A Simple Technique to Sample Pollen From Moths and Its Applications to Ecological Studies

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A SIMPLE TECHNIQUE TO SAMPLE POLLEN FROM MOTHS AND ITS APPLICATIONS TO ECOLOGICAL STUDIES

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Pollen is a physical link between many plant-animal interactions and can be a useful tool for biologists in ecological studies. Pollen found on field collected moths can be sampled and identified. Location of pollen, number of pollen grains, and pollen identification can be recorded for individual specimens. These data can be applied to explore a number of life history phenomena such as nectar plant use and pollination ecology. Further, with the option of using museum specimens, researchers can explore ecological questions from a larger geographic and temporal scale otherwise not easily obtained in a limited field season.

Moths and their interactions with their nectar plants are diverse yet notoriously difficult for researchers to investigate. Apart from larger hovering moths (e.g. Sphingidae), moths that visit flowers at night are often small and cryptic. They are known to seek nectar from small white or pale colored flowers often situated at the tips of branches (Oliviera et. al. 2004; Makholela & Manning 2006; Okamota et. al. 2008; Tasen et. al. 2009). Still, moths nectaring on plants is a phenomenon rarely observed and documenting plant-moth interactions in the field is a formidable task. To complement such studies, investigating pollen found on moth specimens can be applied to further document nectar plants and quantify pollination efficiency.

Researchers have dabbled into exploring these methods to investigate life history questions relating to Lepidoptera pollination ecology (Darwin 1885; Wiklund et. al. 1979; Courtney et. al. 1982; Jennerston 1984; Tasen et. al. 2009), migration (Mikkola 1971; Lingren et. al. 1993), and even overwintering (Berkhouson & Shapiro 1994). Many of these studies used similar techniques to identify pollen with Scanning Electron Microscopy (SEM) and often removed the head of the specimen in order to extract the pollen. The techniques described here offer a less expensive and invasive alternative to using SEM to sample and identify pollen from field collected fresh or museum specimens.

Materials. Stereo microscope (7–15x magnification), hot plate, probe with fine point (see below), glycerin gel stained with pigment (see below), glass microscope slide with cover slips, small weights (~40 grams), slide warmer, reflective microscope with 10–60x objectives, camera attachment and imaging software (AxioVision Rel. 4.5), pollen keys and regional identification manuals, and/or plant samples from the field.

Probe. Wooden dowel 14 cm long and 0.2 cm in diameter, with an embedded tip of a # 2 insect pin.

Glycerin gel with stain. Add 20 g of gelatin (Crescent Chemical Company, Cat. # 23310.02) to 70 ml boiling distilled water, once thoroughly mixed, add 60 ml glycerin (Fisher Scientific, Cat. # 633-4) and 1.2 g phenol (Fisher Scientific, Cat. # G33-4), then after crystals dissolve, add 22 drops of Safranin-O stain (Fisher Scientific, Cat. # S670-25).

Collecting and Preserving Specimens. Field collected specimens should be kept in separate glass vials to prevent pollen contamination. Store moths in vials in a freezer until use in pollen analysis. This will preserve the specimen and help reduce loss of scales or pollen.

Scanning for pollen. To scan a moth for pollen, remove from the vial and pin the specimen through the thorax with an appropriately sized insect pin. Using a small piece of foam to stabilize the pinned specimen, scan the specimen for pollen under a stereomicroscope, paying special attention to the mouthparts (Fig. 1-I). If the proboscis is not visible, use a minuten insect pin to gently bend up from the base of the proboscis until fully exposed. If the specimen is not hydrated, you may need to rehydrate the specimen in a relaxing chamber long enough to rehydrate the proboscis.

Extracting pollen from specimen. Heat the glycerin gel on a hot plate at 52° C in a water bath until it has reached liquid form. Place microscope slide(s) on a slide warmer. Locate pollen on the moth using a stereomicroscope. Pour a small portion of the glycerin gel onto the microscope slide. Gently dip the probe tip into the glycerin gel. For pollen clumps, a small drop of glycerin gel works well. For individual pollen grains, a short streamer of glycerin gel works well, especially for grains embedded between the scales. Once the pollen is adhered to the probe, transfer the pollen from the specimen to the microscope slide into the drop of glycerin gel. Cover the drop with a cover slip, add a small weight on top of the cover slip (Fig. 1-II), and prepare a label for the microscope slide. Allow the slide to rest on the slide warmer long enough to enable the gel to stain the pollen (at least 24 hours). Seal the edge of the coverslip with clear fingernail polish.

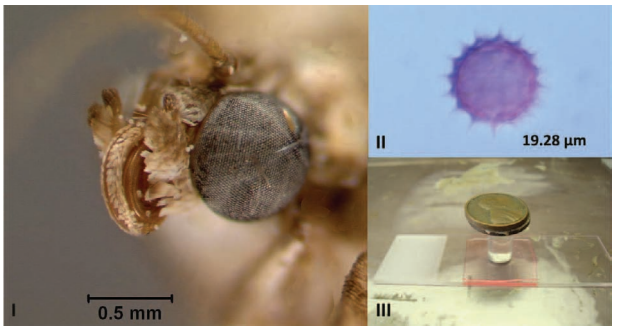

FIG. 1. **I**) Pollen grains found on proboscis and palpi of *Samea ecclesialis* (Pyraloidea). **II**) Photograph of pollen grain, *Eupatorium* sp. (Asteraceae), taken with AxioVision imaging software. **III**) Microscope slide with pollen sample in glycerin gel stain, cover slip, and weight on warming plate.

Identifying pollen: Using a reflective microscope at 40X (objective) and 1.25X (optivar), locate the pollen on the microscope slide and photograph using an attached digital camera and imaging software (Fig. 1-III). Using this image, identify the pollen with either a pollen key (Kapp et al. 2000), pollen identification manual (Jelks 2001), and/or matching pollen with pollen sample extracted from a properly identified plant (i.e. use of pollen collections can be arranged by collection managers at existing palynology institutions).

If the use of a palynology collection is not feasible, it is also possible to create a pollen library by which to match pollen. Pollen can be sampled from flowers observed in bloom within the same time and vicinity that the moths were collected. This is most beneficial when working in a habitat where the majority of plants are not yet represented in a palynology collection. Creating a pollen library with the plants in the area of study is not only beneficial for identifying pollen found on the moths, but can also be preserved in a local palynology library as a resource for future researchers.

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