ECOLOGY

Extensive morphological variability in asexually produced planktic foraminifera

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Marine protists are integral to the structure and function of pelagic ecosystems and marine carbon cycling, with rhizarian biomass alone accounting for more than half of all mesozooplankton in the oligotrophic oceans. Yet, understanding how their environment shapes diversity within species and across taxa is limited by a paucity of observations of heritability and life history. Here, we present observations of asexual reproduction, morphologic plasticity, and ontogeny in the planktic foraminifer *Neogloboquadrina pachyderma* in laboratory culture. Our results demonstrate that planktic foraminifera reproduce both sexually and asexually and demonstrate extensive phenotypic plasticity in response to nonheritable factors. These two processes fundamentally explain the rapid spatial and temporal response of even imperceptibly low populations of planktic foraminifera to optimal conditions and the diversity and ubiquity of these species across the range of environmental conditions that occur in the ocean.

INTRODUCTION

Marine protists are integral members of planktonic communities and important contributors to marine carbon cycling. The protistan clade *Rhizaria*, in particular, plays a major role in the structure and function of pelagic ecosystems. Rhizarian biomass in oligotrophic oceans is estimated to be roughly equivalent to that of all other mesozooplankton (*1*), and they are major players in the biogeochemical cycling of carbon and associated elements. This group, which includes siliceous radiolarians, phaeodarians, Sr-sulfate acantharians, and planktic foraminifera with their calcium carbonate tests ("shells"), is an important component of the biological pump, exporting inorganic and organic carbon from the surface to the deep ocean (*2*).

Despite their importance, limited natural history observations of pelagic rhizarians leave many ecological and evolutionary questions poorly constrained. For instance, the factors promoting, or preventing, diversification across taxa or within a single species are unclear. Two clades of *Rhizaria* (Collodarians and Acantharians) are hyperdiverse with thousands of species, while most rhizarian clades have a genetic diversity of several hundred genotypes (comparable to other micro- to mesoscale eukaryotes) (*3*). The planktic foraminifera are among the relatively nondiverse groups and have remained this way through much of their fossil record, with only ~50 described modern morphospecies and ~250 genotypes (*3*). In addition, rhizarians, like most plankton, exhibit strong seasonality and spatial-temporal patchiness, with populations responding rapidly to favorable conditions for growth. This raises questions as to how disparate populations maintain connectivity from standing stocks that are so low as to preclude efficient sexual reproduction.

Our research focuses on planktic foraminifera, as their shells comprise one of the richest available fossil archives for macroevolutionary studies (*4*, *5*). Paleontologists and paleoceanographers have long linked morphological variation within planktic foraminiferal taxa to environmental factors, supported by repeated observations of plasticity in cultured adult shells (*6*, *7*). However, phylogenetic studies challenge assumptions of ecophenotypy (*9*–*11*). For example, coiling direction in *Neogloboquadrina pachyderma* was once considered ecophenotypic and widely used as a paleothermometer (*12*), but more recent genotyping of *N. pachyderma* revealed that coiling directions are characteristic of genetically distinct species (*10*). Our understanding of the relationship between heritability and morphology is even more complicated, with some genotypes proving morphologically indistinguishable (*11*), such that the role of heritable versus nonheritable factors (like phenotypic plasticity) in producing the array of observed morphologies is enigmatic.

Disentangling heritable versus nonheritable drivers of morphological variation in planktic foraminifera has been further hindered by a lack of life history observations. Planktic foraminifera, brought into culture as adults, have primarily been observed to reproduce sexually through the release of flagellated gametes (*6*, *7*, *13*, *14*). However, gamete release in culture has just once resulted in a second generation (*14*), and only the earliest juvenile states were observed. This leaves major gaps (i.e., diet, microhabitat, life span, and the potential for asexual reproduction) in our understanding of planktic foraminiferal life history and, by extension, potential drivers of ecophenotypic plasticity and speciation pressures during ontogeny.

Here, we present observations of asexual reproduction in cultured *N. pachyderma* and ontogeny and morphology in the resulting second generation. Observation of subadults in culture provides a window into foraminiferal life history and behavior and serves to provide a conservative lower estimate of the degree of phenotypic variability possible in clonal populations of planktic foraminifera. The potential for asexual reproduction and extensive morphological plasticity in planktic foraminifera may explain several outstanding questions about the observed phenology and widespread distribution of the group, as well as the relative lack of diversity in the clade.

RESULTS

Observations of ontogeny in culture

Adult foraminifera were introduced into culture by isolation of single live individuals from plankton tows collected off of the Central

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California coast. Each adult was held at constant temperature (13°C) in an individual flask of filtered seawater exposed to 12-hour light-dark cycles. A single 13-chambered, sinistrally coiled *N. pachyderma* individual, previously filled with colored cytoplasm, was observed empty ~12 hours after introduction into culture (see Materials and Methods). In neogloboquadrinid foraminifera, cytoplasm becomes pale and opaque in a "pregametogenic" period ~1 to 3 days before gamete release, during which rhizopodial activity slows and ceases (*15*, *16*). None of these pregametogenic behaviors nor any gametes were observed. When the shell was noted empty (day 1), several small spheres with typical cytoplasm coloration (red-orange) were present around its outside (Fig. 1). These were determined to be offspring and are referred to as such hereafter.

On day 2, many offspring were still present around the parent shell, with the cytoplasm a lighter orange color (Fig. 1). By day 3, several two-chambered individuals were clearly visible and increasingly motile, propelled by a rhizopodial network (Figs. 1 and 2). In all offspring, the second chamber was smaller than the proloculus (the first "chamber" or stage; Fig. 3), with an average second-chamber diameter ranging between 3 and 8 μm (mean, 6 μm), compared with proloculus diameters between 14 and 21 μm (mean, 18 μm). On day 4, a diatom bloom started in the flask, consisting of *Chaetoceros* spp.

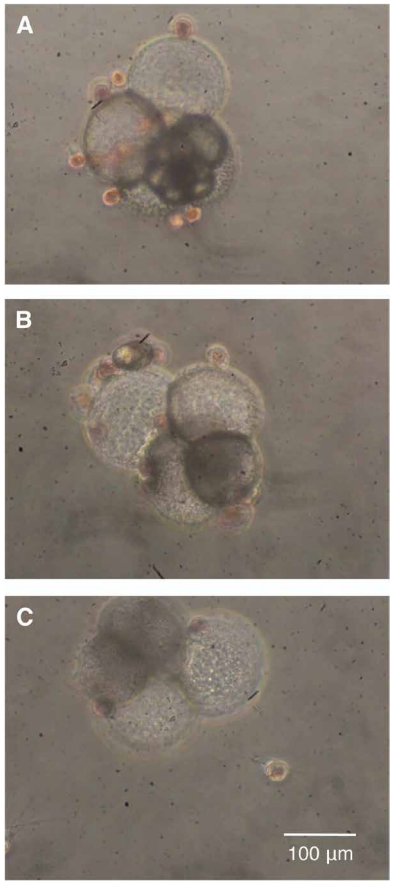

Fig. 1. Images taken of the parent foraminifera and several nearby offspring on the first 3 days that offspring were observed. On day 1 (**A**), offspring appear to be lightly or uncalcified globules, growing into calcified two-chamber forms on day 2 (**B**), with offspring becoming clearly motile with well-developed rhizopodial networks by day 3 (**C**). Scale bar, 100 μm.

and a pennate diatom. At this point, ~1/3 of the offspring were transferred to a 24-hour dark condition, while the remaining 2/3 remained exposed to 12-hour light-dark cycles.

A maximum of 43 offspring were observed in the first 21 days, although the mobility of the offspring and the presence of crevasses and ridges in flasks made accurate accounting difficult. Offspring continued to add chambers for the duration of the experiment, with some mortality at every life stage. After day 27, offspring were commonly observed covered in decaying diatom material (Figs. 2 and 4). Starting on day 66, the shells of some offspring thickened, developing a "crusty" texture typical of the species at maturity, and the cytoplasm became paler. On day 68, at least one offspring underwent gametogenesis, producing free-swimming gametes and leaving the shell empty.

Morphology of parent and offspring

The related species *Neogloboquadrina incompta* (predominantly dextrally coiling) and *N. pachyderma* (predominantly sinistrally coiling) are generally distinguished by shell coiling direction [sensu Darling *et al.* (*10*)]. However, populations of both species contain a small portion (1 to 3%) (*10*) of individuals that coil in the opposite or nondominant direction, and there are no widely accepted morphological criteria for identifying such individuals. At this study's collection site, there is a mixed population of *N. pachyderma* and *N. incompta*, with *N. pachyderma* being the more abundant in late summer, comprising >75% of the August assemblage (*17*). The parent was sinistrally coiled, thus identified as *N. pachyderma*.

Of the second-generation shells, 11 were recovered in adult form, with the longest dimensions between 120 and 181 μm, all with some degree of crusting (Fig. 5). Coiling direction was ascertained in 20 offspring, 13 of which coiled sinistrally (65%). There were differences in coiling direction proportion between the 12-hour light and 24-hour dark treatments (see Materials and Methods): 87.5% were sinistral ($n = 9$) in the 12-hour light treatment (flask "O"), and 50% ($n = 12$) in the 24-hour dark treatment (flask "D") ($P < 0.0001$, by a proportion z test). The number of chambers in the final whorl varied across adults, with six individuals showing a tighter 4-chamber morphology, and five a looser 4.5- to 5-chamber morphology (Fig. 5).

DISCUSSION

Evidence for asexual reproduction in culture

Planktic foraminifera are normally observed to reproduce sexually in culture (*6*, *7*, *13*). In this way, planktic foraminifera have been hypothesized to differ from their benthic relatives, which can alternate between haploid (asexually produced) and diploid (sexually produced) generations [as reviewed in (*18*)]. These observations, in some cases

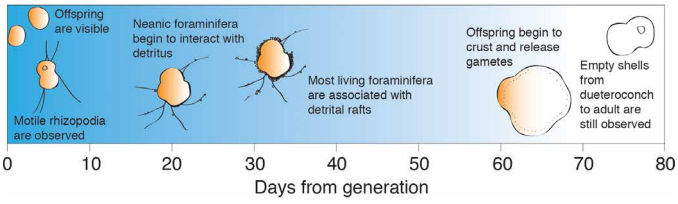

Fig. 2. Schematic of the major events occurring over the observation period. As development progressed at different rates for individuals, the timeline depicted represents observational milestones and general trends but may not be applicable to any given foraminifer.

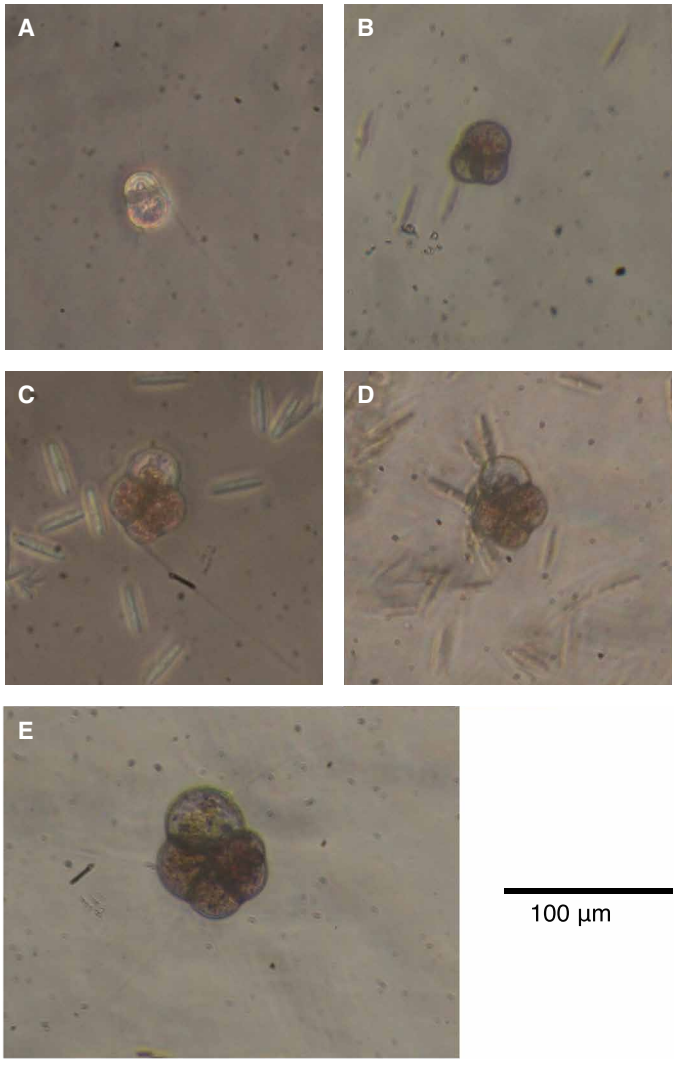

Fig. 3. Chamber growth observed in juvenile foraminifera. The earliest observed calcified stage was two chambers (**A**) after which growth to three (**B**), four (**C**), five (**D**), and six chambers (**E**) was observed. Scale bar, 100 µm.

supported by the absence of bimodality in traits such as the size of the proloculus (*19*), have led several authors to speculate that planktic foraminifera may reproduce exclusively sexually and that evolution of planktic foraminifera from their benthic ancestors may depend on this modification to their life cycle (*7*, *20*).

Our observation of asexual reproduction in *N. pachyderma* allows us to firmly reject the hypothesis that planktic foraminifera reproduce exclusively sexually and supports a single earlier observation of asexual reproduction (*21*). We considered two alternative scenarios for our observations: introduction of gametes or zygotes (propagules) from an outside source and self-fertilization (autogamy). The first process is highly improbable, as the only viable routes of introduction to the cultures are through tow material or filtered seawater. The relatively large mesh (150 µm) used in tows makes the capture of propagules unlikely, although not impossible if entrained in other material. However, in this scenario, over 40 propagules of the same stage would all have had to be transported into a single vial with an adult foraminifer (out of the ~120 individuals isolated from that tow) through two rinses in filtered seawater and a transfer to the culture flask, all without being ingested by the adult or noticed during assessments of viability. The second route for introduction through the filtered seawater intake system is equally improbable. Filtration (0.6 µm) should not regularly allow gametes (1 to 4 µm) to pass. Infiltration by diatoms in a similar size class sometimes occurs, so we cannot entirely discount transport of gametes or zygotes. However, it is routine practice to collect filtered seawater in 2-liter batches distributed across ~26 flasks, once again making it improbable that all propagules would end up in a single flask and be of the same developmental stage.

We also rule out self-fertilization, although it has been reported in benthic foraminifera following gametogenesis [as reviewed in (*18*)]. Self-fertilization, or autogamy, is by definition preceded by gamete release. In our observations, the parent did not exhibit pregametogenic changes, nor were gametes observed. Moreover, self-fertilization in planktic foraminifera has not been documented, despite the frequency with which sexual reproduction is observed.

Observations of asexual reproduction in planktic foraminifera are currently limited to *N. pachyderma*, but there is no reason to consider this species an exception. Rather, observations of asexual reproduction may result from methodological differences between culture of nonspinose species (including *N. pachyderma*) and that of more frequently cultured spinose species. *N. pachyderma* are observed regularly in their culture vessel, usually by inverted microscope, due to their tendency to sink and adhere to the bottom of their culture flask. By contrast, spinose taxa float in culture and are therefore observed in their culture vessel using only a hand lens and transferred into a shallower "viewing chamber" when viewed at higher resolution. The later methodology is not conducive to observing asexual reproduction, as offspring are too small to be readily visible by a hand lens, and asexual reproductive behavior in the parent could potentially be mistaken for death following unobserved gametogenesis. Thus, we argue that the potential for asexual reproduction in planktic foraminifera must be reconsidered more generally.

Planktic foraminifera may be capable of reproducing either sexually or asexually but tend toward sexual reproduction under culture conditions. In some benthic foraminifera, the haploid generation can reproduce either sexually or asexually (*18*, *22*–*24*), and some species display a preference for asexual reproduction when population densities are too low for gamete fusion (*23*). By contrast, other species appear to increase the frequency of sexual reproduction in stressful or unstable environmental conditions (*25*, *26*). Planktic foraminifera are generally cultured in isolation once retrieved by scuba or net tow; thus, a preference for sexual reproduction at high population densities does not explain observations. However, a skew toward sexual over asexual reproduction under suboptimal, stressful, or unnatural conditions, such as in laboratory culture, could explain the dominance of gametogenesis in culture (i.e., up to 90% of individuals reproduce sexually in culture, depending on conditions) (*6*). Thus, we suggest that alternation of generations is facultative and suspect that we may have observed asexual reproduction because the individual in question began to reproduce before capture, with offspring appearing less than 12 hours after introduction to culture.

Ontogeny and feeding in early developmental stages of *N. pachyderma*

Our observations suggest that planktic foraminiferal calcification begins at the two-chamber stage. At first observation, cytoplasm appeared dark and unobscured by a shell, and no empty single

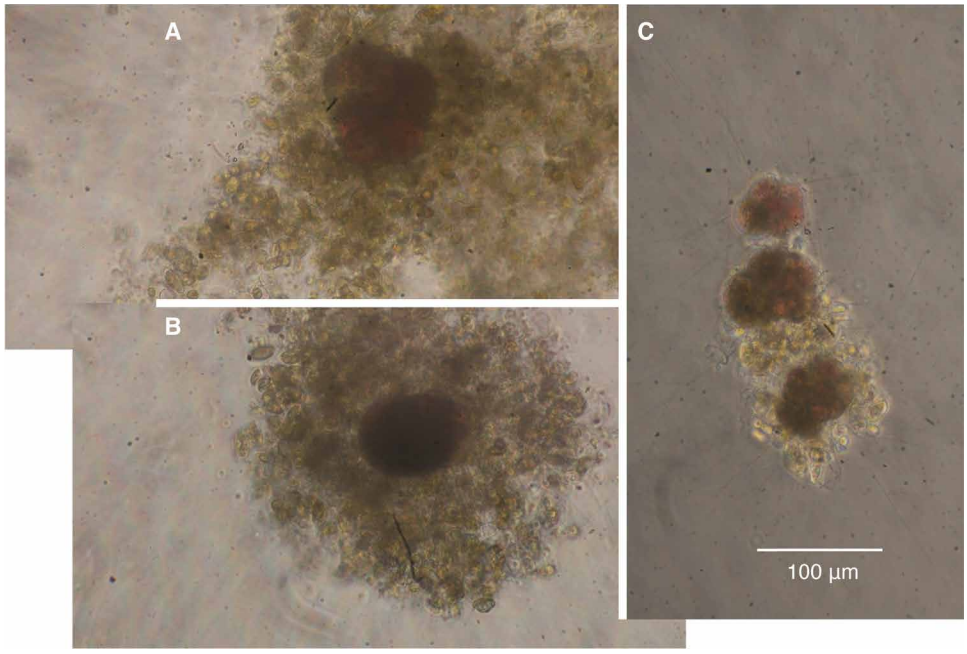

Fig. 4. Examples of neanic and adult *N. pachyderma* entrained in detritus (**A** to **C**), Individual foraminifera are shown (A and B), as are individuals with varying chamber numbers occurring simultaneously (C). Scale bar, 100 µm.

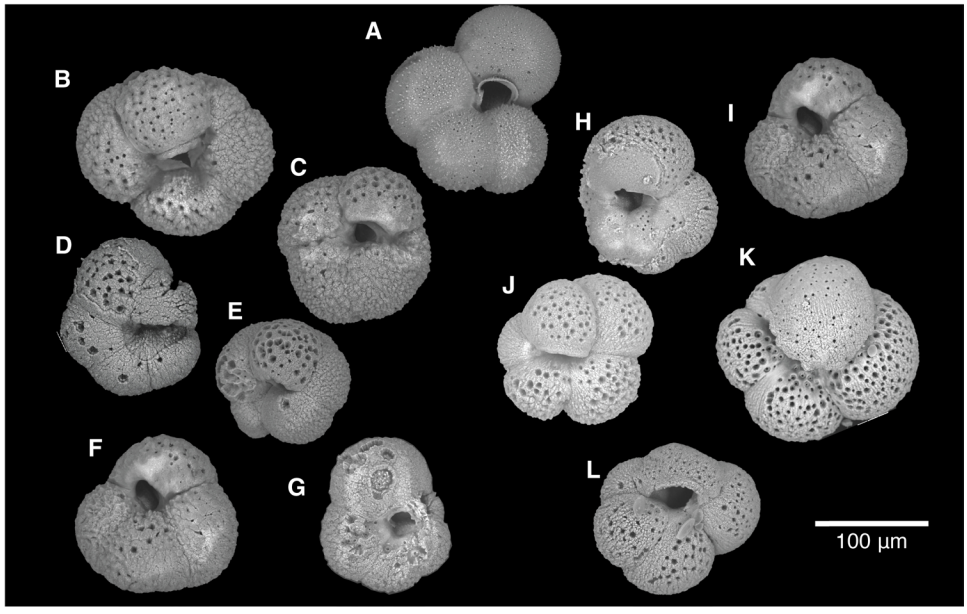

Fig. 5. Scanning electron microscopy images of the shells of the parent foraminifera and all recovered adult shells from offspring. Foraminifera (**A**) is the parent, foraminifera (**B** to **G**) were grown in 12-hour light conditions, and foraminifera (**H** to **L**) were grown in 24-hour dark conditions.

chambers were ever observed. However, empty two-chambered shells were found up to 78 days after the initial reproductive event and 76 days after observations of the earliest two-chambered form, indicating the relatively robust nature of the empty shells to dissolution under these conditions. Thus, we suggest that proloculi were weakly or noncalcified, consistent with similar observations in both planktic and benthic foraminifera (*24*, *27*). Our additional observations of early growth largely support previous inferences about ontogeny (*19*, *27*, *28*). Both the second and third chambers were smaller than the proloculus (Fig. 3) (*27*–*29*), and pores only emerged around the sutures once foraminifera reached ~6 chambers, as described for *Globorotalia inflata* (Fig. 6) (*19*).

Early ontogenetic natural history observations are generally lacking for *Rhizaria*. Despite the frequent observation of gamete release in culture, gametes rarely fuse under these conditions. Only one previous report of a (sexually produced) second generation of planktic foraminifera has been made in the laboratory (*14*), with no offspring surviving into adulthood. We observe distinct transitions

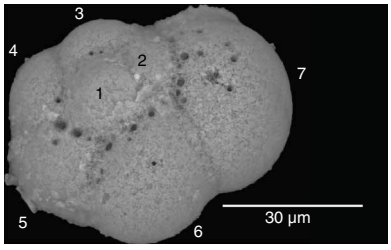

Fig. 6. Scanning electron microscopy image of a seven-chambered *N. pachyderma* shell. The presence of pores along sutures is visible as are a small number of pores beginning on chambers 6 and 7. Scale bar, 30 µm.

in feeding behavior with ontogeny. Juvenile stages of two to six chambers (~30 to 80 µm in length) cast a wide rhizopodial network along the bottom of the flask and appeared to be in constant motion with active rhizopodial streaming (Figs. 2 and 3 and fig. S1). Despite chamber addition, no feeding was observed. Rather, juveniles actively avoided algal cells (fig. S2) and were never seen to attach to intact algae. Despite opportunities for cannibalism (fig. S1), including overlapping rhizopodial networks, no cannibalistic feeding was documented. Thus, we observed juvenile foraminifera discriminating against some food sources and infer that they rely on a bacterial or protozoan diet (*27*).

Upon reaching ~6 chambers (sometimes referred to as the "neanic" stage; >80 µm diameter), foraminifera began collecting decaying algal material in their rhizopodial network (Figs. 2 and 4 and fig. S3). After this point, living individuals commonly floated in or were otherwise encased in rafts of detritus, supporting geochemical and observational evidence that some nonspinose foraminifers, including neogloboquadrinids, may live on and/or in marine snow (*16*), grazing on detritus or bacteria. This selection of microenvironment was typical, and between days 6 and 78, 54% of observations of living foraminifera, inclusive of all life stages, showed them to be associated with detritus. The earliest instance of gametogenesis, proceeded by changing cytoplasm color and crust formation, was observed on day 68, suggesting that the complete life cycle of asexually produced *N. pachyderma* can occur in roughly 2 months (Fig. 2).

Phenotypic variation in a genetically similar population

We observe a range of morphologies in the asexually produced offspring of the *N. pachyderma* individual. There are five recognized morphological types of *N. pachyderma*, previously described as Nps 1 to 5 (*31*, *32*). The parent had an uncrusted Nps-5 morphology, characterized by a relatively open coil and more globular chambers similar to *N. incompta*. The offspring, by contrast, occupied the four other morphologies of *N. pachyderma* (Nps-1 to 4), but not the parent Nps-5 morphology, and differed across major morphological traits including the degree of incrustation, number of chambers in the final whorl, degree of compactness, and presence of an apertural lip. In addition, many of the offspring had the opposite coiling direction of the parent, as also reported from the one previous observation of asexual reproduction in planktic foraminifera (*21*). If these individuals had been collected from tows, then they would likely have been assigned to different species (i.e., *N. pachyderma* for sinistrally coiled individuals and *N. incompta* for dextrally coiled individuals).

Our observations present a case study of the morphological range of clones from the same individual, acclimatized to the same environment through ontogeny, and exposed to minimal variability in macroenvironmental variables. Thus, they act as a lower estimate of the relative importance of phenotypic plasticity in planktic foraminiferal morphology. Temperature and salinity were held constant in our cultures, and the composition of seawater was identical across treatments. The specific particulate masses in which foraminifera entrained themselves varied in size and likely in algal and bacterial concentrations, thereby potentially modifying the chemical (e.g., pH, O_2, and CO_2) and nutritional microenvironment of the clones (*33*). However, foraminifera did not colonize detrital habitats until the neanic phase; thus, these environmental differences likely only influenced adult morphology, not early development such as coiling direction. Utilization of specific microhabitats, thus, may be an important, but not the sole, contributor to the range of adult morphologies observed. The relative abundance of shells coiling in the nondominant direction was greater in the 24-hour dark treatment (50%) than in the 12-hour light treatment (12.5%), indicating that factors such as light conditions or associated changes in pH and O_2 could contribute to coiling direction.

Given the high degree of genetic relatedness and the minimal differences in macroenvironment, neither genetic diversity nor major shifts in physical or chemical environment are necessary to generate substantial morphologic diversity in planktic foraminifera. An additional potential source of the morphological differences between the parent and offspring is dimorphism between the haploid and diploid generations, as described in some (especially larger) benthic foraminifera. Dimorphism in benthic foraminifera includes variation in proloculus size [e.g., (*22*)], as well as chamber arrangement and coiling direction [e.g., (*34*)]. There was no clear evidence for dimorphism in *N. pachyderma*. The average proloculus diameter (18 µm) in cultured offspring was comparable to previously reported ranges of ~16 to 20 µm (*29*, *30*), and coiling direction varied among offspring. The distinctive characteristics of the parent include a larger size than most offspring and a lack of crusted texture (Fig. 5), but gamete release from small and uncrusted individuals is sometimes observed in culture (*6*, *15*). Generations of *N. pachyderma* thus could be described as isomorphic, although the potential morphotypic difference between parent (uncrusted Nps-5) and offspring (Nps-1 to 4) merits future study.

Planktic foraminiferal morphology is plastic in response to environmental manipulations in culture (*6*–*8*), with the extent of this plasticity epitomized by our observations. We show that the full range of morphologies in one species (*N. pachyderma*) and part of another (*N. incompta*) occurs in genetically similar individuals (i.e., clones), without manipulation of major macroenvironmental variables. Our findings support phylogenetic work documenting extensive morphological variability within some genotypes (*31*, *32*) and a lack of a clear genotype/phenotype match. Together, these results demonstrate an important role for phenotypic plasticity and nonheritable factors in driving planktic foraminiferal morphology. Variations in morphology and growth rate have also been observed in clonal communities of benthic foraminifera (*35*). Together, this indicates that morphological heritability may be low across *Foraminifera* or at least in taxa exposed to a high degree of environmental instability such as planktic and shallow-living benthic foraminifera.

Implications for pelagic protistan ecology and evolution

To succeed in the open ocean, pelagic protists must exploit spatially and temporally restricted patches of favorable environmental conditions; use frequently unstable resources; and, if they are limited to sexual reproduction, find mates in a vast three-dimensional environment.

We provide natural history observations that may explain the ability of planktic foraminifera to respond to rapid environmental change and take advantage of optimal environments on seasonal to evolutionary time scales. For *N. pachyderma*, this includes seasonal phenology, inconsistent lunar periodicity, and genetic connectivity between highly disparate populations.

One of the enduring conundrums in planktic foraminiferal ecology is the apparent rapid response of populations to temporally and spatially patchy optimal conditions, with low or imperceptible population densities in between. Asexual reproduction, as reported here, solves this problem, providing species with the means to rapidly increase from population densities too low for reproduction by the fusion of short-lived gametes. Many foraminifera, typified by the neogloboquadrinids, are characterized by one to two distinct annual peaks in abundance, with adult individuals rare or absent for much of the rest of the year (*17*, *36*). The occurrence of alternation between fast-growing asexual generations, rapidly increasing both standing stock and flux when conditions are favorable, and a slower-growing sexual generation favored in unstable or suboptimal conditions could account for this pattern.

On shorter time scales, variation in the frequency of asexual reproduction among planktic foraminiferal species might explain differing responses to lunar periodicity. Lunar periodicity in standing stocks, size, and shell flux has been observed in numerous species of planktic foraminifera (*37*, *38*), attributed to the need for synchrony in gametogenesis to allow gametes to meet and fuse. Therefore, low and varying degrees of lunar periodicity between populations and species, size classes within species, and through time (*39*) are difficult to explain with obligatory sexual reproduction but are readily explained by the occurrence of facultative sexual and asexual reproduction as shown here.

Planktic foraminifera commonly maintain genetic connectivity across great distances, including bipolar distributions [e.g., (*10*)]. How they, and other wide-dispersing plankton groups, manage to disperse efficiently across these distances is a major question. We propose that some species have a slow-growing, sexually produced generation that disperses much farther than their faster-growing asexually reproduced counterparts. This dynamic may be partially analogous to the propagule hypothesis for benthic foraminifera, where juveniles that are more frequently the product of sexual reproduction can maintain dormancy until conditions become suitable for growth (*39*, *40*).

Whether the phenotypic differences exhibited here affect fitness is currently unknown, but isotopic differences between differing morphologies of *N. pachyderma* (*32*) suggest that morphotypic plasticity is associated with underlying physiology and/or adaptation to differing optimal environments. High phenotypic plasticity and variability in planktic foraminifera and the maintenance of long-distance genetic connectivity via facultative alternation of generations may reduce opportunities for ecological speciation, despite the spatial and temporal isolation of favorable conditions and resources in an unstable pelagic environment. Whether high phenotypic plasticity and adaptations to long-distance dispersal are characters that distinguish these low-diversity clades from the high-diversity ones remains to be tested.

MATERIALS AND METHODS

On 7 August 2019, foraminifera were collected by plankton tow from 0- to 100-m depth using a 150-μm mesh net near the shelf break off of North-Central California, USA (38°26′20″, −123°27′01″). Tow material was gently rinsed from the net and kept in the dark at near surface water temperatures during transit to the Bodega Marine Laboratory. Immediately upon return, viable foraminifera were picked from tows and selected for culture based on the presence of colored cytoplasm and rhizopodial activity. Foraminifera were rinsed twice with 0.6-μm filtered seawater and placed into individual Falcon Flasks containing filtered seawater. Falcon Flasks were then stored in a recirculating water bath (13°C) under full spectrum reef lights set to a 12-hour timed light-dark routine.

Following the observed reproductive event, the contents of the Falcon Flask was split into three by vigorous agitation of the water in the original flask and removal of two approximately equal aliquots of seawater. Each Falcon Flask was topped off with fresh filtered seawater to 75 ml. One flask was then moved into dark incubation in an attempt to stem the ongoing diatom bloom (here referred to as flask "D"). Another flask, "A," had three crushed *Artemia* nauplii introduced as a source of alternative food to the blooming diatoms. Last, the original flask, "O," was maintained, as it had been with the empty shell of the parent foraminifer intact. Because of the small size and delicate nature of the shells observed, no manipulation of individuals or alteration of the environment was attempted between this point and the end of the experiment. Shells were regularly imaged, counted, and observed.

On day 52, flasks "D" and "A" were gently washed over 8-μm mesh filter paper (Whatman Grade 2), and shells were picked from the filter and stored in micropaleontological slides. Observations of foraminifera in flask "O" continued for 82 days. No shells we recovered from flask "A," eight offspring and the parent were recovered from flask "O," and 17 offspring from flask "D." All recovered shells were mounted on double-sided carbon tape and imaged using a scanning electron microscope in the Department of Earth and Planetary Sciences at the University of California Davis.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/28/eabb8930/DC1

View/request a protocol for this paper from *Bio-protocol*.

REFERENCES AND NOTES

1. T. Biard, L. Stemmann, M. Picheral, N. Mayot, P. Vandromme, H. Hauss, G. Gorsky, L. Guidi, R. Kiko, F. Not, In situ imaging reveals the biomass of giant protists in the global ocean. *Nature* **532**, 504–507 (2016).
2. R. Schiebel, Planktic foraminiferal sedimentation and the marine calcite budget. *Global Biogeochem. Cycles* **16**, 1065 (2002).
3. C. de Vargas, S. Audic, N. Henry, J. Decelle, F. Mahé, R. Logares, E. Lara, C. Berney, N. Le Bescot, I. Probert, M. Carmichael, J. Poulain, S. Romac, S. Colin, J.-M. Aury, L. Bittner, S. Chaffron, M. Dunthorn, S. Engelen, O. Flegontova, L. Guidi, A. Horák, O. Jaillon, G. Lima-Mendez, J. Lukeš, S. Malviya, R. Morard, M. Mulot, E. Scalco, R. Siano, F. Vincent, A. Zingone, C. Dimier, M. Picheral, S. Searson, S. Kandels-Lewis; Tara Oceans Coordinators, S. G. Acinas, P. Bork, C. Bowler, G. Gorsky, N. Grimsley, P. Hingamp, D. Iudicone, F. Not, H. Ogata, S. Pesant, J. Raes, M. E. Sieracki, S. Speich, L. Stemman, S. Sunagawa, J. Weissenbach, P. Wincker, E. Karsenti, Eukaryotic plankton diversity in the sunlit ocean. *Science* **348**, 1261605 (2015).
4. T. H. G. Ezard, A. Aze, P. N. Pearson, A. Purvis, Interplay between changing climate and species' ecology drivers macroevolutionary dynamics. *Science* **332**, 349–351 (2011).
5. P. M. Hull, R. Norris, Evidence for abrupt speciation in a classic case of gradual evolution. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 21224–21229 (2009).
6. J. Bijma, W. W. Faber, C. Hemleben, Temperature and salinity limits for growth and survival of some planktonic foraminifers in laboratory cultures. *J. Foramin. Res.* **20**, 95–116 (1990).
7. R. Schiebel, C. Hemleben, *Planktic Foraminifers in the Modern Ocean* (Springer-Verlag, Berlin, 2017).

8. J. E. Burke, W. Renema, M. J. Henehan, L. E. Elder, C. V. Davis, A. E. Maas, G. L. Foster, R. Schiebel, P. M. Hull, Factors influencing test porosity in planktonic foraminifera. *Biogeosciences* **15**, 6607–6619 (2018).
9. C. de Vargas, R. Norris, L. Zaninetti, S. W. Gibb, J. Pawlowski, Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces. *Poc. Natl. Acad. Sci. U.S.A.* **96**, 2864–2868 (1999).
10. K. F. Darling, M. Kucera, D. Kroon, C. M. Wade, A resolution for the coiling direction paradox in *Neogloboquadrina pachyderma*. *Paleoceanography* **21**, PA2011 (2006).
11. A. André, F. Quillévéré, R. Morard, Y. Ujiié, G. Escarguel, C. de Vargas, T. de Garidel-Thoron, C. J. Douady, SSU rDNA divergence in planktonic foraminifera: Molecular taxonomy and biogeographic implications. *PLOS One* **9**, e104641 (2014).
12. D. B. Ericson, Coiling direction of globigerina pachyderma as a climatic index. *Science* **130**, 219–220 (1959).
13. A. W. Bé, O. R. Anderson, Gametogenesis in planktonic foraminifera. *Science* **192**, 890–892 (1976).
14. D. R. Ketten, J. M. Edmond, Gametogenesis and calcification of planktonic foraminfiera. *Nature* **278**, 546–548 (1979).
15. C. V. Davis, J. S. Fehrenbacher, T. M. Hill, A. D. Russell, H. J. Spero, Relationships between temperature, pH, and crusting on Mg/Ca ratios in laboratory-grown *Neogloboquadrina* foraminifera. *Paleoceanography* **32**, 1137–1152 (2017).
16. J. S. Fehrenbacher, A. D. Russell, C. V. Davis, H. J. Spero, E. Chu, B. Hönisch, Ba/Ca ratios in the non-spinose planktic foraminifer *Neogloboquadrina dutertrei*: Evidence for an organic aggregate microhabitat. *Geochim. Cosmochim. Ac.* **236**, 361–372 (2018).
17. C. V. Davis, T. M. Hill, A. D. Russell, B. Gaylord, J. Jahncke, Seasonality in planktic foraminifera of the central California coastal upwelling region. *Biogeosciences* **13**, 5139–5150 (2016).
18. S. T. Goldstein, Foraminifera: A biological review in *Modern Foraminifera*, B. K. Sen Gupta, Eds. (Kluwer Academic Publishers, Dordrecht, 1999), pp. 37-55.
19. K.-Y. Wei, Z.-W. Zhang, C. Wray, Shell ontogeny of *Globorotalia inflata* (I): Growth dynamics and ontogenetic stages. *J. Foramin. Res.* **22**, 318–327 (1992).
20. M. Kucera, L. Silye, A. K. M. Weiner, K. Darling, B. Lübben, M. Holzmann, J. Pawlowski, J. Schöenfeld, R. Morard, Caught in the act: Anatomy of an ongoing benthic-planktonic transition in a marine protist. *J. Plankton Res.* **39**, 436–449 (2017).
21. K. Kimoto, M. Tsuchiya, The "unusual" reproduction of planktic foraminifera: An asexual reproductive phase of *Neogloboquadrina pachyderma* (Ehrenberg). *Anuário do Instituto de Geosciências UFRJ* **29**, 461 (2006).
22. C. Dettmering, R. Röttger, J. Hohenegger, R. Schmaljohann, The trimorphic life cycle in foraminifera: Observations from cultures allow new evaluation. *Eur. J. Protistol.* **34**, 363–368 (1998).
23. J. N. Harney, P. Hallock, H. K. Talge, Observations on a trimorphic life cycle in *Amphistegina gibbosa* populations from the Florida keys. *J. Foramin. Res.* **28**, 141–147 (1998).
24. V. Stouff, M. Lesourd, J.-P. Debenay, Laboratory observations on asexual reproduction (schizogony) and ontogeny of *Ammonia tepida* with comments on the life cycle. *J. Foramin. Res.* **29**, 75–84 (1999).
25. P. Hallock, Why are larger foraminifera large? *Paleobiology* **11**, 195–208 (1985).
26. D. N. Schmidt, E. Thomas, E. Authier, D. Saunders, A. Ridgwell, Strategies in times of crisis–Insights into the benthic foraminiferal record of the Palaeocene–Eocene thermal maximum. *Phil. Trans. R. Soc. A* **376**, 20170328 (2018).
27. G.-J. A. Brummer, C. Hemleben, M. Spindler, Ontogeny of extant spinose planktonic foraminifera (Globigerinidae): A concept exemplified by *Globigerinoides sacculifer* (Brady) and *G. Ruber* (d'Orbiny). *Mar. Micropaleontol.* **12**, 357–381 (1987).
28. A. G. M. Caromel, D. N. Schmidt, I. Fletcher, E. J. Rayfield, Morphological change during the ontogeny of the planktic foraminifera. *J. Micropaleontol.* **35**, 2–19 (2015).
29. M. S. Sverdlove, A. W. H. Be, Taxonomic and ecological significance of embryonic and juvenile planktonic foraminifera. *J. Foramin. Res.* **15**, 235–241 (1985).
30. C. Huang, Observations on the interior of some late neogene planktonic foraminifera. *J. Foramin. Res.* **11**, 173–190 (1981).
31. F. Eynaud, T. M. Cronin, S. A. Smith, S. Zaragosi, J. Mavel, Y. Mary, V. Mas, C. Pujol, Morphological variability of the planktic foraminifer *Neogloboquadrina pachyderma* from ACEX cores: Implications for late pleistocene circulation in the arctic ocean. *Micropaleontology* **55**, 101–116 (2009).
32. N. El Bani Altuna, A. J. Pieńkowski, F. Eynaud, R. Thiessen, The morphotypes of *Neogloboquadrina pachyderma*: Isotopic signature and distribution patterns in the Canadian arctic archipelago and adjacent regions. *Mar. Micropaleontol.* **142**, 13–24 (2018).
33. A. L. Alldredge, Y. Cohen, Can microscale chemical patches persist in the Sea? Microelectrode study of marine snow, fecal pellets. *Science* **235**, 689–691 (1987).
34. R. Nigam, N. Khare, The reciprocity between coiling direction and dimorphic reproduction in benthic foraminifera. *J. Micropaleontol.* **11**, 221–228 (1992).
35. M. R. Langer, W. A. Makled, S. J. Pietsch, A. Weinmann, Asynchronous calcification in juvenile megalospheres: An ontogenetic window into the life cycle and polymorphism of *Peneroplis*. *J. Foramin. Res.* **39**, 8–14 (2009).
36. L. Jonkers, M. Kučera, Global analysis of seasonality in the shell flux of extant planktonic foraminifera. *Biogeosciences* **12**, 2207–2226 (2015).
37. M. Spindler, C. Hemleben, U. Bayer, A. W. H. Bé, O. R. Anderson, Lunar periodicity of reproduction in the planktonic foraminifer *Hastigerina pelagica*. *Mar. Ecol. Prog. Ser.* **1**, 61–64 (1979).
38. L. Jonkers, C. E. Reynolds, J. Richey, I. R. Hall, Lunar periodicity in the shell flux of planktonic foraminifera in the Gulf of Mexico. *Biogeosciences* **12**, 3061–3070 (2015).
39. B. J. Ross, P. Hallock, Dormancy in the foraminifera: A review. *J. Foramin. Res.* **46**, 358–368 (2016).
40. E. Alve, S. T. Goldstein, Dispersal, survival and delayed growth of benthic foraminiferal propagules. *J. Sea Res.* **63**, 36–51 (2010).

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