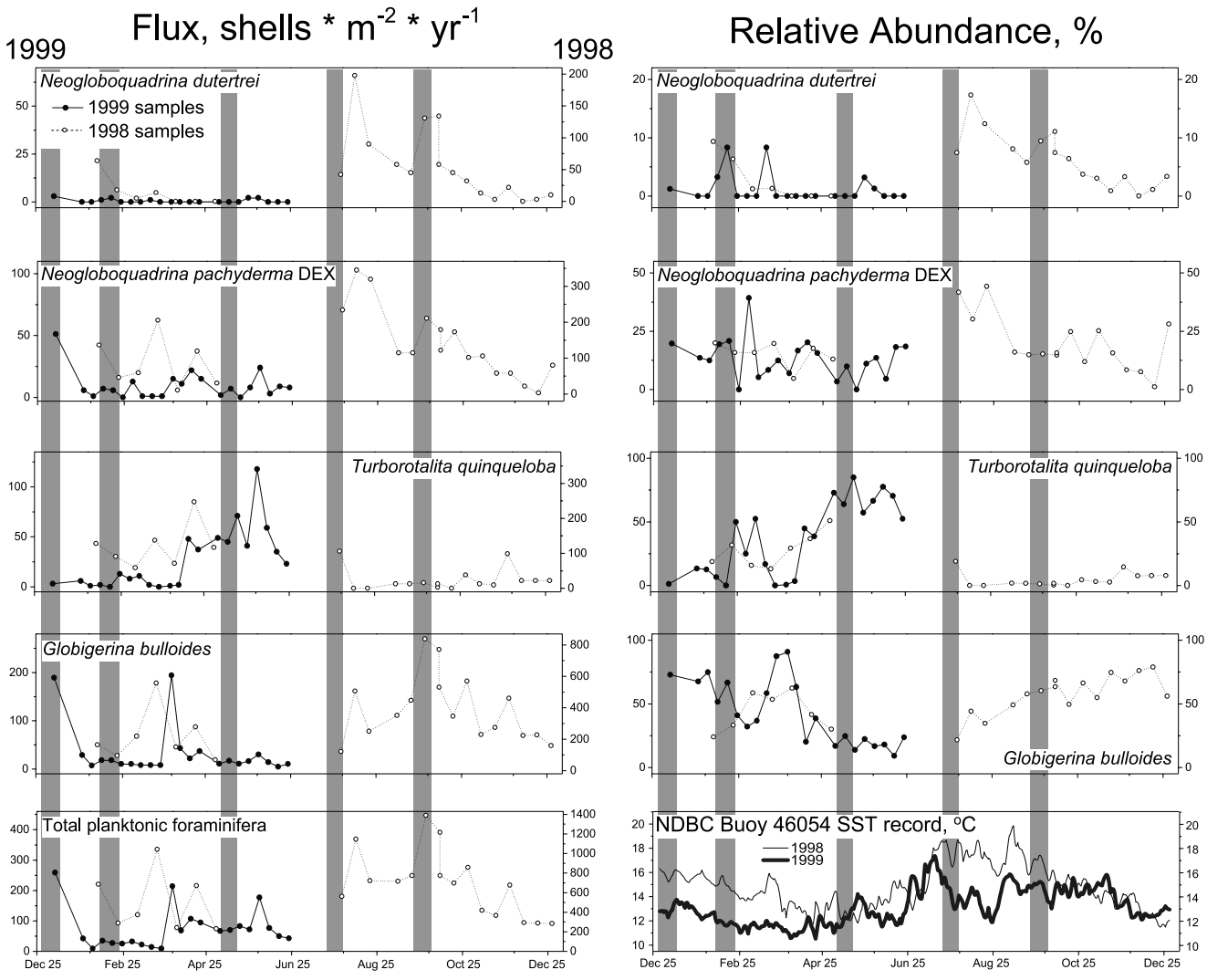

Figure 2. (left) Sediment trap data on fluxes and (top right) relative abundance of planktonic foraminifer morphospecies in the Santa Barbara Channel between January 1998 and June 1999. The gap in data in 1998 was caused by trap failure; the mooring project was finished at the end of June 1999. Note the different scales for flux data for 1999 (left) and 1998 (right). Shaded vertical bands in the right and left panels denote the five sampling intervals used for planktonic foraminiferal genotyping. (bottom right) Daily average sea surface temperature (SST) during 1998 and 1999 as recorded by the West Santa Barbara Channel Buoy (NDBC 46054 in Figure 1).

[17] There are four clear genetic types in the type II cluster (types IIa–IId). To date, type IIa has been found in both the subpolar Arctic and subpolar Antarctic [*Darling et al.*, 2000; *Stewart et al.*, 2001] and now in the SBC. Type IIb has also been found in both the subpolar Arctic and subpolar Antarctic [*Darling et al.*, 2000; *Stewart et al.*, 2001] and type IIc in the subpolar Antarctic alone [*Darling et al.*, 2000]. Finally, type IId has been found in the transitional waters of the Southern California Bight [*Darling et al.*, 1999] and now specifically in the SBC.

[18] With all methods of tree construction, types IIb and IId clearly diverge earlier than types IIc and IIa (84% bootstrap support). The relative branching order of types IIb and IId is not, however, resolved (see asterisk in Figure 3), with their placement being inconsistent among different tree methods.

3.2.2. *Turborotalita quinqueloba* Lineage

[19] The evolutionary relationships within the *T. quinqueloba* lineage are shown in Figure 4. The phylogeny is rooted on the subtropical *T. quinqueloba* type I genotype from the Coral Sea. As for *G. bulloides*, type I has been shown to consistently fall basal within the *T. quinqueloba* cluster in foraminiferal phylogenetic trees [*Darling et al.*, 2000], indicating an earlier divergence than those within the type II cluster.

[20] In the type II cluster, there are four clear genetic types (types IIa–IId). To date, type IIa has been found in both the subpolar Arctic and subpolar Antarctic and type IIb in the subpolar Arctic alone [*Darling et al.*, 2000; *Stewart et al.*, 2001]. Type IIc has been found in the subpolar Antarctic [*Darling et al.*, 2000] and now in the SBC together with a new *T. quinqueloba* genotype, type IId.

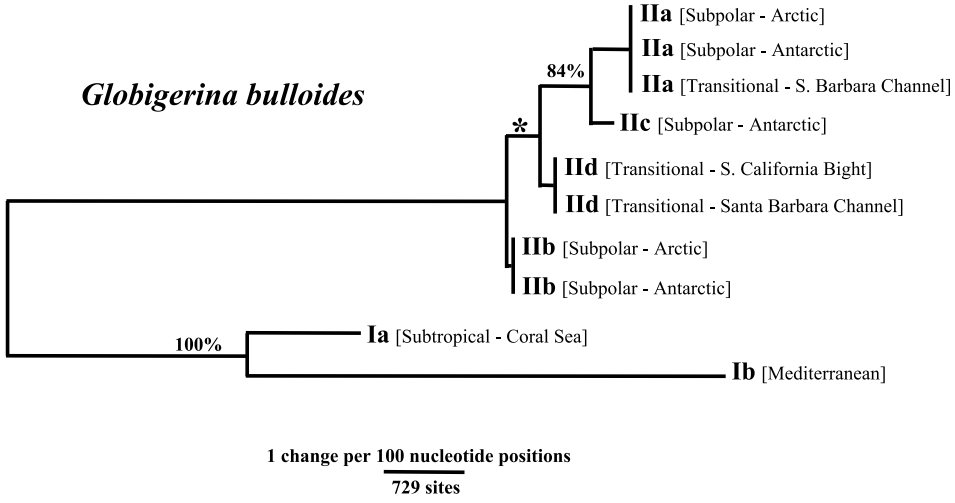

Figure 3. Neighbor-joining small subunit (SSU) ribosomal (r) DNA phylogenetic tree based on 729 nucleotide sites showing the relationship among the genetic types of the morphospecies *Globigerina bulloides*. The tree is rooted on the subtropical *G. bulloides* type I genotypes. Bootstrap values are expressed as a percentage and reflect the degree of support for a particular branch within the tree; only bootstrap values above 70% are shown. Type Ib (Mediterranean) sequence is from *de Vargas et al.* [1997].

[21] The *T. quinqueloba* genotypes, types IIb–IId, fall in a clear group. This is resolved with all methods of tree construction and strongly supported in bootstrap analyses (99% in the neighbor-joining tree). The type IId sequence was incomplete, and its placement within this group was determined from a tree based on analysis of 361 sites. The evolutionary relationships within this group remain unresolved. Types IIb and IIc do, however, show clear genetic differences in the variable regions that cannot be aligned for use in phylogenetic analysis. The *T. quinqueloba* type IIa genotype clearly falls outside of this group.

3.2.3. *Neogloboquadrina* Lineage

[22] The evolutionary relationships within the *Neogloboquadrina* lineage are shown in Figure 5. The phylogeny is rooted on *Globorotalia inflata* [*de Vargas et al.*, 1997], which shared a common ancestor with the *Neogloboquadrina* lineage in the late Oligocene (25–23 Ma) [*Kennett and Srinivasan*, 1983]. The *G. inflata* sequence was chosen as an out-group, as it has the maximum number of complementary nucleotide sites to the neogloboquadrinids, thus providing better resolution in the in-group.

[23] *N. pachyderma* DEX is genetically highly divergent from the other members of the neogloboquadrinid cluster, which effectively reduces the number of alignable sites available for phylogenetic analysis. Therefore only 686 sites could be utilized in the construction of the neogloboquadrinid tree. The *N. pachyderma* DEX genotypes include type I (both subpolar Arctic and subpolar Antarctic)

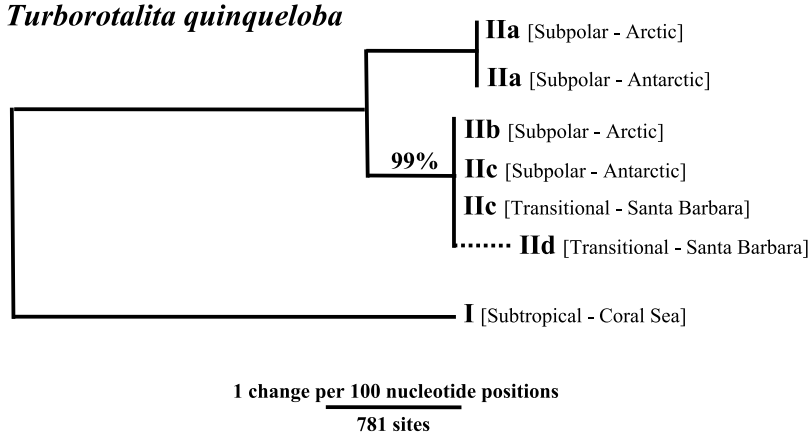

Figure 4. Neighbor-joining SSU rDNA phylogenetic tree based on 781 nucleotide sites showing the relationship among the genetic types of the morphospecies *T. quinqueloba*. The tree is rooted on the subtropical *T. quinqueloba* type I genotype. Bootstrap values are expressed as a percentage and reflect the degree of support for a particular branch within the tree; only bootstrap values above 70% are shown. The new type IId sequence was incomplete (bottom line), and its placement within this group (dotted line) was determined from a tree based on analysis of 361 sites.

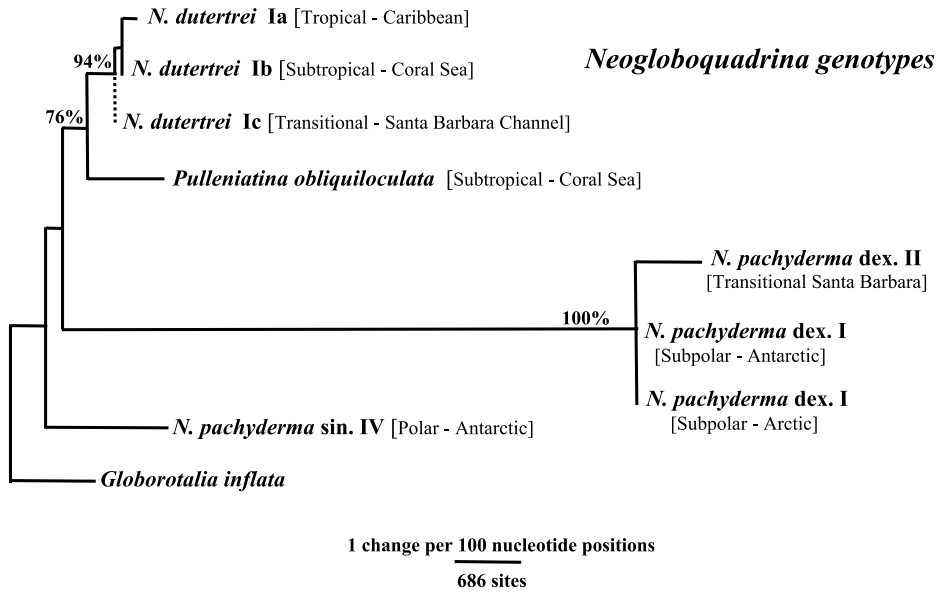

Figure 5. Neighbor-joining SSU rDNA phylogenetic tree based on 686 nucleotide sites showing the relationship among the genetic types within the neogloboquadrinid clade. The phylogeny is rooted on *Globorotalia inflata* (sequence from *de Vargas et al.* [1997]), which shared a common ancestor with the *Neogloboquadrina* lineage in the late Oligocene. Bootstrap values are expressed as a percentage and reflect the degree of support for a particular branch within the tree; only bootstrap values above 70% are shown. The new *N. dutertrei* type Ic sequence was incomplete (dotted line), and its placement within this group was determined from a tree based on analysis of 362 sites.

[*Darling et al.*, 2000; *Stewart et al.*, 2001] and a new type II from the Santa Barbara Channel. They cluster together (100% bootstrap support) and are so divergent from the other taxa in the tree that their placement within the phylogeny is problematic and their relationship to the other taxa remains uncertain.

[24] *N. dutertrei* forms a clear group in the molecular phylogeny, which is resolved with all methods of tree construction and supported in 94% of bootstrap replicates in the neighbor-joining tree. It includes three genotypes: type Ia (tropical, Caribbean), type Ib (subtropical, Coral Sea), and a new type Ic from the Santa Barbara Channel. Although the type Ic sequence was incomplete, its placement within the group was determined from a tree based on analysis of 362 sites. The three *N. dutertrei* genotypes cluster together with *P. obliquiloculata*, with a relatively high bootstrap support (76%).

3.2.4. Rare Species

[25] Apart from the four dominant species described above we have also sequenced specimens of *Globigerinella siphonifera* types IIa and IIb. Both types were found previously in the Southern California Bight [*Darling et al.*, 1999] and are also known to occur in the subtropical Atlantic [*Stewart*, 2000]. One specimen of *Globigerinoides ruber* was sequenced and confirmed as the ''California type'' previously described from the Southern California Bight [*Darling et al.*, 1999]. It is also known to occur in the subtropical Atlantic [*Stewart*, 2000]. In addition, we have confirmed the genetic identity of one specimen as *Globigerinita glutinata* and four specimens as *G. uvula* by comparison against sequences from *Darling et al.* [1999] and *Stewart et al.* [2001].

3.3. Distribution

[26] This study confirms that four of the genotypes of planktonic foraminifer species (*G. bulloides* type IId, *G. ruber* California type, and *G. siphonifera* types IIa and IIb) collected within the Southern California Bight off Santa Catalina Island in 1997 [*Darling et al.*, 1999] were still present in the California Current System in 1999. This suggests that these genotypes are a persistent component of the planktonic foraminifer assemblage in this area.

[27] The seasonal distribution of genotyped specimens of planktonic foraminifera from the SBC is shown in Figure 6. It is important to note that the number of the genotyped specimens does not reflect the true population structure in the water column. This is recorded, with an appropriate time lag, in the sediment trap data (Figure 2). Our data set allows us for the first time to assess the seasonal dynamics of genotypes of the planktonic foraminifer morphospecies *G. bulloides*. Specimens of this morphospecies were genotyped in samples from all five sampling intervals, and we can confirm that *G. bulloides* type IId occurs in the SBC throughout the year. In addition, in the sample from January 1999 we have identified a single specimen of *G. bulloides* type IIa. This genotype has previously been found in both the subpolar Atlantic Arctic and the subpolar Antarctic [*Darling et al.*, 2000; *Stewart et al.*, 2001] and in the the Canary Current system [*Stewart*, 2000]. This confirms that type IIa is also present in the North Pacific.

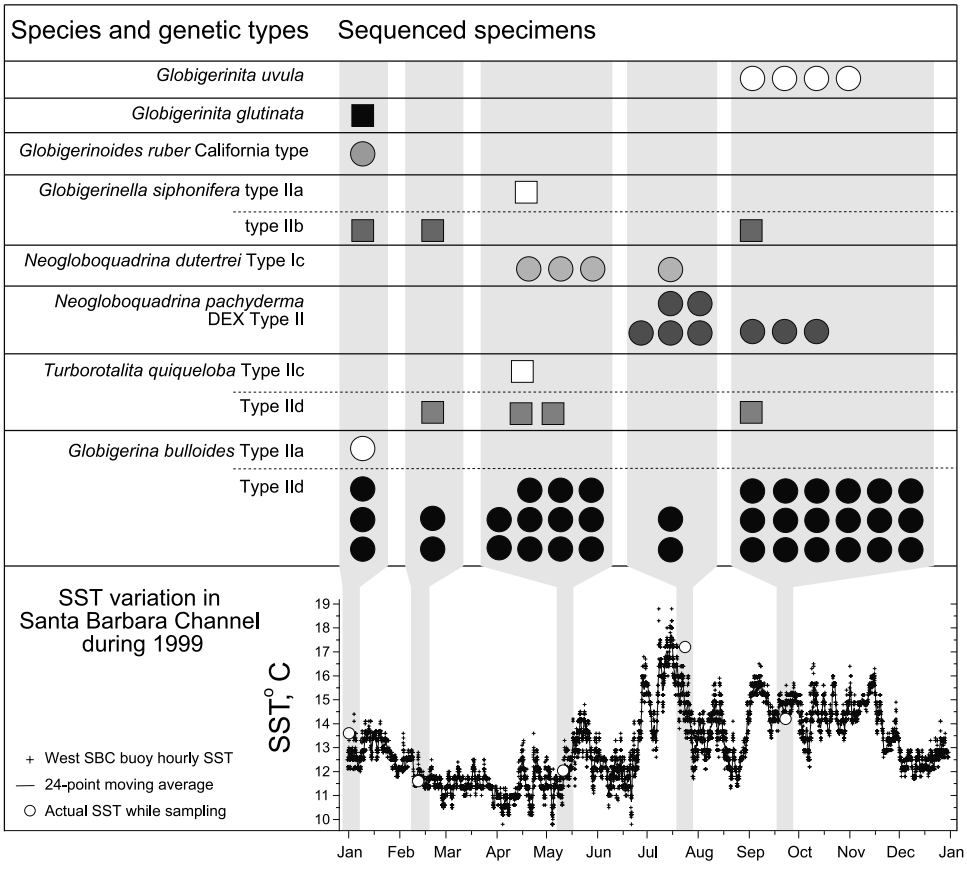

Figure 6. Seasonal distribution of the planktonic foraminifer genotypes collected during five sampling intervals (denoted by vertical shaded areas) in 1999 in the Santa Barbara Channel (SBC). Each symbol represents one specimen; the shading and form of the symbols represent different genotypes. Within each genotype, complete genetic identity throughout the sequenced fragments (usually 500 bp) was observed. (bottom) Hourly SST variation in the SBC during 1999 as recorded by the Mid SBC Buoy (Figure 1). The data were smoothed by a 24 point running average. Also shown are actual measurements of SST taken on board the collection vessel.

[28] In addition, specimens of *G. siphonifera* type IIb and *T. quinqueloba* type IId were identified in samples from three sampling intervals. Although the sampling density is much lower than for *G. bulloides*, the repeated occurrence of these genetic types suggests that they may occur throughout the year also. Our sediment trap data indicate the relatively low abundances of both *N. dutertrei* and *N. pachyderma* DEX in the early part of the year (Figure 2), which reflects the low number of genotyped specimens in our data sets. For both species we have genotyped specimens from two sampling intervals. All specimens of each morphospecies were of the same genotype, but the sampling density is too low to draw any conclusions about the seasonal dynamics of these species in the SBC.

3.4. Coiling Direction of *Globigerina bulloides*

[29] Coiling ratios for *G. bulloides* in all sediment trap samples from 1999 and for the total count of all these samples are shown in Figure 7. Overall, the coiling direction is significantly skewed toward sinistral coiling (63%, N = 441, 21 samples). The coiling ratio was >50% sinistral in 15 out of 21 samples (N between 4 and 170), and in the 6 samples where the coiling ratio was <50% sinistral the number of specimens was consistently very low ($N < 16$). Among the 36 genotyped specimens of *G. bulloides* type IId, 19 were sinistral and 17 dextral. This reflects the fact that we have tried to achieve an equal representation of both coiling types when collecting specimens for DNA analysis. Although the coiling ratio among the genotyped specimens cannot be expected to reflect the coiling ratio of the actual populations, it clearly shows that both coiling varieties of *G. bulloides* type IId are genetically identical with respect to the analyzed SSU rDNA fragment.

4. Discussion and Conclusions

4.1. Genetic Identity and Evolution

[30] Constructing SSU rDNA genetic trees from the highly divergent planktonic foraminiferal genotypes severely reduces the number of nucleotide sites that can be used for phylogenetic analyses (~505 sites of the ~1000 bp in the DNA fragment analyzed). Therefore, to be able to recruit more of the variable sites for analysis, we have constructed genetic trees for individual morphospecies or

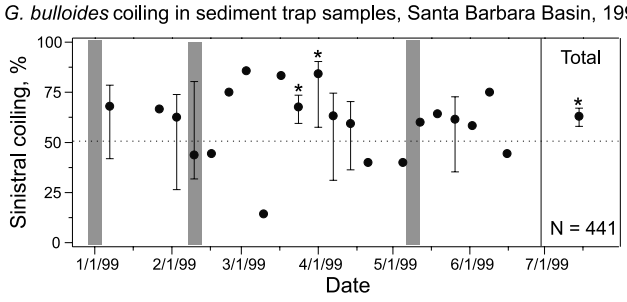

Figure 7. Percentages of sinistral *G. bulloides* in sediment trap samples taken during January–June 1999. The SST during the first half of 1999 varied between 10° and 16°C (Figure 6). The percentages are based on a total of 441 specimens; 95% confidence intervals are shown for all samples with more than 15 specimens. Asterisks indicate samples where the coiling ratio is significantly nonrandom. The total count is significantly skewed toward sinistral coiling ($p < 0.05$). Shaded vertical areas indicate the three sampling intervals during the first half of 1999.

genera (Figures 3–5). This increases the resolution among the different genotypes, allowing us to speculate on the order of their evolution.

[31] The *G. bulloides* tree highlights the large genetic distance between the cool and warm water genotypes, indicating that the divergence of the warm water taxa must have occurred much earlier than the divergence of the individual cool water genotypes. Within the cool water cluster, types IIb and IId clearly diverged earlier than types IIa and IIc. The latter form a well-defined derived cluster with a high bootstrap support. The branching order of types IId and IIb remains unresolved with all tree methods, perhaps indicating that they diverged in quick succession. Given the evolutionary distances within the *Globigerina* cluster [*Darling et al.*, 2000] and the relative antiquity of the divergence between the cool water and warm water clades, it is possible to speculate that the divergences within the cool water cluster occurred relatively recently, perhaps within the Quaternary. The *T. quinqueloba* tree (Figure 4) shows a similar pattern with later divergences within the cool water cluster [*Darling et al.*, 2000]. It could be that the late divergences within the cool water clusters are possible cryptic speciation events triggered by the changing hydrographic regimes of the Quaternary period.

[32] This hypothesis could be tested by calibrating the molecular evolution rates within the *Globigerina* clade. However, lineages within this clade are clearly evolving much faster than those in other parts of the tree [*Darling et al.* 2000], and it would therefore appear inappropriate to use the previously calculated substitution rates, such as those derived from the *Orbulina*/*Globigerinoides* clusters [*Darling et al.* 1999]. Since all divergences within the *G. bulloides*/*T. quinqueloba* clade are cryptic, we can only use the initial divergence between the two morphospecies for calibration. Using the phylogeny advocated by *Spezzaferri* [1994], it can be determined that the divergence between the two morphospecies occurred in the middle Oligocene at ~28 Ma (first occurrence of *G.* aff. *ottnangiensis*). This date combined with the average pairwise distance among the genotypes included in the *Darling et al.* [2000] tree yields an average substitution rate of 7.8 substitutions $\times 10^{-9}$ site^{-1} y^{-1}. Although there is an apparent difference in substitution rates as reflected in the lengths of the *G. bulloides* and *T. quinqueloba* branches [*Darling et al.*, 2000], applying the average rate to both lineages is as of now the only way to estimate ages of the cryptic divergences. Even with these inherent errors the results appear to support our hypothesis. The estimated age of the cold/warm divergence in the *G. bulloides* clade is 5.8 Ma, and in the *T. quinqueloba* clade it is 3.7 Ma, while the estimate of the first divergence within the cold water clade in *G. bulloides* is 0.7 Ma and in *T. quinqueloba* is 0.4 Ma. The absolute errors of these dates are impossible to estimate, but even very large errors would still place the cold water clade radiations well within the Quaternary.

[33] In the neogloboquadrinid tree the three *N. dutertrei* genotypes cluster together with *P. obliquiloculata*. The shared ancestry of both species is supported by the fossil record; the divergence from the common ancestor, *N. acostaensis*, occurred between 10 and 6.2 Ma [*Kennett and Srinivasan*, 1983; *Chaisson and Pearson*, 1997]. While some of the SBC genotypes of *G. bulloides* and *T. quinqueloba* also occur in the Atlantic, the new SBC genotypes of *N. dutertrei* and *N. pachyderma* DEX have not been encountered before. This confirms the suggestion by *Darling et al.* [2000] that there is higher genetic diversity than already recognized within these morphospecies, and it is likely that even more will be discovered as new areas of the world ocean are investigated.

4.2. Geographical Distribution of Genetic Types

[34] Although it is too early to speculate in detail on their geographical distribution, it is interesting to note that four of the six genotypes of the most abundant morphospecies in the SBC have, as yet, only been identified in the California Current System (*G. bulloides* IId, *T. quinqueloba* IId, *N. dutertrei* Ic, and *N. pachyderma* DEX II). In the SBC, *G. bulloides* IId clearly grows and reproduces in SST between 10° and 19°C (Figure 6) in upwelling and nonupwelling regimes and when the thermocline is well developed or absent. Even though it is able to tolerate this wide range of hydrographic regimes, it has to date not been found in the Atlantic Ocean, where equivalent regimes exist [*Stewart*, 2000; *Kucera and Darling*, 2002]. A transect from the United Kingdom to the Azores and the Canary Islands did not reveal the presence of this genetic type, although types IIa, IIb, and Ib were found to occur there. It was found neither in the subpolar North Atlantic [*Darling et al.*, 2000; *Stewart et al.*, 2001] nor in the subpolar South Atlantic [*Darling et al.*, 2000]. Given their high potential for dispersal [*Darling et al.*, 2000] and the fact that there are at least six distinct genotypes of *G. bulloides* worldwide [*Kucera and Darling*, 2002], one can argue that type IId may eventually prove to be endemic to the Pacific.

[35] Unlike the potential endemic genotypes discussed above, *G. bulloides* IIa occurs in subpolar and transitional waters of both the North and South Atlantic [*Darling et al.*,

2000; *Stewart*, 2000; *Stewart et al.*, 2001]. *Darling et al.* [2000] explained the genetic identity of the bipolar populations of this genotype by its ability to cross the tropical zone. This would also explain its occurrence in the North Pacific, where the genotype would have crossed the equatorial Pacific from the Southern Ocean. The transient appearance of this high-latitude genotype in January can be explained by an incursion of the cool California Current into the SBC.

4.3. Seasonal Dynamics of Genetic Types

[36] The hydrographic regime in the SBC changes dramatically throughout the year, and the assemblage composition of morphospecies of planktonic foraminifera varies accordingly [*Kincaid et al.*, 2000]. An interesting phenomenon occurs with *G. bulloides*, as it has two main peak fluxes (March and September) in the SBC, each occurring at times of very different hydrographic regimes. In this situation one might expect that the two flux peaks would reflect the presence of two different genetic types of this morphospecies, each adapted to the different seasonal hydrographic conditions as suggested by *Norris* [2000]. Yet our data surprisingly show that throughout the year the SBC waters were inhabited by the same genetic type IId. Our sampling has covered periods of peak population blooms in the water column and intervals of lower population density of *G. bulloides*. It is important to note that the March peak in export flux of *G. bulloides* as recorded in the sediment trap data actually represents a peak bloom beginning in February. This is because export production of foraminiferal shells occurs on vacation of the shell following reproduction, and our February collection thus should have captured the ''bloom'' specimens or at least their immediate ancestors.

[37] The distribution of present-day morphospecies of planktonic foraminifera has been previously correlated to different hydrographic regimes in the ocean [*Bé*, 1977]. Such patterns can be observed even among the various genetic types [*de Vargas et al.*, 1999, 2001; *Kucera and Darling*, 2002]. However, our data suggest that the physical properties of the water column alone are not the major factor influencing the distribution and growth of *G. bulloides* type IId in the SBC. Perhaps biological interactions are more important for the occurrence in time and space of this genotype [*Verity and Smetacek*, 1996].

[38] Although the data set is clearly not large enough to draw any conclusions, it may be that the transient occurrence in January of *G. bulloides* type IIa, a known cool water dweller [*Kucera and Darling*, 2002], is a seasonally controlled phenomenon. The annual expansion of cold waters in the North Pacific during the northern winter would allow the high-latitude populations of this genetic type to be transported farther to the south.

4.4. Coiling Ratio

[39] Although its exact mechanism was not understood, coiling direction in many species of planktonic foraminifera was traditionally considered to be under environmental control, with coiling ratios regarded as important paleoceanographic proxies [*Ericson*, 1959; *Ericson et al.*, 1954]. Recent genetic data have shown that coiling direction in *N. pachyderma* and possibly also in *Truncorotalia truncatulinoides* is under genetic control [*Darling et al.*, 2000; *de Vargas et al.*, 2001]. There were earlier suggestions that the coiling in *G. bulloides* also responds to environmental parameters [*Boltovskoy*, 1973; *Malmgren and Kennett*, 1976; *Naidu and Malmgren*, 1996]. In all studies, the ratio was found to vary between 50 and 80% sinistral, never showing a preference for dextral coiling. In addition, all of the above studies concluded that the percentage of sinistral specimens increases with decreasing sea surface temperature.

[40] We found that coiling direction in *G. bulloides* IId is not as strongly biased (Figure 7) as it is in *N. pachyderma*, where each genotype has a strongly preferential coiling direction. There are two possible explanations. First, it is still possible that we will find genetic differences within type IId at the population level [see *de Vargas et al.*, 2001], which would split type IIa into two subtypes, each with opposing coiling directions. Second, it could be that each genotype of *G. bulloides* has its own fixed coiling ratio, and the observations by *Boltovskoy* [1973], *Malmgren and Kennett* [1976], and *Naidu and Malmgren* [1996] reflect varying degrees of mixing between these genotypes. Indeed, *Stewart et al.* [2001] reported that out of 32 randomly collected and genotyped *G. bulloides* IIa, 24 (75%, $p < 0.05$) were sinistral. Alternatively, one could still argue that the coiling direction in *G. bulloides* is influenced directly by water temperature. Our sediment trap data are not sufficient to test this hypothesis throughout the full SST range, but they do not seem to support it in any way. During the first half of 1999 the SST in the SBC varied between 10° and 16°C, yet we do not see any significant pattern in the sediment trap coiling ratio counts for this period (Figure 7).

4.5. Implication for Proxies

[41] Our findings on the spatial and temporal distribution of genotypes of the most common planktonic foraminifer species in the SBC have significant implications for the application and development of paleoceanographic proxies. The proxies are based on the assumption that each morphospecies of planktonic foraminifera represents a genetically continuous species with a single environmental/habitat preference. However, the distinct genetic types recognized within a morphospecies have been shown to exhibit different environmental preferences [*Huber et al.*, 1997; *Darling et al.*, 1999, 2000; *de Vargas et al.*, 1999, 2001]. Here we argue that the *G. bulloides* type IId genotype is likely to have a restricted geographical distribution and possibly also different environmental preferences from the other genotypes (types Ia and Ib and types IIa, IIb, and IIc). Therefore there is a distinct possibility that a proxy derived from *G. bulloides* type IId cannot be used on shells of other genotypes of *G. bulloides*. For example, since *G. bulloides* type IId has not been identified yet in high latitudes of the Atlantic Ocean [*Kucera and Darling*, 2002], geochemical and isotopic calibrations based on this genotype may not be valid for *G. bulloides* from this region. Only culturing calibrations based on the different types can unequivocally answer this question.

[42] A further potential complication for the development of foraminifer-based proxies arises from the observed seasonal distribution of the genotypes in SBC (Figure 6). Calibration of geochemical and isotopic proxies from culture experiments relies on the fact that all collected specimens represent the same biological species. While this may be achieved in the SBC for *G. bulloides* type IId for most of the year, a culture collected in January 1999 could potentially contain a significant number of type IIa specimens. For *T. quinqueloba* and *G. siphonifera* the situation may be the same. In 1999, there was a good potential for collecting genetically mixed samples of these morphospecies.

[43] *Sautter and Thunell* [1991] recorded a rare occurrence of an ''encrusted'' morphotype of *G. bulloides* in an April sediment trap collection from the San Pedro Basin. This morphotype showed an isotopic enrichment in ^{18}O and ^{13}C from ''normal'' *G. bulloides* collected during the same month. The stable isotopic values suggested an extreme calcification depth of 250–400 m when the same calibration equation was used for this morphotype as for the ''normal'' *G. bulloides* [*Bemis et al.*, 2002]. A similar ''small, thickly calcified, small aperture'' morphotype of *G. bulloides* has been found to dominate glacial samples in Ocean Drilling Program Core 893 from the SBC [*Hendy and Kennett*, 2000]. This glacial morphotype also shows ^{18}O- and ^{13}C-enriched isotopic values, yielding paleotemperatures colder than those recorded by benthic foraminifera from the same samples [*Bemis et al.*, 2002]. These two facts led *Bemis et al.* [2002] to suggest that the ''encrusted'' morphotype may represent a genetically distinct form of *G. bulloides* that requires a specific calibration equation. It is tempting to suggest that this ''encrusted'' morphotype could, in fact, be the *G. bulloides* type IIa. On the basis of its current distribution in the world ocean [*Kucera and Darling*, 2002] one can certainly predict that the proportion of *G. bulloides* IIa in the SBC was likely much higher during cold periods in the past.

[44] In the fossil record, where only morphospecies of planktonic foraminifera are recognized, the cooccurrence in the same area of different genotypes of a morphospecies could affect the precision of paleoenvironmental reconstructions based on these morphospecies. If it becomes possible to differentiate these genotypes in the fossil record, the amount of information that could be extracted will substantially increase.

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