Cycad Prepollen: Description and Possible Evolutionary Consequences of Zoidogamy

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In the September 2001 issue of *The Cycad Newsletter*, Tom Broome (Broome 2001) wrote the following regarding pollination and fertilization of cycads: "The pollen will be stored in pollen chambers until it is time to fertilize the ovule. This can take as long as four months to occur. At this time of release, the motile sperm swim down a tube and fertilize the ovule." I would like to qualify and clarify those remarks, especially because of the potential evolutionary consequences of cycad pollination

Technically, cycads have prepollen instead of pollen. However, colloquially, both pollen and prepollen are often referred to as pollen. Prepollen has a proximal aperture—proximal to the center of the tetrad of four microspores (incipient prepollen grains) formed via meiosis of the microspore mother cell. In cycads, gingkos, and presumably in all fossil plants with prepollen, the sperm cells are released through this proximal aperture (Poort et al. 1996). Pollen tubes of both prepollen grains and pollen grains emerge ("germinate") through the distal end of the grain.

In cycads and gingkos, the pollen tube forms a highly branched haustorium (Norstog 1993; Friedman and Gillford 1997). This is a parasitic outgrowth, very much resembling the haustoria of parasitic flowering plants such as mistletoe (e.g., *Viscum, Phoradendron*) or dodder (*Cuscuta*). The branched pollen tube parasitizes the nucellus, which is the layer of the ovule just inside of the seed coat and just outside of the female gametophyte. Most importantly, the incipient sperm cells do *not* travel through the haustorial pollen tube. In fact, the haustorial pollen tube typically grows in a direction away from the egg cell(s). Instead, the sperm cells remain in the remnants of the original prepollen grain while the haustorial pollen tube grows. The sperm cells and the enclosing prepollen grain grow considerably in size, being supplied with nutrients by the haustorium, and the sperm cells grow flagella.

When it is time for the flagellated sperm cells to be released from the prepollen grain, they dehisce through the proximal aperture. They then swim through the pollen chamber to the egg cells. The pollen chamber is an open area between the nucellus and the megagametophyte. The nucellus is formed from diploid tissue, i.e., with the same full complement of chromosomes that is contained in roots, stem, and leaves. The megagametophyte is the female haploid tissue, i.e., with half the complement of chromosomes, the same number as in gametes. The egg cell or cells are imbedded in the megagametophyte. Plants with prepollen and flagellated sperm cells are termed zoidogamous. The above-cited references to Friedman & Gillford (1997) and Norstog (1993) provide helpful diagrammatic sketches of fertilization in zoidogamous plants.

It is generally believed that ancestral zoidogamous plants in the Paleozoic produced prepollen with flagellated sperm cells that dehisced through a proximal aperture, but produced no pollen tube—haustorial or otherwise—from the distal end of the prepollen grain. Because of the lack of haustorial pollen tube, sperm cells had to grow to full size *before* being released from their parent's pollen sac ("microsporophyll"). These prepollen grains were often very large (Stewart 1951). Evolution of haustorial pollen tubes and the consequent reduced size of incipient sperm cells are believed to have provided plants like cycads and gingkos with a substantial adaptive advantage over more ancestral zoidogamous Paleozoic seed plants (Poort et al. 1996).

In flowering plants and most other plants with true pollen, a properly functioning pollen tube forms a straight, unbranched structure. This pollen tube only twists or occasionally branches due to incompatibility of the pollen with female tissues, and then fails to deposit sperm in a place where fertilization can occur. A properly functioning (i.e., straight and unbranched) pollen tube in these seed plants can derive some nutrition from the female tissue through which it grows. The sperm cells—which may or may not have yet divided via mitosis from the generative cell—travel through the growing pollen tube, staying at or near its leading and growing edge, and are only released once the pollen tube grows through the micropylar end of the ovule and are deposited at the egg cell (and possibly also at the polar nucleus or nuclei). The pollen grains of such plants have no proximal aperture, and the sperm cells have no flagella. Plants with true pollen and with pollen tubes that deposit nonflagellated sperm directly on the eggs cell are termed siphonogamous.

There exists one extant set of plants that are seemingly intermediates between zoidogamy and siphonogamy: the conifer family Araucariaceae, which contains the extant genera *Agathis* (kauris) and *Araucaria* (Norfolk Island pines). Although these conifers are siphonogamous plants that deposit nonflagellated sperm at the egg cells, their pollen tubes are branched, with most branches growing toward the egg cells. However, a few branches usually grow in other directions, such as toward the chalazal end of the ovule. These branched araucarian pollen tubes grow the length of female cone scales and can take from five to twenty-four months to reach the nucellus and egg cells, sometimes including growing through tissue enveloping the cone scales (Burlingame 1913; Eames 1913; Owens et al. 1995; Owens et al. 1998). These branched pollen tubes are thought to be absorbing nutrients from (i.e., parasitizing) the female cones to a similar degree as zoidogamous plants.

The only other group that apparently had siphonogamy with branched pollen tubes was the Mesozoic cycadeiods, i.e., the order Bennettitales (Rothwell and Stockey 2001; Stockey and Rothwell 2001). Bennettitales always lacked pollen chambers, further evidence of siphonogamy. Although the vegetative portions of these plants superficially resembled cycads, cycadeoids were probably the group most closely related to flowering plants (Axsmith et al. 1998; Doyle 1998).

Siphonogamous plants probably descended from zoidogamous plants with haustorial pollen tubes which, in turn, probably descended from zoidogamous plants without pollen tubes (these assertions are not definitive because of the extreme rarity of fossil pollen tubes). This evolutionary progression is also reflected in increasing selective advantage, as was already partly seen in the advantage that haustorial pollen tubes probably conferred over other zoidogamous plants that did not have pollen tubes. But, what fitness advantage might siphonogamous plants have had over zoidogamous plants with haustorial pollen tubes?

Zoidogamy—with its flagellated sperm and branched haustorial (or nonexistent) pollen tubes—appears to have imposed a macroevolutionary constraint when compared with siphonogamy. Of the lineages that had animal pollination, the siphonogamous ones had periods of great species diversity, while the zoidogamous ones never did (reviewed in Gorelick 2001). Angiosperms (flowering plants), Gnetales (including *Welwitschia, Ephedra,* Gnetales, and several extinct genera), Bennettitales (cycadeoids), and Cheirolepidiaceae (a Mesozoic conifer family) were mostly insect pollinated, presumably all siphongamous, and extremely diverse in either the Mesozoic or Cenozoic. Furthermore, these were the only lineages of seed plants that were ever diverse. The Cycadales (true cycads) and Medullosales (a group of Paleozoic seed ferns) were also mostly animal pollinated, but were zoidogamous and never diverse.

Mulchay (Mulchay 1979) hypothesized that the extraordinary diversity of flowering plants was due to pollen tube competition. Modifying and generalizing this hypothesis, seed plant diversity may be due to a combination of animal pollination *and* pollen tube competition on maternal tissues, but where pollen tube competition only truly occurs in the pollen tubes of siphonogamous plants that carry sperm cells (Gorelick 2001). This provides one explanation for the relative abundances of seed plant lineages, including the paucity of cycad diversity.

There exist other hypotheses for the preponderance of flowering plants and the paucity of cycads, hypotheses that have also not yet been falsified and that do not invoke zoidogamy or siphonogamy. Nevertheless, the possibility exists that the relative lack of cycad diversity may be largely due to zoidogamy, with its haustorial pollen tubes and sperm cells that must use their flagella to swim through the pollen chamber to an egg cell

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