

Sample Filtration Procedure

- Place a filter funnel inside the Erlenmeyer flask. Fold a filter paper (VWR, pore size = 5 μm , diameter = 11 cm) and place it into the funnel. Position a cell filter (100 μm) above the paper filter (Fig. S1A).

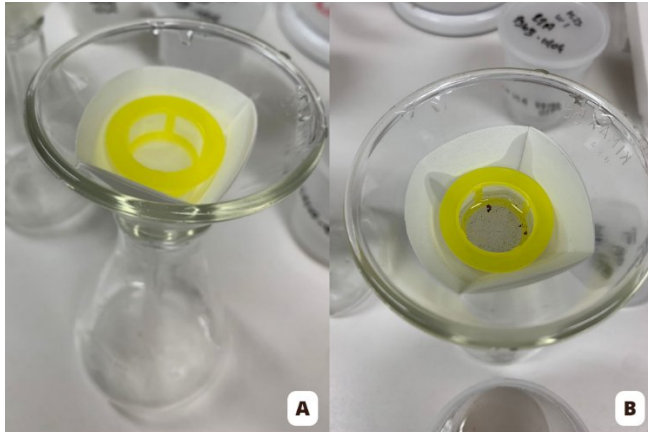


Figure S1: Separation of pollen from debris

- After coarsely grinding the flower units, pour them into the cell filter and rinse them with ethanol (Fig. S1B).
- Transfer the funnel with the paper filter into a labelled test tube (Fig. S2A). Pierce a small hole at the base of the paper filter using a fine-tipped pipette (Fig. S2B) and rinse the pollen from the paper filter with ethanol until the tube is full. The pipette tip should also be rinsed to ensure complete pollen recovery. Use additional test tubes if necessary.

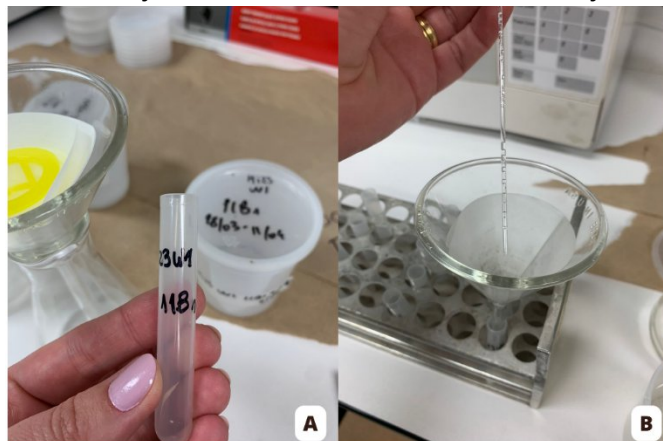


Figure S2: A - Identification of the test tube. B - Making a hole in the paper filter with a pipette.

- Place the test tubes in the centrifuge (settings: 8000 rpm, 20–25 $^{\circ}\text{C}$, 20 min) (Fig. S3A). Ensure that the centrifuge is properly balanced (add water-filled tubes if needed) (Fig. S3B).

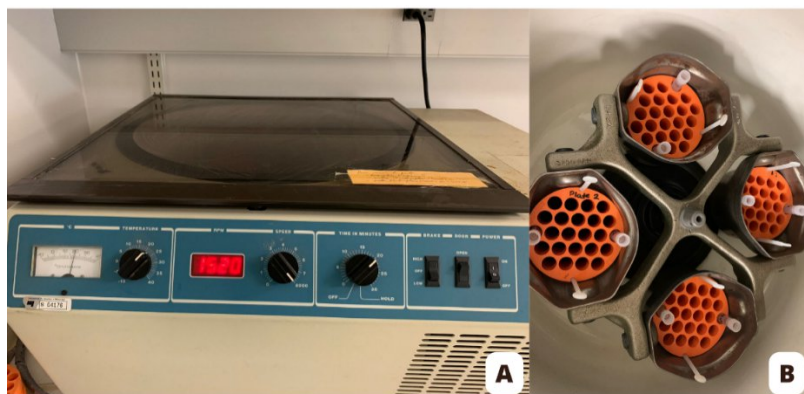


Figure S3: A - Centrifuge settings. B - Balance of the samples inside the centrifuge

- Decant the ethanol into the sink. Stop pouring when the liquid level approaches the pellet (Fig. S4A); it is preferable to retain a small residual volume rather than risk pollen loss.
- Add 200 μ L of PBS solution with a micropipette (Fig. S4B). Use a different tip for each tube not to contaminate the other tubes (Fig. S4C).

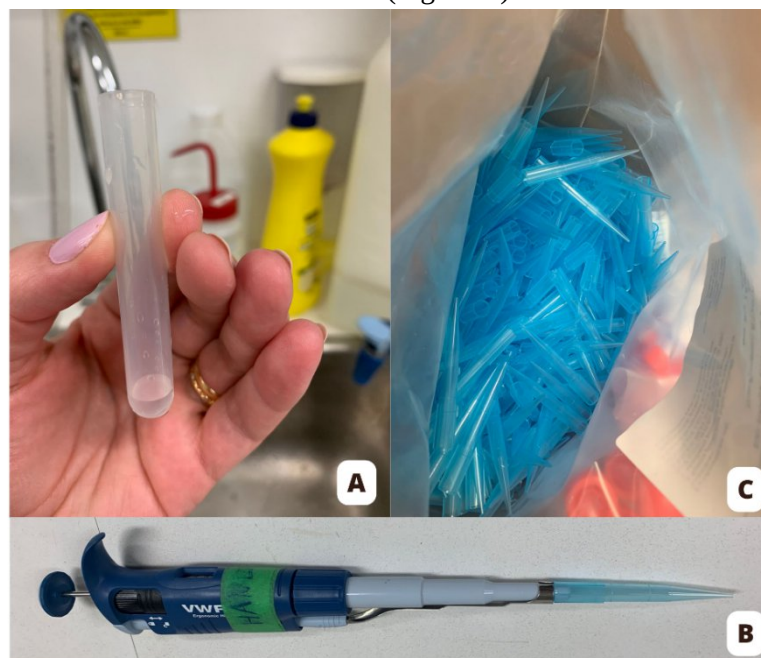


Figure S4: A - Limit to pour the alcohol. B - Micropipette. C - Tips

- Store the tubes in the freezer until analysis.

Note :

The filtrate was suspended in Dulbecco's phosphate-buffered saline (PBS) solution, a standard neutral isotonic buffer commonly used in flow cytometry to minimize aggregation. To further limit pollen grain aggregation, samples were stirred regularly during data acquisition with the cytometer, as pollen grains, particularly the larger ones, sediment rapidly (within less than 30 seconds). Analysis was typically initiated with approximately 200 μ L of resuspended sample and further diluted when an excessively high particle concentration was observed on the flow cytometer. When necessary, multiple tubes were run per sample and the resulting counts were summed to obtain the final value for each sample. Instruments such as the CYTOFlex can accommodate volumes up to 5 mL and allow consecutive tube addition.