Pollen Collection
Information and Protocols
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Pollen Identification, Collection, and Pollen Experts

Existing online pollen libraries (these libraries do not have images of hummingbird plant species of interest). Common Southwestern plant species that pollinators prefer. Pollen experts that may be interested in helping hummingbird banders collecting pollen from birds and other scientists/citizen scientists who are collecting pollen from plants. (All potential collaborators have been contacted).

Making Mounts of Pollen Grains
- Mounting techniques (glycerol wet mounts, air mounts, glycerol jelly, non-water based mounting media)
- Reading Material

An Overview of Mounting Media for Microscopy
http://www.microbehunter.com/an-overview-of-mounting-media-for-microscopy/

Pollen Identification- Discover Life Website
http://www.discoverlife.org/mp/20q?guide=Pollen

Pollen Identification Activity- The Virtual Forest Initiative of the Black Rock Forest Consortium
https://blackrock.ccnmtl.columbia.edu/paleoecology/identification

Pollen Identification- University of Arizona Catalog of Internet Pollen and Spore Images
http://www.geo.arizona.edu/palynology/polonweb.html

Pollen Identification- MicrolabNW Phptomicrograph Gallery
Pollen Under the Microscope
http://www.microlabgallery.com/PollenFile.aspx

Pollen Forecast (Perhaps this could help us identify what kind of pollen is floating around where and when?)
https://www.pollen.com/forecast/current/pollen/80305

Pollen Identification-Pollen Image Library
http://www.saps.org.uk/pollen/pollen/index.htm

The Global Pollen Project: a new tool for pollen identification and the dissemination of physical reference collections- a scientific article

Plant Identification- Lady Bird Johnson Wildflower Center
Native Plant Database, Search for native plants by scientific name, common name or family. If you are not sure what you are looking for, try the Combination Search or our Recommended Species lists.
Pollen Experts

• AASP, The Palynological Society
http://palynology.org
AASP – The Palynological society (AASP-TPS) was founded to promote the science of palynology for and in behalf of the public interest. It is a non-profit organization, to foster the spirit of scientific research among its members and others engaged in this field of science. It also aims to gather information and data on this subject that is disseminated to its members and to the general public. AASP-TPS was established as the “American Association of Stratigraphic Palynologists” following a survey conducted by Herbert Sullivan in spring of 1967. It held its first meeting on December 8, 1967 in Tulsa, Oklahoma.

• Palynology Instruction- List of courses and professors around the world
http://www.geo.arizona.edu/palynology/classes.html

• International Federation of Palynological Societies
http://www.geo.arizona.edu/palynology/ifps.html
The International Federation of Palynological Societies (IFPS) is a federation of regional, national, linguistic, and specialist palynological organizations of the world. Its goals are to advance knowledge in palynology and related subjects by promotion of international cooperation and sponsorship of regular meetings between palynologists of all countries and regions. IFPS publishes the WORLD DIRECTORY OF PALYNLOGISTS irregularly, and PALYNOS Newsletters semiannually.

• University of Florida- Florida Museum
https://www.floridamuseum.ufl.edu/index.php/paleobotany/palynology/about/

• Northern Arizona University
https://nau.edu/research/1000-words/pollen/
The Laboratory of Paleoecology at Northern Arizona University has a state-of-the-art pollen-processing laboratory to identify and count pollen. This laboratory includes five research-grade light microscopes, two binocular microscopes, and a modern pollen reference collection of ca. 2500 specimens. In addition, the facility has all of the field equipment necessary for collecting sediment cores from lakes and wetlands, including coring platforms and coring equipment. Photo: IDEA Lab

Hummingbird Flowers- The best 18 plant families for natural nectar
http://www.hummingbirdsociety.org/hummingbird-flowers/
Anisacanthus- desert honeysuckle
Pollen Experts: Individuals to reconnect with.

Scott Anderson: Scott.Anderson@nau.edu
Extremely helpful, open to the idea of collaborating, would like to digitalize his pollen library but predicts it will be very expensive.

Stephen T. Jackson: jackson@uwyo.edu
Jane M. Beiswenger: jmbeis@uwyo.edu

Owen K Davis: odavis@email.arizona.edu
Chad Lambert Yost (palynologist): chadyost@email.arizona.edu (palynologist)
John M Logan (palynologist): jlogan@email.arizona.edu

Vaughn M. Bryant: vbryant@tamu.edu
Capulin Volcano National Monument Pollen Protocol

Zach Cartmell: zach_cartmell@nps.gov
Cassy Hill: Cassy_Hill@nps.gov

Syringes and glycerin jelly used. Omit phenol or any other antiseptic.

It is suggested that before the pollen samples are of immediate use, there must first be a sufficient pollen library (including native and non-native plants of the area).

First collect from flowering plants to begin building a sufficient pollen library.

1) First step to proper pollen identification is proper flower identification.
   Used the syringe with the glycerin gel. With the exposed gel on the syringe, dab the stamen of the flower. The portion of the gel with the sample can then be removed with a sterilized exacto knife and put into a properly labeled container/baggy.
   If the flower has multiple blooms, it is acceptable to remove a bloom and undergo the pollen collection process in the “lab”.
   If the plant species is unknown, take a picture of the flower and the full plant in order to identify it later. If there are multiple plants, a species sample can be harvested for ID later.

2) Once pollen samples have been collected, it is time to start heat-fixing the samples on a glass slide. If gel has been cut already proceed to step 4.
3) Place the syringe sideways with the gel laying against the center of the slide and using an exacto knife slice off a few mm of the gel.
4) Using sterile forceps, position the gel where the sample side of the gel is flat down on the slide.
5) If the heat fixing process is postponed, place the cover slip on top of the gel and place the sample in the properly labeled plastic baggy. Set aside in a cool dry place.
6) With distilled water was both the exacto knife and forceps, and dry completely. Change out gloves and repeat the process with other samples.

Heat-fixing the pollen sample
1) Heat a 250 ml beaker of water on a hotplate set to 65-75 degrees Celsius/150-170 degrees Fahrenheit.
2) In order to melt the gel on the slide, place the slide over the top of the beaker.
3) Place the cover slip on top of the gel once it has melted. Be careful not to trap air bubbles.
4) Once the cover slip has been placed and is secure, the sample is ready for observation.
5) Once observed and identified, label the slide with species name, date and time of when sample was obtained.
6) Store the slide in the slide book.

It is advised to heat-fix the sample as soon as possible.
Collecting Pollen Samples from Hummingbirds

1) Take the syringe with the glycerol gel to collect the pollen that is around the bill and the head of the bird. Dab the top, sides and bottom along the full length of the bill. Do not let the sides of the gel or the syringe touch the bill. Dab on the head of the bird only where pollen is possible.

2) Repeat steps 2-6 from previous protocol as well as the heat fixing pollen process. If pollen was visible on the bird, write the corresponding acronym/label in order to communicate the color and location of the visible pollen on the bird.

3) Set up your hot plate with a beaker of water on it.

4) Cut the gel from the syringe and place it on a clean slide. Place the slide over the beaker the beaker. Leave the gel on until it has melted. Be careful that the gel does not stay on the heat for too long as it can denature the pollen.

5) Once the gel has melted enough, place a cover slip on top and remove the slide from the heat.

Capulin Volcano National Monument staff member collecting pollen from a Black-chinned Hummingbird’s bill.
To sample pollen, we made fuchsin jelly. The pink dye allows many of the pollen surface features to be visible under a microscope. The recipe can be found here: http://tiee.esa.org/vol/v2/experiments/pollinate/pdf/pollinate.pdf. I would like to note that we did not use the phenol or gelatin from the recipe. Once the jelly was cool, we would pinch out a small piece with forceps and dab over the surface of hummingbird bills.

Once you have done your swab, you can put this in an epitube for later. Put the piece of jelly on a microscope slide and hover 4 inches above a bunsen burner flame until it has liquefied SLOWLY. You don’t want to melt it too fast or too close or you will denature the pollen. You can then put a slide cover on and get to identifying.

There are a couple of problems with using this method:

1) Many types of pollen are the same shape and are thus unidentifiable. Tricolpate pollen seemed to be the most common variety of pollen. If you make a reference collection of pollen collected from flowers in the field, I’m sure you will be able to find a handful of identifiable pollen by species. We found quite a few species that were diagnostic using this method, plus it is cheap!

2) Don’t rely on size of pollen to identify species as there is both variation that exists with respect to size within a species and different pollen grains may have absorbed/lost more water than others.

3) If you want to have a clearer picture of what all of the pollen is, you can do some pollen metabarcoding using the Illumina Miseq. This has finer taxonomic resolution than visual identifications, but not always. Cost is ~$20 / sample without labor, so not cheap, but in many instances better than dealing with a huge mess of pollen slides. You can multiplex 392 samples into a single Illumina run for maybe ~24 total hours of sample prep time. We are going to try this this summer and use tape to collect pollen, then wash the pollen off of the tape using ethanol or buffer.

4) If you decide to use tape, make sure to store in the fridge or freezer.

**Gel Preparation of Basic Fuchsin Gelatin and Pollen Slides:**


**Ingredients**
- distilled water, 175 mL
- glycerin, 150 mL
- gelatin, 50 g
- crystalline basic fuchsin as desired- enough to make solution the color of a fine claret
- crystalline phenol, (Phenol is not used as it can be harmful to the hummingbird).

**Steps to make jelly**
1) Add gelatin to the distilled water in a beaker and heat until the gelatin dissolves.
2) Add 150mL of glycerin
3) Add phenol (usually skip this step)
4) Add basic fuchsin crystals a few at a time until the solution is the color desired. If it is too light then the pollen will not be stained, but if it is too dark the stain may mask the details of the pollen.
5) Filter the solution through glass wool or cheesecloth.
6) Pour into sterile containers such as petri plates. If phenol is not used, refrigerate the plates and slides. If not they will only keep about a month.

**Preparation of Pollen Slides**

1) Keep the prepared slides and the unused jelly out of the sun and in a cool enough place so they do not melt.
2) With a dissecting needle, cute small cubes of the jelly out of the petri plate,
3) Brush the cube of jelly against an anther containing pollen, or on an insect.
4) Place the cube containing the pollen sample on a glass slide
5) Place a cover slip on top of the cube of jelly
6) Gently heat the slide over a candle flame until the jelly melts. Do not overheat.
7) Label the glass slide (date, species sample was collected on).
Our protocol uses police fingerprint-lifting tape, an upgrade from the ordinary Scotch tape provided in the Nabhan/ASDM collecting kit. I lay a narrow pre-cut strip lengthwise down the bird's bill and crown, gently smooth it with a finger to ensure good adhesion to the pollen, then lift it starting at the bill end and apply it to a glass microscope slide labeled with the bird's band number and the date. We do this only on birds with visible pollen and will sample the throat and ventral surface of the bill if pollen is evident there. My collecting bias has been in favor of small but diverse samples over quantity and duplication. We have several hundred slides archived from previous seasons, waiting for someone to help us identify the pollens.

We also note on our data sheets the color and location of any visible pollen on all birds, using alpha codes for colors and numbers for location (1 = bill tip, 2 = mid-bill, 3 = bill base, 4a = forecrown, 4b = chin/throat). If we can ever get the pollen colors and locations correlated to specific flowers, it will help us understand the phenology and relative importance of the nectar resources along the San Pedro River.
**Moth Pollen Collection by Atwater 2011**

A simple technique to sample pollen from moths and its applications to ecological studies by Atwater 2011.

Materials needed:
- Stereo microscope (7-15x magnification)
- hot plate
- probe with fine point
- glycerin gel stained with pigment
- glass microscope slide with cover slips
- small weights
- slide warmer

Glycerin Gel with Stain
1) Add 20 g of gelatin to 70 ml boiling distilled water
2) Once thoroughly mixed, add 60 ml of glycerin and 1.2 g phenol
3) After crystals dissolve, add 22 drops of Safranin-O stain

To scan a moth for pollen, remove from the vial and pin the specimen through the thorax. Scan the specimen for pollen under a stereomicroscope. Extracting pollen from specimen. Heat the glycerin gel on a hot plate at 52 degrees C in a water bath until it has reached liquid form. Place microscope slides on a slide warmer. Locate pollen on the moth. Pour a small portion of the glycerin gel onto the microscope slide. Gently dip the probe tip into the glycerin gel. 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. 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.

Identifying pollen:
Pollen key, Pollen and spores, Kapp et al. 2000 or a pollen identification manual, Allergy pollen keys with images Jelks 2001, and/or matching pollen with pollen sample extracted from a properly identified plant. 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 resources.

Pollen analyses for pollination research, unacetolyzed pollen –Gretchen D. Jones
Collecting pollen internally and externally
For best results use slides that have a thickness of 0.93-1.05mm.
18mm square cover slip easily fits onto a 15X75 mm glass slide so that the slide can be labeled and the covers slip sealed to the slide. Cover slips should be a 1 or 1.5 with a thickness of between 0.13 to 0.19mm. Thinner cover slips are more fragile and make crack more easily.
Glycerol Jelly Mounting Media for Pollen According to Kisser

### Materials

- Glycerin
- Unflavored gelatin powder
- Distilled water
- 250 mL beaker
- 100 mL graduated cylinder
- Hot plate w/magnetic stirrer w/stirbar
- 1 mL syringes
- Red food coloring

### Instructions

**Step 1:** Gather Equipment

**Step 2:** Put 10 g of gelatin powder into a 250 mL beaker

**Step 3:** Add 35 mL of distilled water to the gelatin

**Step 4:** Add 30 mL of Glycerin
**Step 5:** Set the beaker on a hotplate and allow the water to warm and dissolve the mixture (About 40 degrees Celsius). Place a stirbar in the mixture to stir.

**Step 6:** Add enough drops of red food coloring to get the mixture to turn dark red.

**Step 7:** After the gelatin is completely dissolved (after about 10-15 minutes) remove from the hotplate.

**Step 8:** Allow mixture to cool slightly.
Step 9: Use the syringes and draw 1 mL of the gelatin mixture into each of them. Do this until there is no mixture left.

Step 10: Place the syringes in the refrigerator to solidify.

Helpful tip for making gel:

Use a hot plate if available and set it to the suggested heat of setting of 40 degrees celsius. We improvised and used a single burner. The heat must have been too hot and concentrated and it actually burned our mixture. It smelled terribly and we had to start over again. When we remade the mixture, we hovered the beaker and placed it on the corner vs. the center.
Pollen Collection Using CAVO’s Method

Step 1: Gather Equipment
Make sure to cut the tip of your syringes so that the gel is exposed to the pollen evenly.

Step 2: Dab the stamens of the flower with the gel. Make sure to get it as centered as possible and avoid getting pollen on the edges.

Step 3: Place the syringe with pollen into a labeled baggy. The bag should be labeled with location, date, initials, plant species or description of the plant.

Step 4: If you are unfamiliar with the plant and would like to identify it later, make sure to take pictures of the flower, leaves, and overall shape of the plant.

Helpful tip for collecting pollen: If you are in a place that allows you to collect the flowers and if there are plenty of blooms on a plant, you could also take the flower, place it in a labeled ziploc bag and collect the pollen in the lab and immediately make pollen slides from them. You may reuse syringes until you are out of gel.

Materials

- Scissors
- Gel syringes
- Ziploc bags
- Sharpie
- Camera to document flowers
Creating Pollen Mounts

**Materials**

- Scissors
- Gel syringes
- Tweezers
- Beaker full of water
- Hot plate
- Glass slides
- Cover slips
- Sharpie

**Step 1: Gather Equipment**

**Step 2: Using sterile scissors, cut off as much of the gel that is needed to make a slide mount (only a few millimeters).**

**Step 3: Using sterile forceps take the piece of gel from the scissors to place on the slide.**

**Step 4: Position the gel where the sample side of the gel is flat down on the slide.**
Step 5: Have a 250 mL beaker with water being heated on a hotplate to 65 - 75 degrees C or 150 - 170 degrees F. Melt the gel pollen sample taking the slide and suspend it above the hot water.

Step 6: Allow the gel to melt.

Step 7: Once the gel has been melted, remove from the beaker and place a cover slip over the melted sample. Be careful not to trap air bubbles.

Step 8: Once the cover slip has been placed and is secured, the sample is ready for observation. Make sure all slides are labeled correctly and stored away in a cool and safe place.

Helpful tip for making pollen mounts: In order to avoid air bubbles in slides, try placing the cover slip on one end of the sample and letting it fall on the other end instead of dropping it down evenly or you can slightly press down on the corners of the cover slip for a few seconds to help fix it in place.
Pollen Observation

**Step 1:** Use a light microscope to observe your slides.

**Step 2:** A standard microscope has 3 to 5 objective lenses that range in power. Start with the lens with the least magnification and work your way up. Be careful that the objective lens doesn't touch the slide as it could destroy the specimen.

**Helpful tip for observing slides:**

*In order to be successful in your pollen identification, make sure to create proper pollen mounts. Below is a slide with too many air bubbles.*

*We would like to thank the University of Colorado Museum of Natural History for allowing us to borrow their microscopes and space that helped us greatly in moving forward with this pollen project. A special thanks to Cathy L. Regan and Rebecca Coon.*
<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
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<td>Aconitum columbianum</td>
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<td>Plant Name</td>
<td>Common Name</td>
</tr>
<tr>
<td>----------------------------</td>
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</tr>
<tr>
<td>Ribes aureum</td>
<td>Golden Currant</td>
</tr>
<tr>
<td>Ribes cereum</td>
<td>Squaw Currant</td>
</tr>
<tr>
<td>Ribes inerme</td>
<td>Whitestem Gooseberry</td>
</tr>
<tr>
<td>Ribes montigenum</td>
<td>Mountain Gooseberry</td>
</tr>
<tr>
<td>Ribes sanguinium</td>
<td>Pink-flowered Currant</td>
</tr>
<tr>
<td>Ribes spp</td>
<td>Various Currants</td>
</tr>
<tr>
<td>Ribes viscosissimum</td>
<td>Sticky Currant</td>
</tr>
<tr>
<td>Robinia neomexicana</td>
<td>New Mexico Locust</td>
</tr>
<tr>
<td>Rosa woodsii</td>
<td>Woods’ Rose</td>
</tr>
<tr>
<td>Rubus parviflorus</td>
<td>Thimbleberry</td>
</tr>
<tr>
<td>Rubus spectabilis</td>
<td>Salmonberry</td>
</tr>
<tr>
<td>Salvia dorri</td>
<td>Hairy Sage</td>
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<tr>
<td>Sambucus racemosa</td>
<td>Red Elderberry</td>
</tr>
<tr>
<td>Symphoricarpos albus</td>
<td>Common Snowberry</td>
</tr>
<tr>
<td>Symphoricarpos occidentalis</td>
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</tr>
<tr>
<td>Symphoricarpos rotundifolius</td>
<td>Round-leaved snowberry</td>
</tr>
</tbody>
</table>

Plants from Colorado, Wyoming, South Dakota, Idaho, Montana, and North Dakota

Black-chinned, Ruby-throated, Calliope, Broad-tailed, and Rufous Hummingbirds
Pollen images shared by: Nicolas Alexandre- nalexandre@berkeley.edu
Irina Chen- chenirina.2014@gmail.com

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Aconitum columbianum/ Western Monkshood

Allium geyeri/ Geyer’s Onion

Apocynum cannabinum/ Indian Hemp
Aquilegia caerulea. Rocky Mountain Columbine - Colorado Blue Columbine

Aquilegia caerulea

Aquilegia elegantula. Western Red Columbine
Aquilegia elegantula.3 / Western Red Columbine

Campanula parryi.2 / Parry’s Bellflower
Campanula parryi / Parry’s Bellflower

Castilleja sulphurea / Sulphur Indian Paintbrush

Castilleja sulphurea.2 / Sulphur Indian Paintbrush
Castilleja miniata/2/Giant Red Indian Paintbrush

Castilleja miniata/ Giant Red Indian Paintbrush

Castilleja rhexifolia/Split Leaf Indian Paintbrush
Chamerion angustifolium/Fireweed

Claytonia lanceolata/Western Springbeauty

Claytonia lanceolata
Corydalis aurea/Golden Corydalis

Corydalis caseana.2/Sierra Fumewort

Corydalis caseana.3/Sierra Fumewort
Corydalis caseana

Delphinium barbeyi.2/Tall Larkspur

Delphinium barbeyi.3/Tall Larkspur
Delphinium barbeyi.4/Tall Larkspur

Delphinium barbeyi

Delphinium nutallianum/Nuttall’s Larkspur
Delphinium nutallianum.2/Nuttall’s Larkspur

Dodecatheon pulchellum.2/Shooting Star

Dodecatheon pulchellum/Shooting Star
Erythronium grandiflorum / Glacier Lily

Frasera speciosa / Elkweed
Gentianopsis crinita/Greater Fringed Gentian

Geum.2/Avens- in the rose Family

Geum.3
Hydrophyllum fendleri/Fendler’s Waterleaf

Image Not Available

Hydrophyllum fendleri.2/Fendler’s Waterleaf

Image Not Available
Ipomopsis aggregata.2/Scarlet Gilia

Ipomopsis aggregata/Scarlet Gilia

Ipomopsis tenuituba/Slendertube Skyrocket
<table>
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<tr>
<th>Image</th>
<th>Description</th>
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<tbody>
<tr>
<td><img src="image1.png" alt="50 µm" /></td>
<td>Iris missouriensis/Western Blue Flag, Rocky Mountain Iris</td>
</tr>
<tr>
<td><img src="image2.png" alt="50 µm" /></td>
<td>Lathyrus leucanthus/Aspen Pea</td>
</tr>
<tr>
<td><img src="image3.png" alt="50 µm" /></td>
<td>Linaria.2</td>
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Linum lewisii/Wild Blue Flax

Lonicera involucrata/Twinberry Honeysuckle

Lupinus argenteus/Silvery Lupine
Mimulus guttatus -> Erythranthe guttata - Common Yellow Monkeyflower

Oenothera flava / Yellow Evening Primrose

Pedicularis bracteosa / Bracted Lousewort
Pedicularis procera/Giant Lousewort

Penstemon strictus/Rocky Mountain Penstemon
Penstemon strictus

Penstemon whippleanus/Whipple’s Penstemon
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<tr>
<td><img src="image2.png" alt="Phacelia heterophylla/Varied Leaf Phacella" /></td>
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</tr>
<tr>
<td><img src="image3.png" alt="Phacelia sericea/Blue Alpine Phacelia" /></td>
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Note: The images are not available.
<table>
<thead>
<tr>
<th>Polemonium foliosissimum/Towering Jacobs Ladder</th>
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</thead>
<tbody>
<tr>
<td>Polemonium pulcherrinum.2/Showy Jacob’s Ladder</td>
</tr>
<tr>
<td>Polemonium pulcherrinum.3</td>
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</table>
Polemonium pulcherrimum/Showy Jacob’s Ladder

Ribes cereum/Wax Currant

Ribes montigenum.2/Mountain Gooseberry
Ribes montigenum/Mountain Gooseberry

Symphoricarpos rotundifolius/Round Leaved Snowberry

Taraxicum officinale.2/Common Dandelion
Taraxicum officinale/Common Dandelion

Trifolium repens.2/White Clover

Trifolium repens.3/White Clover
Trifolium repens/White Clover
Beardlip Penstemon (Penstemon barbatus)

Gumbo Evening Primrose (Oenothera cespitosa)

Platte Lupine (Lupinus plattensis)

Wholeleaf Indian Paintbrush (Castilleja integra)

Columbine (not native to CAVO)

Indian Blanket (Gaillardia pulchella)

Scarlet Gaura (Gaura coccinea)

Pollen Images Shared by Capulin Volcano National Monument (CAVO)
Acknowledgments

We would like to thank all of the hummingbird researchers and their organizations for sharing their methods and pollen images with us in order to help make this project possible.

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