Which came first, bi- or tricellular pollen? New research updates a classic debate

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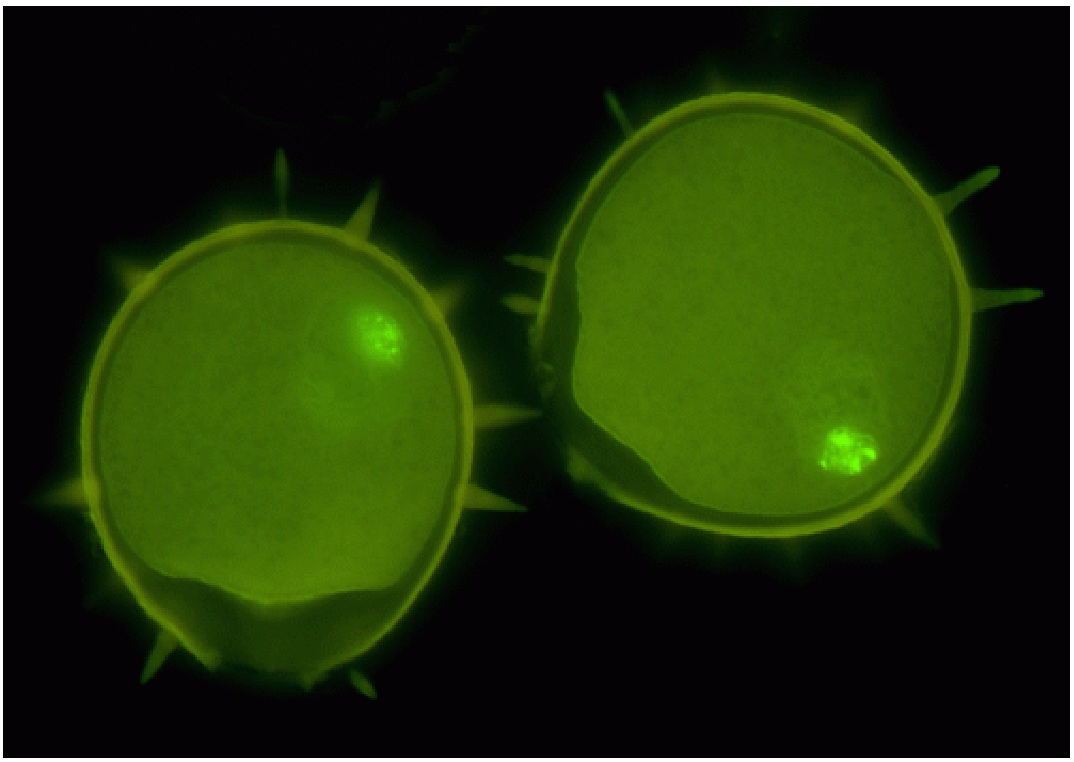

Bicellular pollen of the pond lily, *Nuphar advena* at the time of its dispersal by insects. The vegetative body of the gametophyte consists of a single cell and takes up the entire space within the pollen grain. A separate "generative" cell, that will eventually give rise to the two sperm cells, is free within the cytoplasm of the "vegetative cell." Shown are the nuclei of these two cells stained with a DNA-binding fluorochrome -- the bright one is the generative nucleus and the large, faint vegetative nucleus is directly adjacent to and surrounding it. Credit: Joseph Williams.

With the bursting of spring, pollen is in the air. Most of the pollen that is likely tickling your nose and making your eyes water is being dispersed in a sexually immature state consisting of only two cells (a body cell and a reproductive cell) and is not yet fertile. While the majority of angiosperm species disperse their pollen in this early, bicellular, stage of sexual maturity, about 30% of flowering plants disperse their pollen in a more mature fertile stage, consisting of three cells (a body and two sperm cells). And then there are plants that do both.

So which is the ancestral state, why did the earlier onset of maturity (the tricellular state) evolve so often, and is the tricellular state an evolutionary "dead end"? These questions, and others, were tackled in the classic work by James L. Brewbaker in 1967 and have been revisited in a new study, drawing upon an impressive database of over 2000 species, to determine which came first, tri- or bicellular pollen, and which leads to greater species diversity.

In the 1920s it was proposed that tricellular pollen had evolved independently within angiosperms numerous times and was an irreversible state. These predictions were supported by a classic, elegant, and notable study conducted by Brewbaker and published in the *American Journal of Botany* in 1967. Brewbaker used data from 1,908 species in one of the first large-scale tests of an evolutionary developmental hypothesis. He mapped pollen state (bi- vs tri-) onto a phylogenetic tree and found that tricellular families always appeared to be nested within bicellular families. He thus concluded that bicellular pollen was ancestral and had given rise to tricellular pollen multiple times. He also concluded that tricellular pollen never seemed to give rise to bicellular pollen.

Joseph Williams, an associate professor at the University of Tennessee, has had a long-standing interest in the reproductive biology of flowering plants, and is particularly interested in the evolution of development of ancient flowering plants. As part of the Centennial Celebration of the *American Journal of Botany*, Williams and co-authors (University of Tennessee and Creighton University) decided to re-examine the core questions that Brewbaker tested using modern, updated phylogenies and many more species than Brewbaker had available to him 50 years ago (http://www.amjbot.org/content/101/4/559.full.pdf+html).

"I think many of us who did their graduate work during the '60s through the early '90s saw our first flowering plant phylogenetic tree when we opened the October 1967 issue of *American Journal of Botany* to Jim Brewbaker's two-page-wide tree comprising 265 families," comments Williams (http://www.jstor.org/stable/2440530?seq=4). "That he had only constructed the phylogeny to answer a question about the evolution of pollen development was even more impressive."

"Our Centennial Review paper moved from being a straightforward review to a research paper," explains Williams, "because new methods for studying evolutionary rates of binary traits had just come out, and I thought: Why not redo Brewbaker's analysis with modern methods? Coincidentally, we had been collecting data on pollen cell number for the last seven years, so we had a great dataset in hand. To his credit, Brewbaker had already anticipated all the important questions, so you could say that our paper just added clarity to the nearly 50-year-old answers he suggested."

Indeed, Williams and co-authors expanded the Brewbaker dataset by including 2,511 species for which they modeled trait evolution (tri- vs bicellular pollen) using a modern (2013) seed plant phylogeny and two different sets of analyses.

Much to their surprise, the results from their analyses did not strongly support a bicellular ancestry, contrary to Brewbaker's findings, and, in fact, were ambiguous as to the ancestral state. While one analysis pointed to a tricellular ancestry, another analysis—which allowed evolutionary rates of the traits to vary across a phylogeny—found more uncertainty at the base, with a tricellular ancestor only slightly more likely than a bicellular ancestor.

Interestingly, they also found that both bi- and tricellular lineages gave rise to each other. Thus, their analyses debunked the long-standing assumption that pollen states could only evolve in one direction, namely from bi- to tricellular, and that tricellularity was a "dead end."

"Furthermore, our study showed that despite the recurrent evolution of tricellular pollen, those lineages with tricellular pollen actually had slower evolutionary rates," adds Williams. "Tricellular lineages had both reduced net speciation rates (speciation minus extinction) and reduced rates of reverting to the bicellular state."

In other words, even though tricellular species are formed often, suggesting an advantage to this dispersal state, tricellular lineages evolve slowly. And the net effect is that bicellular species are more common than tricellular species.

The authors speculate further that ecology plays an important role in these findings.

"Tricellular pollen develops rapidly after pollination, and so it would be favored in many of the unique lifestyles of angiosperms that demand rapid reproduction, such as herbs, annuals, and herbaceous aquatics," Williams notes.

"But acquiring those kinds of habits has consequences. The pattern of tricellular lineages rarely re-evolving the bicellular state suggests a reduced ability to respond to changing pollen dispersal conditions over evolutionary time, which in turn has slowed their rate of diversification."

One of the ideas that Williams is interested in continuing to pursue is the conflict between the ecology of pollen dispersal (the free-living phase of pollen ontogeny) and the ecology of pollen tube growth after pollination (where pollen is protected and competes with other pollen for fertilization success).

"I'm currently working with large datasets that will allow me to look for correlations between dispersal traits—such as pollen dimensions, DNA content, cell number, pollen energy reserves, water content, pollination syndromes—and pollen tube performance traits—such as tube dimensions and elongation rates, style lengths and duration of growth," concludes Williams.

More information: Joseph H. Williams, Mackenzie L. Taylor, and Brian C. O'Meara. Repeated evolution of tricellular (and bicellular) pollen. *American Journal of Botany* April 2014 101:559-571. www.amjbot.org/content/101/4/559.full.pdf+html

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