A compilation of methods and techniques used by graduate students in the Canadian Pollination Initiative as presented during a professional development training workshop at Simon Fraser University, July 29-Aug 1, 2013.

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How you do it:

- Bundle tubes made of paper straws, dried bamboo or reed grass, or holes drilled into untreated wood. Create enclosure so water sheds away from the nesting tubes.
- Tubes should be about 15cm long and plugged at one end (can use papier mâché). Bees won’t use tubes not closed at one end.
- Trap nests should be set above the flower line and out of large trees having many leaves providing shade. Too much shade will encourage cavity nesting wasps (also very interesting!).
- Fasten trap nests to a stake, fence post, or any fixed structure using cord, twine, or zip-ties.
- Nesting tubes can be opened (usually wait until the fall), and the larva reared and identified. Larvae can be stored in a cool walk-in fridge (not less than 5°C) to simulate winter conditions. At home, trap nests can be stored in an unheated parking garage or shed. In wooden blocks, vials can be attached to the entrance to the nesting tube.
- When rearing after winter, be careful to keep larva as separate as possible or you will risk transmitting hyper abundant parasites such as Mellitobia and Monodontomerus. If the bees cannot be carefully sorted, leave them in the trap nests and clean the tubes out after they’ve emerged.
- For the homeowner, in this case, it is good to have an alternate trap nest – or extra tubes – so that there are enough available tubes in early spring. Some filled tubes won’t have bees emerge until early summer.
- Not all cavity-nesting bee species will use trap nests; be careful not to enhance populations of exotics or one species exclusively.
- Bee genera using trap nests include Megachile, Osmia, Heriades, Hopolitis, Chelostoma, Stelis, Coelioxys, Hylaeus, and Anthidium.

Additional resources:

Mader et al 2010, Managing Alternative Pollinators
Native Buzz Website
Xerces Society - How to build a nest box
NEST EXCAVATION FOR THE STUDY OF GROUND NESTING BEES
JENNIFER ALBERT, YORK UNIVERSITY

Background information: Ground nesting is the most widespread nesting strategy among bees. One might want to excavate bee nests to collect juvenile bees or to study nest architecture, juvenile development, phenology, brood size and/or sociobiology.

How you do it:

Steps for excavation of bee nests:

1. Block the nest entrance either before sunrise on the day of the excavation or after sunset the day before to ensure no bees are out when you dig.

2. Blow talcum powder down the hole using a straw or tube until the tunnel is full. This will make it easier to follow the tunnel(s).

3. Insert wire or similar into the hole as far as it will go.

4. Dig a trench to the side of the nest that is deeper than a typical nest and far enough away so as not to disturb the actual nest (e.g., for H. farinosus, which makes narrow and deep nests, dig around ~30 cm deep and try to start at least 30 cm away).

5. Extend the trench slowly toward the nest with the trowel.

6. When you are very close switch to the paint scraper and slowly remove one layer of dirt at a time until you hit the nest.

7. You will begin to see cells belonging to the nest, likely with juveniles in them, and will eventually come to the main tunnel or tunnels where the talcum powder will be.

8. Work your way down the tunnel from the top by slowly scraping away the dirt with your scraper until you come to a point where there are no more cells.

9. Continue excavating even below the apparent end of the tunnel to ensure that you haven’t missed anything.

Additional tips

• Keep your net close in case any adults escape.

• Many bees nest in aggregations and nest contents might get mixed up. Juveniles are often laid with their head toward the tunnel, this may help you tell which offspring belong to which nest.

• Foundresses often dig down, if you can’t find her keep digging!
Background information:

Bee tags are small plastic dots numbered 1 through 100 and coloured in one the five international colours. They come with glue and applicator. The tags are sold for a range of applications including marking mussels, crabs and snails. In honey bees and bumble bees, the tags are attached to the thorax, allowing a numbered approach to bee marking.

How you do it:

By orienting the tags at 90 degree angles, you can increase the number of possible combinations to almost 400 for just one color of tag. However, keep in mind that some numbers will be ambiguous and will therefore need to be eliminated. One general problem with bee tags is that certain numbers (6 and 9, for example) are identical and can only be distinguished if you adopt a consistent orientation. It is possible to paint bees on the abdomen with different colors in addition to tagging them to increase the number of possible marking combinations.

How to tag the subject bee:

• To collect a bee for marking, open the hive in a darkened room lit only with a red light (bees won’t fly under red light).
• Put the bee in the a refrigerator for about 5 minutes.
• If the bee has a hairy thorax, you may wish to use a fine razor blade to remove the hairs.
• Apply the adhesive on the thorax.
• Use forceps to carefully apply the tag.
• Adhere the tag directly to the thorax cuticle (so it is not lost if the hairs fall out).

Additional resources:

Sources of bee tags:
www.thebeeworks.com/catalog
(search for "queen marking kits")

Instructional videos on Youtube:
http://www.youtube.com/watch?v=YD2cCOyUatE
http://www.youtube.com/watch?v=VIY4LYxGFq8
Background information: Why identify insects in ethanol? 1) Identify while the specimen and key characters are in pristine condition, as the pinning process can take a toll; 2) Specimens retain some flexibility and body parts can be moved to ensure characters are clearly visible; 3) Get preliminary data quickly rather than wait for completion of pinning, labelling & entry into a database.

What you need: Insect specimen in ethanol with unique code (GUI), 70% ethanol, petri dishes (~5 cm / 2” diameter), Plasticene, dissecting probes and pins, soft tweezers, dissecting microscope and light source, fumehood

How you do it:

• Safety first! Work in a fumehood, as ethanol fumes can be irritating after prolonged periods. Consult your MSDS to become familiar with toxicity symptoms. Work in a fumehood comfortably by removing shelves in the cupboard underneath so your knees fit inside while seated, and lowering the sash on top of the microscope, so eyepieces protrude (see photo A at right).

• To reduce glare, fill petri dish with enough 70% ethanol to just cover the specimen. Too much ethanol will cause specimens to float around the petri dish.

• It is more challenging to get insects in the right position in ethanol than with pinned specimens, so use Plasticene shapes to position insects such that key characters are visible. Be creative and find the shapes that work best for your taxa.

• The Plasticene shapes I’ve found most useful for bees are logs (narrowed in the middle so one log works for insects of many sizes), wedges (with a depression in the top centre) and horseshoes (see photos B & C).

• The hardest body part to position so it is visible is the facial surface. Use a probe to press down lightly on the upper abdominal sterna to get a clear view of the facial features (photo D).

• Use your light source to your advantage – shift the light back and forth over features like sutures to make them easier to see. Shine your light source sideways across the microscope stage to backlight and emphasize wing venation or hairs around the edges of the body (photo E).

Other great uses for Plasticene:

○ create dividers within your petri dish to aid in sorting specimens
○ make a 3-step pinning block out of Plasticene in a petri dish
○ pinch a piece of Plasticene onto an insect pin to create a handy marker when removing specimens from a unit tray

Other resources:
MSDS for 95% ethanol:
POLLEN IDENTIFICATION
GAIL MACINNIS, CONCORDIA UNIVERSITY

Background information: To aid in the identification pollen grains caught with wind sampling devices, or from insect bodies.

How you do it:

1. Collect your pollen.
   a) To make a reference collection: ideally remove partially open buds (to avoid contamination) or using tweezers, grab several anthers and gently pull and twist. Place anthers or flowers in a labelled bag or envelope (paper best). Clean instruments with ethyl alcohol after collecting each specimen. Even if you don’t make a reference collection, be sure to take note of vegetation in your sampling area that flowers during your study.
   b) For insect-dispersed pollen: Collect pollen directly from insect bodies. (see page 8)
   c) For wind-dispersed pollen: use specially designed equipment (e.g., a Rotorod) or simple silicon greased slides facing prominent wind direction.

2. Place pollen grains on microscope slide and stain using Caberla’s solution:
   - glycerin - 5.0cc
   - 95% ethyl alcohol - 10.0cc
   - distilled water - 15.0cc
   - saturated basic fuchsin - 2 drops (or 50mg powder)

   Place cover slip on microscope slide and seal edges with clear nail polish.

3. Look at grains under microscope and record:
   a) Size of grains. Most wind-dispersed grains are between 20 to 60µm, but certain insect-dispersed pollens can be as large as 250µm.
   b) Shape of grains. Generally have elliptical or spherical shape, but can also be square, wedge, triangle, football, Mickey Mouse.
   c) Surface structure. The majority of pollen grains have pores (holes) furrows (slits). The number and types of pores and furrows varies between plants, providing the primary means of identification. For example, grasses have 1 pore, birch grains have 3 pores with a ring around each pore (annulus), and some weeds such as amaranth can have as many as 80 pores.

4. Use Google or the search engine of your choice. Once you have recorded the number and type of pores and furrows and any other visible characteristics (thickening around pores, arcs, rings) you can use these characteristic to find grains fitting your description. You can also compare grains to the reference collection in (1).

Other resources:
Canada Plants: http://www.canadaplants.ca
Australian Pollen and spore atlas: http://apsa.anu.edu.au
Saint John’s University, Center for Pollen Studies: http://www1.csbsju.edu/pollen/
University of Minnesota tools for Microscopic identification: https://tmi.laccore.umn.edu/organic/list/pollen
Medical University of Vienna, Aerobiology and Pollen information: http://www.polleninfo.org

Shapes of pollen grains (not all same scale). A. spherical monoporate monad (grass, Graminae); B. wedge shape (bulrush, Scirpus); C. square shape (ash, Fraxinus); and D. grain with two air bladders (pine, Pinus) Weber 1998.
Background information: Plant-pollinator interactions are important to ecosystem functioning. But how can we know if a visit from a potential pollinator actually leads to pollen deposition? Sampling pollen from visiting insects helps us understand the effectiveness of a pollinator, how pollen transportation may vary by taxa, and how pollen transport compares between wild and managed habitats.

How you do it:

1) Fuchsin jelly¹: This will be your medium for pollen sampling and staining.
   a) Pipette some liquid jelly onto a smooth, flat surface to cool and set. Microscope slides work well, and extra slides can be covered with Parafilm to prevent desiccation (1).
   b) With a razor, slice the jelly into consistent 3mm cubes. Cubes should be handled with clean forceps to prevent picking up debris.
   c) Extra jelly can be kept in a sealed jar and refrigerated for years. Simply melt the jelly in a water bath as needed.

2) Pollen swabs:
   a) Run a clean insect pin through a jelly cube, using the forceps to position the cube. This will act as a pollen swab (2).
   b) If you are sampling in the field, place the swab in a small vial. The vial will keep the cubes moist, will keep your swabs separated, and can be labeled to correspond with the sampled insect.
   c) Be aware of field conditions. Hot weather can melt the jelly, so vials should be kept in a cooler.

3) Sample an insect:
   a) Euthanize/immobilize your insect and place it in a vial.
   b) Use the swab to sample pollen. Target the ventral surface of the thorax and abdomen, legs, tarsi, and mouthparts (3). Corbiculae should be avoided if sampling is related to floral reproduction: this pollen will not contact the stigma.
   c) Return the swab to the vial for transportation.
   d) Note: For insects stored in ethanol, pollen can also be sampled from the ethanol. Filter it, then swab the filter paper after drying.

4) View your sample: A pollen library from the focal habitat is recommended. (see page 7).
   a) Place the cube on a microscope slide, under a cover slip.
   b) Gently heat the slide, either using a lighter or a hot plate. The cube will melt and spread thin. Do not boil your pollen.
   c) Seal the edges of the cover slip with clear nail polish to protect the jelly. The pollen can now be counted and identified (4).

Additional resources:


**Background information:** I often quantify pollen grains to measure the single visit contribution of a pollinator to plant reproductive success. Counting pollen grains on stigmas can determine how many grains a pollinator deposits in a visit, and comparing unvisited and visited anthers can determine approximately how many grains a pollinator removes.

**Quantification of pollen on stigmas: Can count manually or using digital photos and ImageJ**
Totally manual: gives you more control over focus. Using ImageJ: keeps track of grains already counted and keeps a record--useful for quality control and going back to check your data. Can also keep track of multiple grain types.

**Using ImageJ to manually count pollen grains:**
Download ImageJ
Download Cell Counter plugin
Open photo
Click “initialize”
Click “Type 1”
Click on each pollen grain
Click on “Type 2” to switch types

**Quantification of pollen from anthers, bees, etc:**
Particle counter: Established protocol, and fewer issues with pollen clumping. ImageJ: Can count the whole sample, or a larger subsample, have a record of the count, potentially faster, potentially more accurate, no special equipment needed.

*Caveat:* When pollen clumps it is difficult to count in ImageJ.

**Using a particle counter:**
Vortex sample
Weigh vials
Transfer sample to vials (rinse with saline 3x)
Fill vial halfway with saline
Sonicate
Pick anthers out. Check to make sure no pollen is left in anthers
Add more saline
Weigh vial again
Run each sample three times

**Additional resources:**
Background information: Pollen limitation occurs when an inadequate quantity or quality of pollen leads to reduced reproductive success in plants. Pollen limitation can be calculated for any reproductive measure (i.e., fruit set or seed set). Measures of pollen limitation inform us about the health of both the plant and pollinator communities.

How you do it:

- Mark pairs of stems (or flowers). Ideally whole plants will receive treatment or control, but for large plants like lowbush blueberry, you should instead be sure to have both treatment and control on the same genetic individual.

- Can use flagging tape, but beware of colours that may fade to similar shades. To avoid possible effects of tape colour on bee visitation, use same colour of tape with treatments written on tape in permanent marker. Metal tags can also be used, and a metal detector can aid in finding marked stems once fruit is mature.

- Designate one of the paired stems for supplemental pollination, and the other for open (or ambient) pollination.

- Collect pollen from a different genetic individual of the same plant species. (If the plant requires buzz pollination, electric toothbrushes work well to dislodge pollen.)

- Use this pollen to hand pollinate the flowers on the stem designated for supplemental pollination.

- Pollinate the supplemental stems as often as necessary while the flowers are open to ensure maximum possible fruit set.

- Measure fruit and/or seed set of both supplemental and open stems when fruit matures.

- Pollen limitation can be reported as a pollen limitation index or a simple difference in reproductive measures.

- Pollen limitation index is calculated as:
  - $L_{\text{(limitation)}} = 1 - \frac{\text{open}}{\text{supplemental}}$
  - Value should always be between 0 and 1

- Difference in reproductive measures is calculated as:
  - $L_{\text{(limitation)}} = \text{supplemental} - \text{open}$
  - Value should always be a positive number

Other resources:


RECRUITING HOMEOWNERS FOR FIELD WORK

JULIE WRAY, SIMON FRASER UNIVERSITY

Background information: I am investigating how the quality of the surrounding landscape influences pollinator communities in fragmented oak-savannah habitat. We specifically wanted to know if pollinators are using urban gardens surrounding the fragment for floral resources. To do this we needed homeowners to volunteer to give us access to their property. This recruitment process could be used in any survey involving biodiversity in urban areas, or for studies involving citizen participation.

How you do it:

• Select your study sites. I was interested urban-associated oak-savannah fragments, based on percentage of forest-cover in surrounding 400-meter radius.

• If possible, attend any relevant meetings by naturalist or gardening groups, regional conferences, etc. to make personal contact. Personal contact is key.

• Connect with group leaders or others associated with relevant organizations with who are willing to pass on information about your study and your request for volunteers. (For example, I established connections with the District of Saanich, the Victoria Horticultural Society, and the Native Plant Study Group - there are always organizations like this in different municipalities). Getting access is easier once you have a few people on board and can ‘name drop’.

• Develop an advertisement describing the basic purpose, methodology, and goals of your study, your contact information, and (if needed) specific locations for where you are looking for volunteers and distribute to your partner organizations. Often natural history groups have a website or newsletter and may be willing to post your ad for free.

• Advertisements can also be dropped off at individual houses in specific locations where you hope to get volunteers. Return to individual houses the following day to ask for access to property; doing this right away increases the chance they will remember you.

• One challenge with field work in urban areas: how to randomize your samples. For vegetation sampling, I overlaid a grid transparency on online aerial photos to establish permanent sampling plots.

Additional tips:

• Recruit more volunteers than you think you will need as you can usually expect some level of attrition.

• Consider heterogeneity. “Master gardeners” may be keener to volunteer but may not reflect the true urban landscape. It’s worth considering the goals of your study and how you can ensure an appropriate sample.
INCORPORATING POLLINATORS INTO HABITAT RESTORATION PLANNING

KYLIE MCLEOD, UNIVERSITY OF WATERLOO

Background information: My thesis research explores pollinator response to habitat restoration in southern Ontario’s Carolinean life zone. Restoration activities were carried out by the Grand River Conservation Authority (GRCA) 5-6 years prior to sampling on land previously used for agricultural purposes. Restoration activities included seeding with wildflower, sedge, and grass mixes, planting saplings, broadcast spraying for invasives, and digging pits and mounds. Key areas to consider when incorporating pollinators in habitat restoration planning are reviewed here.

How you do it:

1. Goal Setting: Ideally, pollinators should be incorporated into habitat restoration at the planning and goal setting stage. This would allow ecosystem managers to (1) specifically target pollinators, (2) identify how ‘success’ will be assessed, and (3) align pollinator restoration goals with overall restoration goals and to reconcile potential conflicts such as cost or ease of plant establishment.

2. Restoration of forage resources: To attract the greatest diversity of pollinating insects, forage resources should span the entire flight season and include a range of shapes, colours, and sizes. Wildflower seed mixes used by the GRCA included flowering plants with a diverse mix of floral traits and bloom times.

3. Restoration of nesting resources: Nesting resources include nest sites and materials. Bare ground is common in early restoration providing an abundance of potential nest sites for ground nesting species. Stem nesters such as Ceratina can find nest sites in quick to establish plants such as teasel and raspberry. Cavity nesters are relatively uncommon in early stages. Adding artificial nest sites or woody debris could increase the number of potential nest sites while woody plant species are still establishing.

4. Considering the role of exotic plant species: Exotic plants are incorporated into most plant-pollinator communities as forage and nesting resources. In restored habitats invasive exotics may need to be removed. Planning for functional replacement of these species will help fill any resource gaps left by their removal.

5. Evaluating restoration success: Metrics for assessing restoration success can be structural (e.g. abundance, diversity, species composition) or functional (e.g. indicator groups, plant-pollinator interactions). Restoration goals will help determine the most appropriate metric(s). Identifying appropriate reference sites is a significant challenge as comparable remnant habitats may be uncommon or absent. As a result, evaluating community function is often more useful than trying to replicate existing communities.

Additional resources:


GIS AND LANDSCAPE CATEGORIZATION
LINDSEY BUTTON, SIMON FRASER UNIVERSITY

Background information: Geographic Information Systems (GIS) are a powerful tool when looking at landscape scale ecological questions. It’s probable that work has already been done to map out the landscapes around your field sites, however, these resources may not be exactly what you’re looking for. Here I look at what your options are in terms of landscape categorizations, and one way to deal with shapefiles that may not match your research criteria.

What is your research question, and how will you go about analysing it?: I’m interested in how landscape influences pollinator abundance. I had abundance data from 19 field sites, which gives me enough power to look at 3-4 different landscape types, but obviously not 20. Knowing how many landscapes you have the power to look at will affect how you collect your data, as well as how you categorize it. You also want to make sure that all of your field sites are a reasonable distance apart so that your results are not confounded by spatial autocorrelation. I looked at a 1km radius surrounding each site, so I made sure my sites were at least 2km apart.

GIS Software: Decide on which one you want to use for your maps and learn it! I used ArcGIS, which is one of the more powerful programs out there at the moment. It can be expensive, but your university library should have a single use license that you can access. Alternatively you can use a program called GRASS, which is open source (and therefore free!). Although I haven’t used it before, I’ve heard very good things.

You can download landscape shapefiles (.shp) from a number of sources online: These sources contain everything from digital elevation data, land cover types, the national road network, satellite imagery, and aerial photos. Find what you want, download it, and import it to your chosen program. (See links below). Once opened, check that your chosen shapefile matches recent aerial photos. You can very easily get these for free off of Google Earth. ArcGIS also has a Bing Maps baselayer that works well for this. If they don’t match with you may have to create your own shapefiles. ALWAYS ground truth at least a subset to check file accuracy.

Make sure your categorizations are useful to answering your question: If landscape categories are not useful, you’ll have to ground truth. The land cover shapefile from GeoBase for example does not go into detail as to types of crops that would provide forage for bees (flowering vs. non flowering) though does distinguish between annual and perennial crops.

Ground truthing tips: Record everything! You can always group landscape types and reduce later. I printed out a page for each of my field sites showing the aerial photo and a 1km radius around the site, which you can fill in while some one else does the driving.

DISCLAIMER: This kind of ground truthing is more useful for urbanized landscape with easy road access. It’s less useful for remote locations that are difficult to compare to road networks.