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Observation of Early Pollen Exine Patterning by Scanning Electron Microscopy

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In angiosperms, the protective wall of pollen is composed of an outer layer called the exine and an inner layer called the intine [1]. The exine is composed of sporopollenin which is produced by the haploid microspore as well as the surrounding tapetum during pollen wall development. This unique compound is responsible for the pollen grain's physical strength and resistance to chemical, physical and biological attack. The reticulate pattern of the mature exine (Figure 1) is made up of rods called baculae or columella, and the over arching tectum (Figure 2). This patterning is often species specific and can vary greatly among angiosperms [2].

Numerous studies of pollen wall development have been conducted, but the factors and circumstances under which the reticulated exine forms remain unclear. It is understood that the exine pattern is established during meiosis of the microsporocyte [3], although the pattern is not visible by transmission electron microscopy (TEM) until the tetrad stage of development. This stage is characterized by the presence of four individual 1N microspores, which resulted from the meiotic cell division of the 2N microsporocyte, grouped together in a tetrahedral arrangement in a common callose wall. The callose wall originates from the primary callose wall of the microsporocyte [4].

To expose the microspore surface of tetrad stage microspores and observe early exine (proexine) components, termed protectum and procolumella, fixed anthers from tetrad-stage flower buds of Wisconsin Fast Plant (*Brassica rapa)* were squashed between two coverslips coated with poly-l-lysine to release their contents. The callose wall was digested away using cellulase, pectolyase, and cytohelicase in a solution of 1.5% sucrose and 1% polyvinylpyrolidone for a minimum of 2 hours. Samples were then prepared for scanning electron microscopy (SEM) by ethanol dehydration and critical point drying. A series of SEM micrographs show microspores at successive developmental stages (Figure 3A-C). As development progressed, sporopollenin appeared to accumulate on the surface in connection with sporopollenin acceptor particles (SAPs), ultimately producing procolumellae structures of the proexine. Later in the tetrad stage, the procolumellae and tectum become more prominent as sporopollenin deposition continues. This development of procolumellae and protectum are absent in the areas where apertures form [5]. The microspores ultimately exhibit a pattern that is similar to the pattern of mature pollen, although compressed.

In several instances, squashing forced a portion of a microspore from the callose wall prior to enzymatic digestion and we observed different patterns on single microspore (Figure 3D). This suggests that the proexine has the potential to expand immediately upon release from callose, and is elastic in nature. This is not unexpected since Rowely and Skvarla have reported that mature pollen grains returned to a natural looking state after being forced through a small aperture. Additionally, developing pollen grains demonstrate their plasticity through dramatic increase in volume following release from the callose wall. Pollen grains of *Zea maize*, for example, show an increase in volume of 1,200 times following release of microspores from callose [6]. Based on the established elasticity of the exine and the appearance of the procolumellae and protectum on the surface of developing microspores, it may be that the mature exine pattern results by expansion of the proexine established during tetrad development. Now that we have

demonstrated a method for observing proexine in three dimensions, we plan to capture images of later developmental stages to document and measure, through fractal analysis, how the pattern of the wall changes as microspores develop into mature pollen grains.

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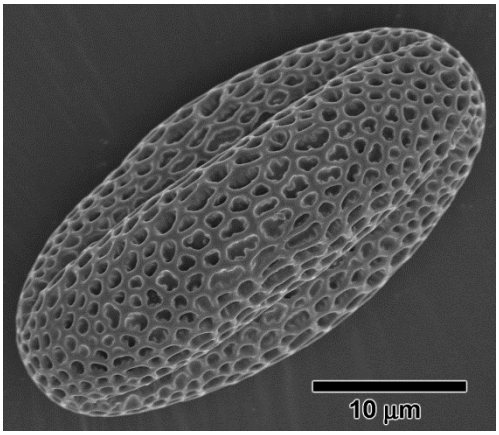

Figure 1: SEM micrograph of a mature pollen grain of *B. rapa*. Size bar = 10 µm.

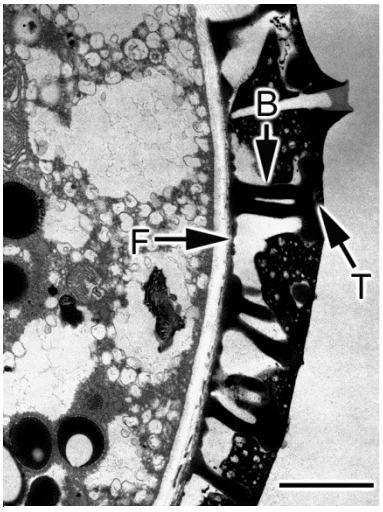

Figure 2: TEM micrograph of mature exine showing tectum (T), bacula (B) and foot layer (F). Size bar = 1 µm

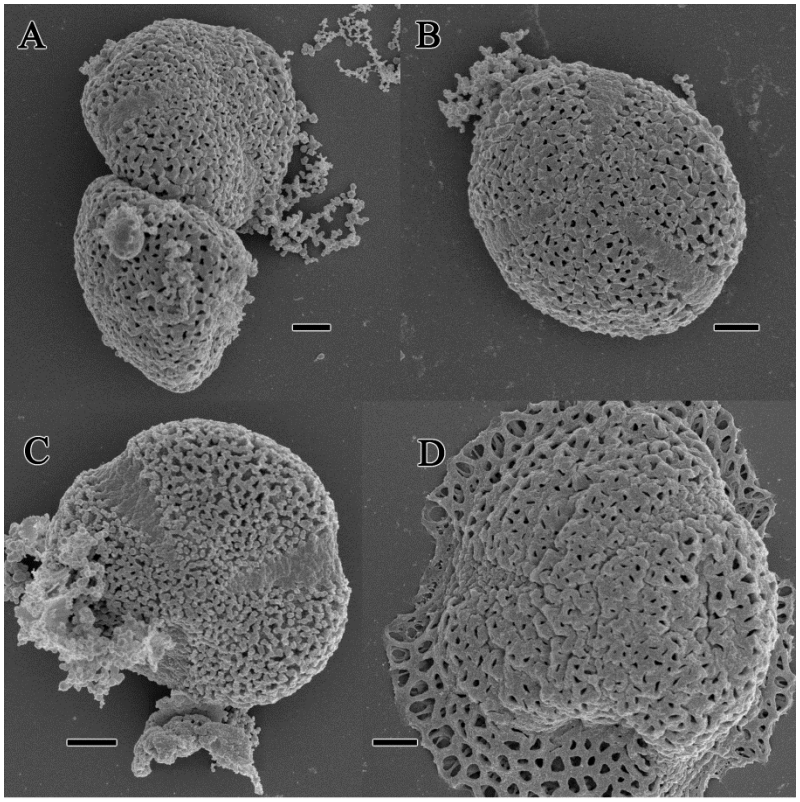

Figure 3: A series of scanning electron micrographs (A-C) of microspores released from the callose at various points in the tetrad developmental stage as well as a squashed tetrad microspore and the apparent elastic nature of developing exine (D). Size bars = 1 µm.

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