A pollen library to study plant-pollinator interactions in changing climates

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Abstract:

The effects of climate change on native bees may depend on the flexibility of their associations with plants: generalists may be more indifferent to shifts in plant flowering times. However, traditional field observations for measuring specialization can be time consuming and difficult to do for more than a few species. An alternative approach is to compare pollen grains collected by bees to positively identified pollen images. To facilitate this approach with native bee communities near Laramie, WY, I created a pollen library of SEM images for 93 distinct entomophilous plant species in three sites in southeastern Wyoming. Specimens have corresponding pollen samples and vouchers and are stored in the Dillon Lab, Biological Sciences room 437, at the University of Wyoming. The information collected in this study can be used as a basis for future studies in these sites involving both plant and pollinator data and can help us determine how well bees might respond to climate change.

Introduction:

Mutualisms between pollinators and the flowers they visit provide vital ecosystem services in both natural and agro-ecosystems (Berenbaum et al., 2007). In the case of bees, successful pollination depends on synchrony between flowering and bee flight time. Climate change, however, can differentially affect bee and plant phenologies (timing of life-history events), leading to potentially disastrous plant-pollinator mismatches (Memmott et al., 2007). Increased specialization in plant-pollinator communities may increase susceptibility of those
communities to phenological mismatch. Specialist pollinators visit very few, or sometimes only one, plant species. Similarly, some plant species may rely on a single bee taxon for pollination. Changes in climate and land use may particularly harm specialists which are already limited in their available resources because of their tight mutualisms, leading, in the worst case scenario, to local extinctions. Generalists, however, may be more resilient because of their broader resource base.

Although estimating specialization in plant-pollinator networks is critical for identifying species and communities most susceptible to decline, it is rarely attempted because it is so time consuming and difficult. The standard approach is to record flower visitations by pollinators of interest. However, missing plant-pollinator interactions may often be false negatives because the observer just didn't watch for long enough. Also, observations can only be verified for bees that are easily identifiable in the field, but most native bees are impossible to identify without the aid of a microscope. An alternative approach is to identify pollen collected by bees by comparison with a library of pollen of positively identified plant species. Species composition and relative quantity of pollen grains sampled from foraging bees and their nests can provide an excellent characterization of the local plant-pollinator network structure (Dorado et al., 2011). This approach requires a “pollen library”, which is a positively identified collection of preserved pollen grains that can be compared with unknown pollen collected from bees and their nests.

In this study, a pollen library was created to facilitate characterization of plant-pollinator networks at two sites near Laramie, WY. This study is particularly valuable because it allows for comparison with an extensive historical dataset on bee and flowering plant abundance and phenology. Tepedino (1980, 1981, and 1982) sampled bees and flowers at two sites southwest of Laramie, WY throughout the season in 1975 and 1976. The Dillon lab is re-sampling these sites
following Tepedino’s standardized protocols to understand how native bee and plant communities might be responding to climate change. The pollen library was created for all entomophilous (with known insect interactions) flowering plants found in these three sites throughout the summer season.

This project lays the groundwork to determine which plants are pollinated by which bees and the degree of specialization of the bees and plants in these networks. This knowledge will be crucial in determining the susceptibility of native bees and plant species, as well as the community as a whole, to anthropogenic threats (e.g. climate change, habitat loss, agriculture).

**Material and Methods:**

Plant samples were collected weekly from May 7th to August 8th, 2012 at three sites in the Laramie Basin in Albany County, Wyoming: Red Buttes (N 41.1703 W 105.5801; 2237 m), Dirt Farm (N 41.2090 W 105.5462; 2282 m), and Boulder Ridge (N 41.0409 W 5658; 2456 m). Plants were collected as they were encountered, and only collected twice if original samples were insufficient to constitute a voucher and pollen. Plants were identified to genus and species using plant guides (Dorn, 2001; Williams, 1992; States & States, 2004) and by personal communication with Dr. Ronald L. Hartman, University of Wyoming Herbarium Curator, or B. Ernie Nelson, Herbarium Manager.

We followed Hartman & Nelson (2009) in plant collection and voucher preparation. Plants were removed from the substrate by first loosening roots with a hammer and then placing entire plant samples in one gallon plastic bags. Flower heads were removed and kept separately in plastic bags in the freezer until processing the pollen. Plants were pressed immediately or after no more
than three days in the refrigerator. After removing excess soil from the roots, plants were pressed in 11.5” x 15” sheets of newspaper, separated by sheets of corrugated cardboard. After drying in a plant-drier (Rocky Mountain Herbarium), plant vouchers were glued (Elmer’s multi-purpose white glue) to 16” x 10” x 1/8” thick, acid-free foam core (UW Bookstore, CAT 4:HTB900-361). Voucher information was laser-printed onto 2” x 4” labels which were affixed to the voucher sheet prior to shrink-wrapping the entire sheet (Window insulating kit shrink wrap). All vouchers are stored in alphabetical order based on genus and species name in two 46.6 quart, watertight storage boxes housed in the Dillon Lab at the University of Wyoming (Biological Sciences 437).

Pollen was extracted following procedures in Kearns & Inouye (1993), with some modifications. Flower heads were removed from freezer, left to thaw for 15 min, and then anthers were removed and rubbed across a 250 micrometer wire mesh sieve (A.S.T.M. E-11 Specification, Advantech US Standard Testing Sieve, No. 60). For flower-heads that were too small to allow separation of the anthers, the entire flower was rubbed across mesh. Particles were caught in a watch-glass and the mesh was rinsed with 75% and 95% ethanol. Excess liquid was then pipetted out of the watch-glass and the remaining particles were rinsed from the watch-glass, through a funnel, into glass sampling jars. The sieve, watch-glass, funnel, and pipette were thoroughly rinsed between flower samples to prevent contamination of subsequent samples.

To image pollen, I extracted small samples from glass sampling jars using disposable micropipets (Wiretrol II) by dragging horizontally-turned pipettes through settled sediment at the bottoms of the jars. I placed the samples on black, double-sided tape on scanning electron
microscope stubs. I was careful not to touch the tape with the pipettes so as not to confound the resolution in subsequent pictures. The tape made re-using stubs possible, as pollen grains are otherwise very difficult to remove when dried to surfaces. SEM Images were taken at 600-6000X resolution and 10-100 μm magnification on a Hitachi TM 1000 Scanning Electron Microscope housed in the Robert A. Jenkins Microscopy Facility at the University of Wyoming. Labeled and cross-referenced images are stored in the Dillon Laboratory.

**Results:**

Over the course of 4 months, I collected 127 plant specimens during 50 days in the field. These collections allowed me to make 108 vouchers for 93 flowering plant species. 54 voucher specimens were collected from Boulder Ridge, 30 from Dirt Farm, and 24 from Red Buttes. All vouchers are stored in the Dillon Lab, with the exception of one voucher, *Liatris lugilistylis*, which is stored in the Rocky Mountain Herbarium at the University of Wyoming, as it is a rare find in Albany County. 119 pollen samples were collected from 93 species. 66 pollen specimens were collected from Boulder Ridge, 30 from Dirt Farm, and 23 from Red Buttes. A total of 509 SEM pollen images were taken from plants collected at the three sites: 269 images of Boulder Ridge plants, 136 images of Dirt Farm plants, and 104 images of Red Buttes. Plant vouchers, pollen samples, and SEM images are all cross-referenced to facilitate future expansion, modification, and use of the pollen library. Unique codes for all specimens (based on site and collection order) facilitate rapid localization of SEM images from plant vouchers and vice versa. All collected specimens were initially labeled by their site and then the number collected.

**Discussion:**
Although I was able to create a nearly exhaustive pollen library for these sites, future continued work with the library is necessary in several areas. I was unable to make a voucher of flax (*Linum lewisii*) because it was so fragile that the dried plant fell apart immediately when I touched it. I was able, however, to collect pollen for this species. Two pollen vials were accidentally dropped and broken: DF 17, *Linum lewisii*, and DF 26, *Sphaeralcea coccinea*. Another sample of pollen, BR 13, exists to represent *Linum lewisii*, but there was no duplicate for *Sphaeralcea coccinea*, so this pollen data for this species was lost. Voucher samples BR 38 (*Allium* spp.) and RB 12 (*Agoseris glauca* and *Crepis runcinata*) had contaminated pollen because I was unaware that these were distinct species and thus lumped them together into one sample. Once all three species from BR 38 were identified, they were labeled and now are useful as a taxonomic tool to help differentiate future *Allium* samples. Only one of these three species was collected separatedly, *Allium cernuum* (RB 6), leaving us without data for the other two *Allium* species. RB 12’s voucher was separated into two parts, A, *A. glauca*, and B, *C. runcinata*, and I was able to use additional samples of these two species to prepare proper pollen samples. Fortunately, I had a previous pollen sample (RB 5) for *Agoseris glauca* and I collected RB 15 to provide a pollen record for *Crepis runcinata*.

All specimens were identified to species where possible. It was not possible to identify 9 of the specimens to the species level, but, in addition to vouchers for all of these, we have pollen and image data for 8 of these 9 species. When the taxonomy of these groups is resolved we will be able to add them to the database. The nine specimens are as follows: RB 10 was an unknown species of *Potentilla*, but this sample was otherwise lost and contaminated, so it is no longer part of the data set. BR 17 and DF 6 were both unknown *Erigeron* species. DF 23 and BR 33 were both unknown *Cryptantha* species. *Cryptantha* appeared uniform in the field, so these may be the
same species, and are cross-listed in all of the samples. This can be changed if they are, in fact, two distinct species. RB 3 is an un-identifiable species of *Dodecatheon*. BR 2, *Erysimum spp.*, BR 3, *Thermopsis spp.*, and BR 5, *Lesquerella spp.*, were all collected too early in the season to be identified to species level, because they were not yet sexually mature. Still, pollen and images were recorded for all three. Their “duplicate” specimens are thought to be BR 24, *Erysimum capitatum*, BR 23, *Thermopsis rhombifolia var. montana*, and BR 5, *Lesquerella parviflora*. It is likely that these specimens are the same species, especially as they were all collected from the same area where they were originally found in the site but later in the season when they were sexually mature. However, they could be different species such that their current co-listing on labels in vouchers and pollen samples will have to be changed in the future.

Pollen samples were usually mildly contaminated in some way, so that the overwhelming majority of grains were clearly the same, but there were a few outliers. I expect that these discrepancies can be explained by pollen contamination in the field and also by potential contamination during pollen extraction. Rinsing the sieve thoroughly between uses may not have eliminated cross-contamination of samples. In some cases, a sample of pollen did not have an overwhelming majority of grains that all had similar morphologies. In these cases, the specimen would be sampled again and imaged a second time. If this “re-do” did not clear up the problem, then images for this specimen were all kept, but they are clearly not as useful as they could be if they had been less diversified.

**Conclusion:**

The research that has been done here is useful just as it is, without any further work or modification. Currently, researchers in the Dillon Lab are working on projects that involve plant
identification. Because these three sites are so well-used by the Dillon Lab, the vouchers that have been collected will be a wonderful tool that Dr. Dillon’s students can use to compare plant pictures or samples from the field to. In addition, the pollen samples from all of these species can have myriad uses in the Dillon Lab’s current projects. Researchers will be able to consult them, and take sub-samples of them to stain and to examine under the microscope.

However, with some modification, this data will become even more applicable. The information collected will be stored in the Dillon Lab and it is expected that a future researcher will be able to sort the pictures and create a database to catalog pollen grains based on shape, size, date collected, and taxonomy. Once this is completed, the “pollen library” will provide a useful tool for quick pollen identification. Studies can then be conducted on insects, mostly bees, foraging in the three sites mentioned. Researchers will be able to remove pollen off of bees’ legs and then take images of this pollen and compare those images with those in the pollen library to deduce which species of plants the bees have been visiting. Data on bee visitation has proved very difficult to collect in the past. Using pollen to chart bee activity is a way to solve this problem. Now, researchers will be able to see which bees are on which plants and be able to find to what levels they are specialists or generalists. This information will allow us to better predict how bees will respond to climate changes and potential loss of host plants.

References:


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