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Matching Anatomies - Correlating Pollen Tube Anatomy With Pistillar Geometry

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Plant cells are surrounded by a stiff matrix, the cell wall, which is a feature that distinguishes them from animal cells. The plant cell wall is composed primarily of polysaccharidic polymers; the most abundant being cellulose (β-(1-4) glucan) and pectins (polysaccharides rich in galacturonic acid) [1]. The relative spatial and temporal distributions of the different cell wall components and the constant regulation of their chemical composition during morphogenesis confer to the individual cells, tissues and organs their final shape [2,3]. The shape and geometry of a plant cell is crucial as it is correlated with specific functional and structural roles. For example, the vascular cells that transport water and nutrients across the plant have a cylindrical shape and very rigid walls. The cells of aerenchyma are star-shaped to allow for air to pass through. Cells with invading function, like root hairs or pollen tubes form cylindrical protuberances that elongate exclusively at their apex. This mode of growth is termed tip growth. The pollen tube, a very fast growing cell that is able to navigate obstacles and orient towards a target, is formed by the pollen grain (or male gametophyte) of flowering plants. It is responsible for the fertilization of the female gametophyte and is thus a requirement for subsequent fruit and seed formation. To reach its target, the tube needs to traverse a series of tissues composing the pistil of the receptive flower. Upon contact with the stigma, the pollen tube emerges from the pollen grain and elongates through the stigma to find its way to the ovary and deliver the two sperm cells. Tip growth requires the growing apical region of the cell wall to be softer than the non-growing cylindrical shank. A precisely regulated spatial gradient in the mechanical properties of the cell wall are necessary to produce a perfectly cylindrical cell. These mechanical properties are determined by the chemical composition of the cell wall [2,4-6].

The cell wall of most plant cell types is rich in cellulose, the major load-bearing component that reinforces the plant extracellular matrix. By contrast, pollen tubes have a very low amount of cellulose in their cell wall. Less than 10% of the *Nicotiana tabacum* [7] and approximately 6-7% of the dry weight of the *Lilium longiflorum* pollen tube cell wall is composed of cellulose [8]. While cellulose is always present in the cylindrical shank, its presence in the growing, apical region of the tube is species-dependent. While in *Arabidopsis thaliana* and *Lilium orientalis* it is present at the tip [5,9] it is absent in other species such as *Camellia japonica* [10]. The relatively low overall amount of cellulose in the pollen tube cell wall suggests that this polymer does not play a major role resisting turgor pressure induced tensile stress in the pollen tube shank. Instead it seems to play an important role in determining and regulating pollen tube diameter in the sub-apical portion of the tube. What if any role cellulose plays in those tubes that display the polymer in the growing apex is completely unknown. The species-specific differences are intriguing as they might provide an answer based on the specific challenges the tube encounters in the pistillar in vivo environment of the respective species. The path through which the tube has to grow varies significantly between species in terms of length, abundance of mechanical obstacles, and availability of nutrients. We therefore wanted to assess whether any correlation between the cell wall mechanics of the pollen tube and the geometry of the pistillar tissues in individual species can yield information on the functionality and the growth mechanism. We analyzed more than a hundred species collected at the Montreal Botanical Garden (Quebec, Canada). To correlate pollen tube

morphology and pistil anatomy, we used a combination of light and fluorescence based microscopy. Pollen grains were germinated in artificial liquid and solid medium, chemically fixed and labeled for cellulose using Cellulose Binding Module 3a. Styles were fixed, resin-embedded, and sectioned. Observations were made using confocal microscopy. The results showed a correlation between (1) the diameter of the pollen tube and the geometry of the style and (2) between the pollen tube cell wall composition and the anatomy of the style (Fig. 1).

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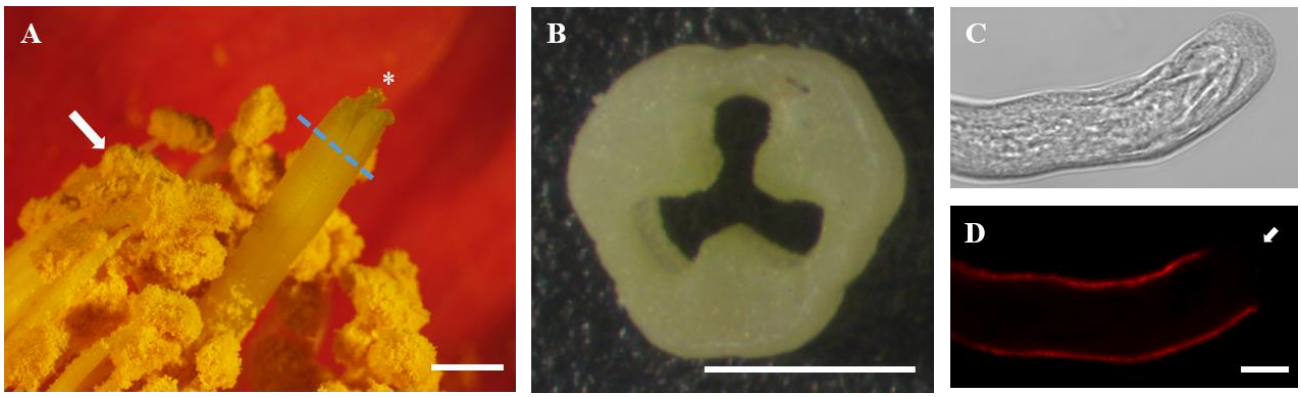

Figure 1. *Camellia japonica* flower anatomy. The pollen grains (arrow in A) are produced in the anthers and deposited on the stigma of a receptive flower by wind or insects (star in A). Upon contact, the pollen grains germinate and form a pollen tube that delivers the sperm cells to the ovary. A transverse section of the style (dashed blue line in A) shows that the style of *Camellia japonica* is of the hollow type and divided into 3 locules (B). Pollen grains were germinated in a liquid culture medium, chemically fixed and crystalline cellulose was labelled using Cellulose Binding Module 3a coupled to anti-mouse Alexa fluor 594 (C,D). Differential interference contrast image of a *Camellia* pollen tube (C). The corresponding fluorescence micrograph (D) shows that the *Camellia* pollen tube is devoid of cellulose at the apex (arrow in D), but displays abundant cellulose in the distal portion. Scale bars in A and B: 0.2 cm. Scale bar in C and D: 10 μm.

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