PERSPECTIVES: PLANT BIOLOGY

Pollen Tube Guidance—Right on Target

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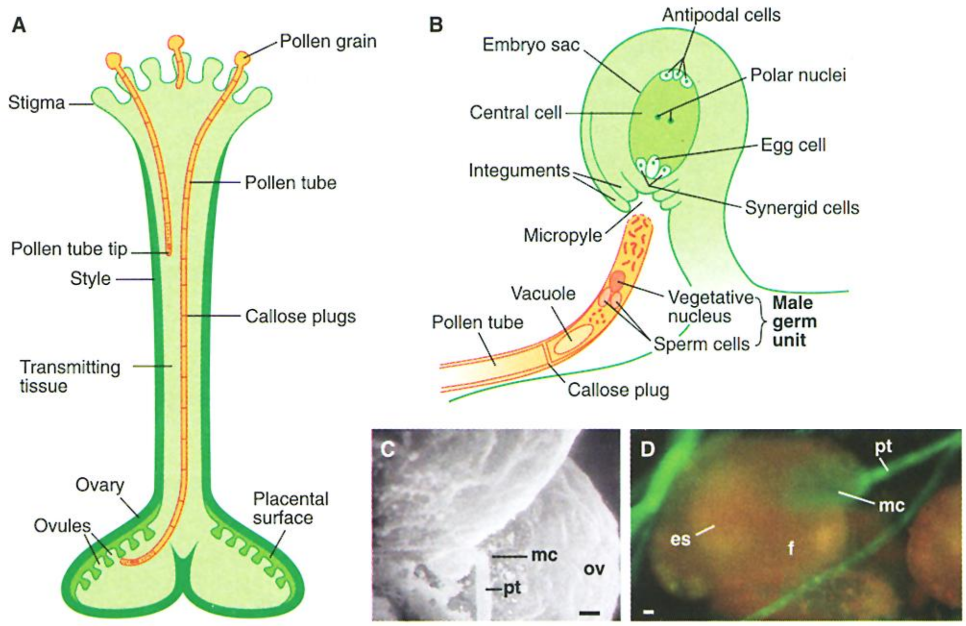
Like mating in other organisms, attraction between the opposite sexes in plants is important for reproduction. Chance brings pollen grains (the male gametophytes) to the surface of the pistil (the female reproductive organ), but it takes active male-female interplay within the pistil to achieve fertilization. Biologists have long puzzled over the guidance cues produced by the pistil that direct the tube growing from the pollen grain toward its target, the embryo sac where the egg resides. On page 1480 of this issue, Higashiyama *et al.* (*1*), working in the flowering plant *Torenia fournieri*, show that the paired synergid cells of the embryo sac are the source of guidance cues that entice pollen tubes to enter the ovules of the ovary.

The pistil is composed of a pollen receptive stigma that is connected to the ovary by a structure known as the style (see the figure). Each haploid pollen grain develops a pollen tube that transports two male gametes from the stigma to the ovules of the ovary. The pollen tube elongates through the female diploid tissues of the style by extending its tip, reminiscent of the elongation of axons during neural development in mammals (*2*–*4*). The female gametophyte (embryo sac) is composed of seven haploid cells including the paired synergid cells and resides in the ovule (see the figure). Upon arrival in the ovary, the pollen tube adjusts its growth trajectory to gain access to an ovule through an aperture called the micropyle (see the figure). Although multiple pollen tubes may approach a micropyle, usually only one successfully penetrates the embryo sac. As it does so, the pollen tube ruptures, releasing the male gametes. In a double fertilization process, the gametes then fuse with the egg cell and the binucleated central cell producing the embryo and the triploid endosperm, respectively, of a future seed (*5*).

Pollen tubes elongate through several cell layers in the style, precluding live observation of pollen tube navigation. Unfortunately, pollen tubes cultured entirely in vitro on chemically defined media have not been optimal for studying pollen tube guidance. Nevertheless, morphological, cytological, and genetic studies have consistently suggested that pollen tubes are attracted by guidance cues that originate from the ovule or, more precisely, from the embryo sac (*2*, *3*, *5*). Adhesive interactions between diploid ovular tissues and the haploid pollen tube also have been implicated in the targeting of pollen tubes to *Arabidopsis* ovules (*3*).

In their study, Higashiyama *et al.* (*1*) adopt a semi–in vivo pollen tube growth assay to examine pollen tube guidance cues in *Torenia.* Unlike most angiosperms, where the embryo sac is embedded within layers of ovular cells, the "naked" *Torenia* embryo sac protrudes out of the ovule, thus making possible direct observation of the pollen tube entering the embryo sac. The egg cell, the two synergid cells, and about half of the central cell of the *Torenia* embryo sac are exposed to mucilaginous materials in the ovary chamber. In the semi–in vivo pollen tube growth system, pollen tubes that have already journeyed through a pollinated style grow into growth medium and are then cultivated in vitro. In previous work, Higashiyama *et al.* showed that pollen tubes emerging from a pollinated *Torenia* style are attracted to the embryo sacs of excised *Torenia* ovules cocultivated in the growth medium (*6*). They observed that a single pollen tube penetrated a synergid cell and discharged its two gametes into the embryo sac as the synergid cell ruptured.

In their new work, Higashiyama and colleagues, with the help of laser ablation, determined which of the four haploid cells in the exposed region of the embryo sac provide the guidance cues. First, they showed that almost 100% of excised intact embryo sacs were competent to attract pollen tubes. Then they demonstrated that



The lure of the pistil. **(A)** Pollen tubes emerging from pollen grains on the surface of the stigma grow through tissues of the style toward the ovary (the stigma, style, and ovary are collectively called the pistil). **(B)** As a pollen tube reaches the vicinity of an ovule, it is attracted by ovular signals to approach an opening (micropyle) and to penetrate the seven-celled embryo sac via one of the paired synergid cells at the entrance. The pollen tube contents (two male sperm cells) are then discharged into the ovule. In a double fertilization process, the egg is fertilized by one sperm and the central cell by the other, resulting in the formation of the embryo and endosperm, respectively, of the future seed. **(C)** A scanning electron micrograph of a pollen tube (pt) that has entered the micropyle (mc) of an ovule (ov) within the ovary of a pollinated tobacco pistil. **(D)** A fluorescent micrograph of a green fluorescent protein–labeled pollen tube (pt) that has entered the micropyle (mc) of an excised ovule in the semi–in vivo pollen tube growth culture assay. The diffuse green fluorescence immediately inside the micropyle reflects the path of the pollen tube within the ovule. es, embryo sac; f, funiculus (ovule stalk). (C and D, size bars are 10 μm).

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ablation of diploid ovular cells, the central cell, the egg cell, or both the central and egg cell simultaneously did not affect the ability of the embryo sac to attract pollen tubes. However, ablation of both synergid cells did abolish the competence of the embryo sac to attract pollen tubes; elimination of a single synergid cell weakened the attraction. When twice the normal number of pollen tubes were cultivated, almost 100% of embryo sacs with one of the two synergid cells left intact were still capable of attracting the pollen tubes. These observations establish that a single synergid cell is necessary and sufficient for pollen tube attraction. This work also resolves the debate, at least for *Torenia*, about the equivalence of the paired synergid cells in attracting the pollen tubes. The investigators went on to demonstrate that a fertilized embryo sac no longer attracts pollen tubes despite the presence of a remaining intact synergid cell. This is consistent with the idea that fertilization prevents additional pollen tubes from entering the embryo sac, thus avoiding the problem of polyspermy [see also (*7*)].

Recent observations of *Arabidopsis* pollen tubes labeled with green fluorescent protein show that the pollen tube also penetrates an *Arabidopsis* embryo sac through one of the synergid cells (*8*). Attraction of pollen tubes by synergid cells is likely to be very common if not universal among flowering plants. However, specificity must exist among guidance cues because pollen tubes from one species do not usually target ovules of a related species (*7*). The guidance cues released by synergid cells provide plants with a final checkpoint to prevent interspecies fertilization. But what are these cues? Observations from different plants suggest that pollen tube guidance is likely to rely on attractant and repellent molecules. Such long- and short-range signals may be similar to the chemotropic factors and their receptors that govern neural cell migration during embryonic development in mammals (*2*–*4*).

Architectural and biochemical diversity among pistils and pollen from different plant species and interspecies incompatibility strongly argue that a universal guidance system is unlikely to account for pollen tube growth in all flowering plants. Differences in the physical and chemical properties of the pistil tissues of a single species and distinct pollen tube behaviors at different phases of growth within the pistil of some plant species suggest that multiple factors contribute to guiding the pollen tube on its long journey (*2*–*4*, *9*). For example, the interface between lipid and water molecules on the surface of the wet stigma may provide the initial directional cue to the pollen tube tip emerging from the pollen grain, guiding it toward the stigma surface (*10*). The organized rows of cells within the "solid style" of some plant species appear well suited for the job of directing elongating pollen tubes along the correct track. Moreover, sugar concentration gradients created by glycoproteins secreted by the style, such as tobacco plant TTS proteins (*2*, *11*, *12*), may also contribute to directing pollen tube migration toward the ovary. The "hollow style" of other plant species, however, provides little structural confinement for the pollen tube. In this case, pollen tubes may bind to surface adhesive molecules expressed by cells lining the stylar canal, which may provide them with both support and guidance (*4*).

Carbohydrate-rich molecules secreted by the ovule (*9*), a high calcium ion concentration in synergid cells (*5*), and adhesion between ovular tissues and pollen tubes have all been suggested as possible chemotropic or contact guidance cues for pollen tubes as they approach the ovules. Biochemical characterization of a heat-labile guidance factor (*6*) from *Torenia* synergid cells may soon reveal the nature of the short-range diffusible molecule that attracts the pollen tube to the ovule. Together with a molecular and biochemical characterization of *Arabidopsis* mutants in which pollen tube targeting to the ovule is defective (*3*), these approaches should help to unveil the mystery of pollen tube guidance. The findings of Higashiyama *et al.* should invigorate efforts to understand the diverse guidance strategies that plants have evolved to ensure reproductive success.

References

1. T. Higashiyama *et al.*, *Science* **293**, 1480 (2001).
2. P. K. Hepler, L. Vidali, A. Y. Cheung, *Annu. Rev. Cell Dev. Biol.*, in press.
3. R. Palanivelu, D. Preuss, *Trends Cell Biol.* **10**, 517 (2000).
4. E. Lord, *Trends Plant Sci.* **5**, 368 (2000).
5. S. D. Russell, *Sex. Plant Reprod.* **9**, 337 (1996).
6. T. Higashiyama *et al.*, *Plant Cell* **10**, 2019 (1998).
7. K. Shimizu, K. Okada, *Development* **127**, 4511 (2000).
8. J.-E. Faure, personal communication.
9. M. Herrero, *Ann. Bot.* **85**, 79 (2000).
10. M. Wolters-Arts, W. M. Lush, C. Mariani, *Nature* **392**, 818 (1998).
11. A. Y. Cheung, H. Wang, H.-M. Wu, *Cell* **82**, 383 (1995).
12. H.-M. Wu, H. Wang, A. Y. Cheung, *Cell* **82**, 395 (1995).

PERSPECTIVES: EARTHQUAKES

Himalayan Seismic Hazard

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Five major earthquakes have visited India in the past decade (*1*), culminating in the devastating Bhuj earthquake of 26 January 2001. That earthquake in particular called attention to the hazards posed by buildings not designed to withstand major but obviously probable earthquakes. It also focused the eyes of the public away from a part of India where even worse damage and loss of life should be expected—the Himalayan arc (see the figure). Several lines of evidence show that one or more great earthquakes may be overdue in a large fraction of the Himalaya, threatening millions of people in that region.

A wealth of geophysical evidence demonstrates that south of the Himalaya, the top surface of India's basement rock flexes and slides beneath the Himalaya—not steadily but in lurches during great earthquakes (see the inset in the figure) (*2*, *3*). This pattern resembles that found where lithospheric plates beneath oceanic regions converge rapidly: At deep-sea trenches, where the ocean floor flexes down seaward of the trench, the entire oceanic lithosphere plunges deep into Earth's mantle, and great earthquakes occur most commonly. Extreme examples are the great earthquakes in Chile in 1960 and in Alaska in 1964. Only during such earthquakes does the entire plate boundary rupture.

Second, Global Positioning System (GPS) measurements show that India and southern Tibet converge at 20 ± 3 mm/year (*4*). A 50-km-wide region centered on the southern edge of the Tibetan Plateau strains to absorb about 80% of this convergence. This region also shows localized vertical movement (*5*), and small earthquakes are most common here (*6*). The surrounding Himalaya accommodates the remaining 20%. Two meters of potential slip in earthquakes thus accumulate each century. In contrast, control points in southern India and southernmost Nepal approach each other no faster than a few millimeters per year (*7*). As the Bhuj earthquake shows, this deformation, although slow, is far from negligible.

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