EISI Plant-pollinator project Standardized Protocol Proposal 2015

The flower survey is part of the plant-pollinator protocol. The purpose of the protocol is to (1) count interactions among plants and pollinators, and (2) estimate the abundance of flowers available to be pollinated.

From 2011 to 2015, flower abundance data has been recorded according to the methods from Helderop 2015:

"At the beginning of each watch, every flowering plant in the plot was identified to species, the number of stalks (that contained at least one flower) was counted, and the numbers of flowers on each stalk were counted for each species (up to ten stalks per species). Thus, flower abundance of each species was counted or (in the cases of species with more than ten stalks) estimated based on the average number of flowers per stalk multiplied by the number of stalks present. Compound flowers and inflorescences were counted as a single flower."

However, several additional decisions are necessary to implement this procedure:

- (1) what defines a stalk,
- (2) what defines a flower,
- (3) how to tell if a flower is in anthesis, and
- (4) how to use these rules to fill in the data sheet

1. What defines a stalk

A stalk is defined as a plant unit growing from the ground <u>or</u> from the base of a plant, no matter how close it is to another stalk. The objective is to find how many separate units an insect sees (helpful statistically), not necessarily how grouped they are. For example, the plant below has several different stalks and its grouping into one plant is never documented. On paper, this means a pollinator has several different opportunities to visit a plant unit in whichever arrangement it happens to be dispersed across the plot. Plenty of species grow along root lines (called rhizomes) so one single plant may have hundreds of stalks and may be difficult to discern. Figures 1a and 1b show a few common species with several stalks in what could be in one plant.



Figure 1a. *Erigeron foliosus* sometimes grows in an aggregate as shown. Each stalk has one flower and in this photo there are upwards of 200 stalks.



Figure 1b. *Orthocarpus imbricatus* can grow in clusters, but each stalk is counted separately. This photo shows about 30 stalks.

2. What defines a flower

This question is not necessarily answered in Helderop 2015 so I propose a new standardized method that should minimize the uncertainty. What we perceive to be a flower is probably the area of most ambiguity because there are several different perspectives of what a "flower" looks like. Also there is so much variability in flower morphology so one rule cannot govern all. An average person would look at *Eriophyllum lanatum*, the wooly sunflower, and see one yellow unit as one flower. A botanist would look it and see hundreds of tiny flowers that are grouped together to form what is called an inflorescence. A flower like this will open up microscopic flowers, each with their own reproductive parts, gradually from the outer ring towards the center over the course of the season. Most species in the family Asteraceae have two types of flowers, ray flowers along the exterior and disc flowers in the interior. The petals we see are each actually a part of individual flowers.



Figure 2. Eriophyllum lanatum in anthesis



Figure 3. The anatomy of an Asteraceae inflorescence

Another complication is when a species with clusters of lots of flowers appears in the data to dominate a plot against a species with larger flowers. For example, *Ligusticum grayi* (Figure 4) has a very high number of tiny flowers in the peak of its season compared to the fewer larger flowers on a species such as a *Penstemon cardwellii* (Figure 5), so designating each tiny flower as "1" could bias

the outcome, making *Ligusticum grayi* appear more abundant than *Penstemon cardwellii*, even though the opportunities for pollinators are rather similar for these two examples (Figure 4, 5).



Figure 4. Ligusticum grayi in anthesis



Figure 5. Penstemon cardwellii in anthesis

Thus, although the protocol instructs us to count every "flower" for our survey, not only is this extremely strenuous, it also does not make sense in the context of how we are using the data. Therefore, I propose that flowers should be counted in units according to the pollinator's perspective. Insects are the vast majority of pollinators in this system. When a honey bee lands on an *E. lanatum* inflorescence, it theoretically gains the same reward as it would be on one umbel of *L. grayi* or one flower of *P. cardwellii*. Therefore, it would make sense to count one umbel, one inflorescence, and one flower each as one "flower." Using what is visible in Figures 2, 4, and 5 as our plot, the data sheet could be filled out as illustrated in Table1. The photos for each species do not show all flowers so some values are estimated.

Species name	Number of Stalks	Number of Flowers per Stalk
Eriophyllum lanatum	1	1
Ligusticum grayi	1	12+8=20
Ligusticum grayi	1	11+6=17
Ligusticum grayi	1	9+7=16
Ligusticum grayi	1	8+6=14
Ligusticum grayi	1	7
Penstemon cardwellii	1	4
Penstemon cardwellii	1	4
Penstemon cardwellii	1	12

Flower Survey Data Sheet

Table 1. This is an example of how a data sheet should be filled out according to the new protocol usingvisible flowers from Figures 2, 4, and 5. Notice that each stalk in the plot is allocated one line (if under10) and total flowers per stalk are added up from each cluster on the same stalk. The photo of*Eriophyllum lanatum* had 1 stalk, *Ligusticum grayi* had 5 stalks, and *Penstemon cardwellii* had 3 stalks,which match their respective number of rows.

As mentioned before, the diversity of flowering species in the Andrews prevents us from establishing one hard-and-fast rule. Here are more specific examples:

Rumex acetosella (Figure 6) has a stalk-like structure with tons of microscopic flowers covering stems that branch off of the central stem. However, visiting insects usually land on one branch on the stalk and rarely visit each flower. One "flower" would be one branch on the stalk. In Figure 6, this specimen has 1 stalk and 10 or 11 flowers. This one can be tricky because it sometimes branches off further, but the standard method is to count a flower as a unit that an insect could visit in one encounter.



1 flower

Figure 6. *Rumex acetosella* flowers on one stalk are on the left. This photo has about 12 flowers. On the right, the photo has 2 separate stalks, the first with about 5 flowers, the second with about 10.

Boykinia major (Figure 7) has a stalk/umbel structure and its flowers are small, white, and abundant around the top of the plant. From observation, we learned that a bee lands on one flower then will navigate around the plant to continue visiting other flowers. One "flower" unit would be one small, white flower because an insect invests in each reward. It is not uncommon for some of these individuals to have 40 or 50 flowers on each stalk, but this makes sense with how popular they tend to be.



Figure 7. *Boykinia major* flowers individually attract pollinators so each white flower is recorded. The photo on the left has about 25 visible flowers that are all on the same stalk. The photo on the right has two stalks with the left exhibiting about 28 flowers and the right exhibiting about 45. Notice all flowers on all clusters on the same stalk are added together.

Achillea millefolium (Figure 8) is a difficult species to count, but under the new standards of the protocol, it should not be too difficult to break into units. Each of the small yellow points rising from each small flower is considered by a pollinator to be a point of nectar/pollen access. A pollinator is only so small, though, and will usually land on an umbel the size of a quarter or a naturally-forming small cluster. Larger clusters can be broken up into quarter-sized clusters counted as "flowers."



Figure 8. *Achillea millefolium* flowers form small platforms that pollinators can land on. The photo on the left shows how small the individual flowers actually are. However, in a survey, one "flower" would be one umbel. The photo in the middle has 1 stalk with about 10 flowers. The photo on the right shows how stalks are counted; this photo has 6 visible stalks.

Phlox gracilis (Figure 9) has exceptionally small flowers, but they are so spread out that each flower would be considered one flower. One insect would visit one flower.



1 flower

Figure 9. *Phlox gracilis* flowers are counted individually, not in clusters. Therefore, this photo shows 6 stalks with 1-4 flowers each.

Eriogonum umbellatum (Figure 10) flowers form in circular clusters with several small flowers in each. Each circular unit is counted as one flower because an insect often lands on the top and visits the whole cluster at once.

Technically several flowers, but recorded as 1 flower



Figure 10. *Eriogonum umbellatum* umbels are counted as flowers. The photo on the left shows about 5 flowers. The photo on the right has 3 stalks, the first with 5 flowers, the second with 3 flowers, and the third with around 4 flowers. Sometimes it helps to look at how the stem branches off into flowers to count them.

4. How do I know if a flower is in anthesis?

A period of anthesis occurs when a flower is living and its reproductive parts are exposed. This can be determined by finding the stigma and style of the inside of a flower. It will be helpful to brush up on the anatomy of a flower in order to find and assess the viability of the style (female part receiving pollen) and the stamens (male part producing pollen). In some species, these parts are very small, so a hand lens is necessary. To complicate things a little more, some species, like *Ligusticum grayi*, have gendered flowers so looking for both reproductive parts is not helpful for determining anthesis. A flower that no longer has petals is usually past anthesis and has moved on to the fruit-production stage, but this is not always true. E. lanatum often loses its leaves early while the disc flowers in the center are still producing pollen. In addition, in some species, the flower turns a shade darker, which usually means it is desiccating and nearing the end of its season.

Observers can also use the time of the season to identify anthesis. Later in the season, most of the early flowers will start to wither and become debatable. If the reproductive parts are no longer accessible because the flowers have wilted, then the flower probably is not producing much nectar anyway and should not be counted as a flower. Observers will notice when a plant's fruit is starting to develop and the ovaries are swelling, which is an indication that the flower already has enough reproductive material and will not have any resources for pollinators anymore.

Basic Flower Structure



Figure 11. When identifying flowers and deciphering if they are in anthesis, it will be imperative to know the anatomy and how the parts can change over time.

When counting an umbel that contains some flowers that are alive, and some that are dead, assess whether at least 10% of the umbel is still offering or accepting reproductive material. For the flowers that have both disc and ray flowers (Erigeron foliosus and Eriophyllum lanatum), count the specimen if at least 4 disc flowers are still in anthesis.

Dandelion-looking plumes are actually dispersal mechanisms for mature seeds in species that consist of only ray flowers, so they are not considered flowers. Grasses are wind-pollinated and not included in the survey either.

4. How to fill out the data sheet

The first thing an observer should do when they approach a plot for a survey is <u>write down their</u> <u>name and the date</u> on both sides of every sheet of paper they will use. This guarantees that a hard copy of the data will always be accessible for future reference. Second, the observer should start the flower survey by identifying all species in the plot or taking photos/notes of those that are indecipherable. Details that are important for identification are color, flower size, number of petals, leaf arrangement, leaf shape, plant size, where it was found, and any special features. Photo identification numbers should also accompany the description.

Next, all species must be counted and recorded. In Helderop's methods section, "the numbers of flowers on each stalk were counted for each species (up to ten stalks per species)" implies that each individual stalk is recorded as well as its respective number of flowers (2015). In the case that there are more than 10 stalks of one species, the observer averages the number of flowers per stalk and records the total number of stalks. The data sheet has a column each for "Species", "Number of Stalks", and "Number of Flowers per Stalk". Therefore the data sheet should be filled in as follows:

Species	Number of Stalks	Number of Flowers per Stalk
Achillea millefolium	1	5
Achillea millefolium	1	6
Achillea millefolium	1	7
Eriophyllum lanatum	1	1
Gilia capitata	25	Avg(1,2,2,1,1,1,3,2,1,2)

Flower Survey Data Sheet

Table 1. Example of data sheet entries for species illustrated in this protocol.

Note: The HJ Andrews Forest has incredible flower diversity, which can leave counting techniques up for interpretation. Since there is not one rule that governs all flowers it is <u>VERY IMPORTANT</u> that observers communicate about counting techniques and record these techniques at the end of this guide for species that were confusing. Data from the past years will always be available to reference how other observers have been counting. The code that was used in the data consistency analysis is also available so counting can be visualized across multiple years. Remember: inconsistent flower survey data is almost as helpful as no data at all.

References:

Helderop, E. 2015. Diversity, Generalization, and Specialization in Plant-Pollinator Networks of Montane Meadows, Western Cascades, Oregon. Unpublished master's thesis. Oregon State University, The College of Earth, Ocean, and Atmospheric Science, Corvallis, OR.