Standard Operating Procedure
Pollen Extraction from Honey and Staining

Laboratory: Environmental/Bioresearch **Location**: Rms 169/222

SOP prepared by: O. Pflaumer/R.Sanders **Last Revision:** 8/28/23

Background: Analyzing pollen found in honey is known as **melissopalynology**. This lab provides experimenters the opportunity to extract pollen from one or more samples of honey and analyze what is found under a microscope. Analyzing pollen present in different honey samples allows inferences to be made about the geographical locations and genera of plants present where bees were pollinating. This information might also be used in forensics to help solve crimes. Microscopic pollen grains from plants of a certain area are assembled in ratios to one another that are unique to that area. When compared with pollen samples from a suspect's clothing, shoes, or vehicle these "pollen prints" can point criminal investigators to a specific geographical location, prove or destroy alibis, and link a suspect to the scene of a crime.

Safety: Eye protection, gloves

Materials
honey sample
beaker
hot plate
2 micro tubes
70% isopropyl alcohol
vortexer
disposable pipettes
centrifuge
flat tipped toothpick or scoop
fuchsin basic stain crystals
disposable gloves
microscope
microscope slide
cover slips

Procedure

Prepare fuchsin jelly stain Day 1 (makes enough for 4-5 lab technicians)

1. Use a 150mL beaker to mix 7g of gelatin powder, 24mL of distilled water, 21mL of glycerin and 0.1g fuchsin crystals. *Note: Wear gloves while making stain.
2. Stir and warm the mixture on a hot plate to make a pinkish gel-like solution.

Prepare honey sample for pollen count and staining

1. In a labeled micro tube, add 1 mL honey to 1 mL warm (60 °C) distilled water.
2. Centrifuge the micro tube of honey solution for 10 minutes at 2500 rpm, making sure the centrifuge is balanced before turning on.
3. Decant (pour off) the supernatant off the top of the pellet of debris in the bottom of the test tube.
4. Add 0.5mL of 70% isopropyl alcohol into the microtube with the pellet; invert to mix then allow to sit for 10 minutes.
5. On a clean glass slide, label one edge with sample number, date and initials.
6. In the middle of the glass slide, add 1-2 drops of the honey solution from the micro tube.

7. Place the slide on the edge of the warm hotplate for 1-2 minutes to dry the pollen sample.
8. Add 1 drop of the fuchsin glycerin jelly to a coverslip and lower the coverslip gently at an angle onto the slide to remove air bubbles and bring the jelly stain in contact with the honey sample.
9. Allow the slide to dry for 5 minutes. This allows the fuchsin glycerin jelly to penetrate pollen grains.
10. View the slide under the microscope at 10X magnification and use the following link to help identify plant source. http://www.microlabgallery.com/PollenFile.aspx or http://apsa.anu.edu.au
11. Repeat steps for each honey sample.