In vivo pollen germination: Staining of pollen tubes

A useful method for monitoring pollen fertility is to stain and visualize pollen tubes as they grow on the stigma and through the transmitting tract. We will observe the growth of pollen tubes on self-pollinated plants as well as on cross-pollinated wild-type and *opr3* plants.

1. Remove open flowers from wild-type plants. Newly pollinated flowers are best to use, but you can also look at flowers with elongating siliques. Before placing the pistils in fixative, remove the petals, and (if you wish) the anthers, as these will often obscure the view of the pistil.

2. Fix the pistils for at least 1.5 hours (can go overnight) in 1 ml of:

    10% acetic acid
    30% chloroform
    60% ethanol

3. Pipet off fixative and suspend in 1 ml 4N NaOH for 10 min.

4. Neutralize with 3 washes of 50 mM potassium phosphate buffer (pH 7.5).

5. Move pistils to a slide and add a drop of 0.05% aniline blue in 50 mM phosphate buffer (pH 7.5). Gently apply a cover slip and view pollen tubes on the fluorescent microscope (use the DAPI filter). To see the tubes on the interior of the pistil, you will need to squash the tissue firmly with the cover slip. With this stain, pollen grains (but not pollen tubes) can also be visualized with the rhodamine filter.