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Gametogenesis and calcification of planktonic Foraminifera

THE processes of gametogenesis and development in benthic Foraminifera are well known[1,2]; however, only recently has there been substantial success in the culture of planktonic species[3]. As part of an investigation of planktonic life cycles, we have maintained in laboratory culture mature foraminifers of *Hastigerina pelagica* and *Orbulina universa* for up to 68 d with more than 80% of the individuals producing gametes. Juvenile stages were subsequently cultured up to initial calcification and regular chamber formation. Although other workers[4-6] have cultured adults and induced gametogenesis, this is the first known success in the maintenance of gametes and the culture of second generation Foraminifera.

Live, adult, planktonic foraminifers were obtained from pelagic waters off Bermuda by hand capture according to a technique of Bé *et al.*[3]. Although this method limits specimens to those visible to a diver in 3–20 m of water, it yields healthy individuals undamaged by net tows or conventional samplers. Each organism was isolated in 500–700 ml of filtered natural seawater and aerated lightly. Normal ambient temperatures (19–23 °C) and lighting (12 h, 40–50 foot candles, fluorescent source) were used. To maintain axenic cultures, the seawater was filtered through successive 0.45-μm and 0.1-μm Millipore filters before the introduction of the foraminifers and repeated periodically to remove accumulated by-products. Adult specimens were removed to separate Petri dishes for biweekly feedings of *Thalassiosira fluviatilis* and nauplii of *Artemia* sp. grown in sterile conditions. *H. pelagica* readily accepted both, suggesting that it is an indiscriminate omnivore in nature. *O. universa* did not accept crustaceans, although carnivorous diets have been reported for it as well as for other spinose species[1,3,5,7]. In any case, our results indicate that planktonic Foraminifera can be maintained readily on an exclusively carnivorous diet although healthiest individuals result from a combined diet of phytoplankton and live Crustacea.

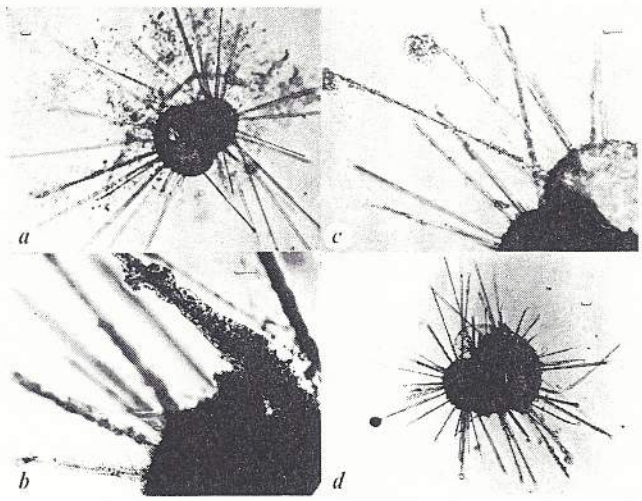

Fig. 1 Light micrographs of major gametogenic stages of *H. pelagica*. Scale bars, 25 μm. *a*, Onset of explosive release. Gametes and cellular residue are emitted only from major apertures and are densest near remains of terminal chamber. *b*, Masses of gametes flowing along spines in cytoplasmic strands during early to middle stages of gradual release. A few remnants of the parent bubble capsule are evident. *c*, Gamete clumps encased in hyaline material on spines near aperture. Unoccupied spines are dissolving inside a sheath formed by the parent organism. *d*, Terminal stages 10–12 h after release began. Few gametes remain in vicinity of parent test; most spines are resorbed leaving only membraneous sheath or base.

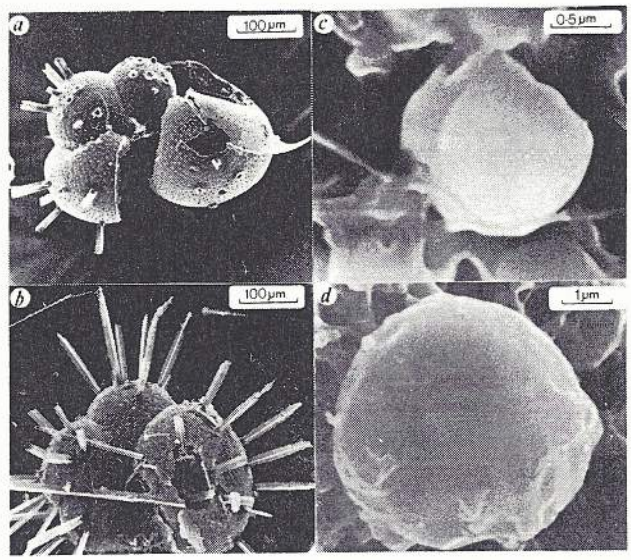

Fig. 2 *a*, Glutaraldehyde-fixed adult at 8-h stage of gamete release. Remains of organelles and developing gametes are found near lip of major aperture. *b*, Post-gametogenic test left 3 weeks in culture. Spines show dissolution pattern. *c*, Biflagellated gamete of *H. pelagica*, fixed 12–15 h after release. The flagella measure 7–10 μm and 12–15 μm respectively. *d*, Initial chamber (proloculus) formed 28 h after gamete release.

Based on observations of 29 *H. pelagica* and two *O. universa*, gametogenesis can be said to be a predictable development preceded by several characteristic morphological changes. Between 24 and 36 h before gamete release, the normally expanded calymma decreases until no bubbles or only a single bubble layer is visible around the chambers. Simultaneously, all pseudopodial filaments are retracted into the calcareous test and the main body of cytoplasm constricts into the inner chambers until the last and typically largest chamber is evacuated. The cytoplasm progressively darkens from pale orange or tan to deep orange or golden brown. Previous workers have attributed intense orange-red hues to redistributed lipid storage products[6]; however, in our specimens, orange pigmentation is a dietary phenomenon. While cytoplasmic coloration intensified in all specimens, only those fed high proportions of *Artemia* were deep orange. Others fed exclusively *Thalassiosira* tended to brown and, in one case of gametogenesis within 5 d of capture, to dark grey or black. The latter coloration may be due to local pelagic species of Crustacea in Bermuda, including the copepods *Candacia ethiopica*, *Euchaeta marina* and *Harpacticus* sp., which have black pigments and are sufficiently large to be a feasible food source for these foraminifers.

In *O. universa*, cytoplasmic regression and loss of the calymma coincided with a loss of all major spines. In contrast, in no *H. pelagica* were all spines shed and the degree of spine loss or disintegration varied between individuals; remaining spines occasionally served to transport gametes to the periphery (Figs 1*b*, 3*b*). Spines in *H. pelagica* may be lost by resorption of their external bases[5], by release of the spine from its base, or by progressive disintegration of the spine from its peripheral end (Fig. 1*c*). In five *H. pelagica*, spines began disintegration after onset of gamete release and were effectively dissolved in 4–6 h. The process is assumed to be autolytic because surface seawater is supersaturated in $CaCO_3$ and would not induce rapid dissolution. Most spines were encased in a residual, membraneous sheath which, as the spine disintegrated, thinned, curving at the peripheral end. More fibril spines were often held in place by secondary mucoid strands. Experiments now in progress indicate that patterns of disintegration within the sheath resemble those of empty foraminiferan tests; however, the triradiate spines of vacated tests require up to 6 weeks to

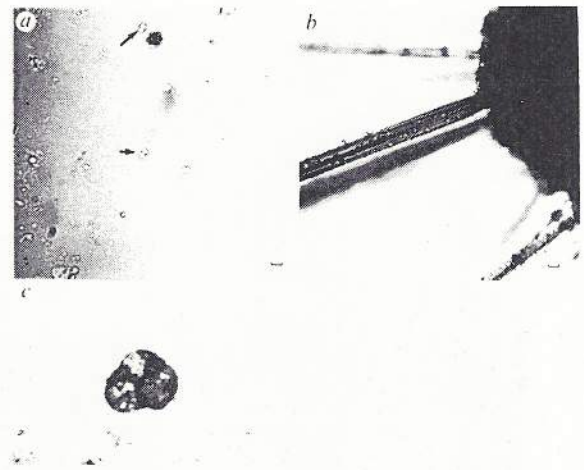

Fig. 3 *a*, Syngamy found in culture water 36 h after release, and *b*, on spines of parent test. A pair in which the process is nearly complete can be seen at top centre of *a*. Scale bar, 5 μm. *c*, Three-chambered *H. pelagica* produced in culture within 15 d of gametogenesis. Chambers shown range 27–40 μm in diameter.

produce the same degree of etching as can be produced in 4 h in a gametogenic individual (Fig. 2*b*). The fragility of *Hastigerina* tests was noted by Berger[8] and a similar spicule dissolution phenomenon shown for other pelagic protozoans[9]. These factors along with limited latitudinal distribution, may explain the relative scarcity of *Hastigerina* in marine sediments.

The actual release of gametes may be explosive or gradual and occurs 2–3 h after the test surface becomes rugose and the cytoplasm dense and granular. In dehiscence, cytoplasm fills the final chambers and gametes are discharged with protoplasm. The final chamber, formed 4–10 d before the onset of gametogenesis in 20% of our cultures, is lost entirely or part of its outer wall destroyed by forcible expulsion of its contents (Fig. 2*a*). Cytoplasmic residue is later released in a characteristic, streaming pattern (Fig. 1*a*), as reported for *Globigerinella aequilateralis*[5], and closely resembling phases described for *Elphidium crispum*[1]. In gradual release, intensification of the cytoplasm is followed by gametes being extruded along the spines. In spite of rapid flagellar movement, the gametes are normally held in a line by a mucoid coat similar to that covering dissolving spines (Fig. 1*b*). Substantial clumps form at the spine tips within 2 h of the onset of release (Fig. 1*c*) and the flow may continue for 8–10 h. Most gametes, however, reach the periphery and separate from the clumps within 3 h. At this stage, the gametes are dark, ovoid bodies, 2–5 μm long, and have two distinct, unequal flagella with a common base (Fig. 2*c*). The gametes can move just after release although rapid motion does not begin until they are 50–100 μm from the surface of the parent test. Movement is typically a stationary oscillation with rapid flagellar beating or rotation as though due to spinning flagella. Flagellar movement was too rapid to be clearly discerned during swimming. Mobile flagellated gametes survived in culture up to 12 d although most of them calcified or died within 5 d.

Syngamy was rare, observed among gametes of only four *H. pelagica*. No single parameter was common to the cultures in which copulation took place, nor were there apparent morphological differences from other gametes. An estimated maximum of 10% of the population formed syngamous pairs, and frequency of copulation was not increased by combining gametes from two specimens reproducing within the same 36 h. The pattern of copulation was invariable, one gamete remaining relatively still while a second moved rapidly around its periphery, periodically withdrew 10–15 μm, and then forcibly pushed against it. Contact generally occurred between the antiflagellar ends of the gametes with adherence evident at the point of impact (Fig. 3). While thus attached, the two pairs of flagella straightened and beat rapidly, and the gamete pair whirled in the water. The more mobile of the two then broke off, withdrew, and repeated the battering process. Separation became progressively more difficult due to coalescence. This activity continued up to 30 min, ending in a single perceptible organism, about 5 μm in diameter, with two pairs of inactive flagella (Fig. 3*a*). Nuclear material could not be resolved in the living material, but a transfer or fusion is assumed.

Calcification to a single chamber, presumed a proloculus, took place as soon as 9 h after gamete release although most cultures did not show calcification for several days. Single-chambered organisms thus formed were smooth spheres, 8–20 μm across, with few or no apertures perceptible (Fig. 2*d*). The degree of calcification varied, with some individuals forming overlapping layers; however, that may have been a culture abnormality. In only one of our cultures have we found chamber formation beyond prolocula. Two polythalamic individuals were produced (Fig. 3*c*), each forming four chambers within 15 d of gametogenesis, the last chamber appearing within 2 d of the third. The organisms measured 65–80 μm in diameter with the largest chambers 35 and 40 μm in diameter. Both were planispiral with irregularly arranged pores about 1 μm wide and a single 10-μm aperture in the final chamber. No spines have been produced on either individual. None of our second generation specimens has matured beyond this stage and the main difficulties foreseen in further culturing are the provision of appropriate food sources and the formation of culture aberrants with a small sample size.

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