Station 1: Pollination Syndromes

The world contains roughly 300,000 species of flowering plants (angiosperms), with many more yet to be discovered. While diversity in shape, size, and color of these flowers is staggering, many species have converged on similar floral **"pollination syndromes,"** suites of characters (color, scent, nectar, floral shape) adapted to specific pollinators. These can be abiotic (such as wind or water), or biotic (such as hummingbird or moth). It turns out you can make a pretty good guess as to what pollinates a given flower if you carefully examine the flower's *form*- and that's what we'll be doing today!

Activity Directions

1. Examine live flowers and herbarium specimens to determine their pollination syndromes (wind, hummingbird, bee, fly or moth).
    a. Do NOT look at every plant; pick plants which have not yet been color-coded so that all are examined we can observe patterns of convergence in form at the end of the lab.
        i. *Handling Herbarium Specimens: Several samples must remain in their plastic covering (noted on the sample), but the rest are part of the teaching collection and may be taken out and handled. (Detailed information on the specimens is provided at the end of this activity.)*
            1. *Always keep the sheets horizontal, and if you pick them up, do so with two hands.*
            2. *The plant material can be examined with a hand lens;*
            3. *In some cases, the sheet will include an envelope that contains loose pieces from the plant- these can be picked up and placed under a dissecting scope or examined with a hand lens.*

    b. Use hand lenses, dissecting equipment and microscopes as you desire. Sometimes it's useful (and fun!) to dissect a flower and pop it under the dissecting scope (we especially recommend doing this with the mums, as they have a multitude of surprises to reveal).

    c. Refer to our guiding questions below to help you investigate each flower.

    d. In the space provided below, draw one or two examples of each pollination syndrome and indicate the special features of that flower that support your classification. As a refresher, a diagram of a generic flower is provided below.

2. Place color-coded post-its on the plants once you've deduced their pollination syndrome *(see board for color code).*

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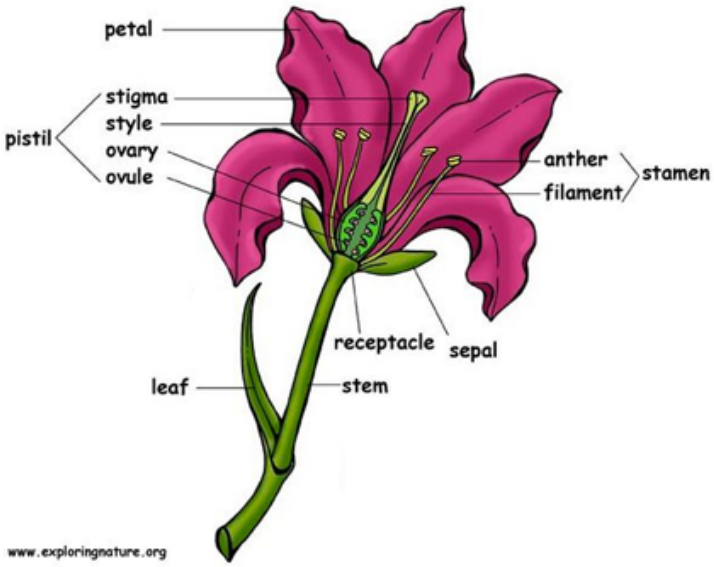
References and Guiding Questions

Generic Flower for reference



Guiding questions for pollination syndrome investigation

Things to think about from the pollinator's perspective:

- What time of day are they active?
- How do they move around?
- What are the shape and size of their mouthparts?
- What are they attracted to?

Things to think about from the flower's perspective:

- What strategies is the flower using to attract pollinators (color, scent, etc)? Are they deceptive (ie, is the pollinator getting tricked into coming to the flower)?
- What rewards is the flower producing for its pollinators, if any?
- What shape and size would a pollinator's tongue/beak have to be to access the reward?
- Where will the pollen land on a pollinator's body?
- Does a pollinator have to be moving or stationary to access the flower?
- What would a flower look like if it weren't trying to attract a pollinator?

Pollination Syndrome Drawings

Wind:

Hummingbird:

Bee:

Fly:

Moth:

Materials and Resources:

Live flowers*:
(Note that horticultural hybrids are denoted by a common variety name after the latin name; they often have confusing pollination syndromes because they've been bred to look pretty to humans, but we've done our best to find ones that still have strong syndrome characteristics)

Anthurium "Red"
Tillandsia "Spirit"
Abutilon "Miss Marmalade"
Brugmansia "Angel's Summer Dream"

Pentas lanceolata "Ruby Glow"
Streptocarpus "Maasen's white"
Chrysanthemum spp (aka 'mums')

Herbarium specimens (please review how to handle herbarium samples below)**:
Trillium erectum
Aquilegia canadensis
Quercus ilicifolia
Poa laxa
Symplocarpus foetidus
Fuchsia boliviana
Aristolochia macrophylla
Aquilegia coerulea

*We used our favorite tropical plant supplier, Logee's, for live specimens because this workshop is happening in the fall and many nurseries aren't stocking local flowers. They are available for mail order and often provide educational discounts, but the plants do require greenhouse facilities to keep them happy and flowering. We realize this might not be feasible if you want to recreate this workshop for your classroom, so we recommend doing this lab in the spring when many local varieties will be easily available in nurseries or in your own backyard! Many of the herbarium samples are local plants and can be accessed by your students online at https://huh.harvard.edu/pages/digital-resources (click "specimens" and it will take you to the herbarium's database, where many of these specimens are scanned at high resolution). We've also included a list of easy-to-find spring plants in the answer key.

**We are using herbarium samples from the Harvard University Herbaria, a research collection of 5 million dried plant specimens. Several samples must remain in their plastic covering (noted on the sample), but the rest are part of the teaching collection and may be taken out and handled. Always keep the sheets horizontal, and if you pick them up, do so with two hands. The plant material can be examined with a hand lens; in some cases, the sheet will include an envelope that contains loose pieces from the plant- these can be picked up and placed under a dissecting scope or examined with a hand lens. There is a wealth of data associated with each specimen- who collected it, where, and when- you'll notice that some of the samples were collected in the 1800s and still look great! We'd be happy to chat more with you about how to use the digital herbarium collections in a lab activity with your students.

Station 2: Fruit/Seed Dispersal

Since plants can't move, it's up to their fruits and seeds to 'leave the nest' – and they've come up with some elaborate strategies to do so! Today we'll be looking at how fruit and seed forms are adapted to different strategies of dispersal, such as wind, water, and animal ingestion. The species provided are not closely related to one another, so in most instances their dispersal mechanism are evidence of *convergence*; in other words, they independently evolved similar forms to adapt to the same function. Note some examples of the convergent forms you see.

Here are some definitions as a refresher:
The **carpel** is the female reproductive unit of a flower; it contains a stigma, style, and ovary.
A **fruit** is the mature ovary of a plant.
A **seed** is a mature ovule of a plant, and develops inside the ovary.
There can be multiple seeds per ovary, or just one. There can be multiple carpels per flower, or just one. (There is a whole botanical language for describing fruit types., but we don't want to get bogged down in that today. We just want to focus on how the forms of the fruits and/or seeds are adapted to dispersal methods)

Activity Directions

1. On the following pages you will find different fruit/seed dispersal mechanisms. Match the samples to the dispersal mechanism.
    a. Do NOT look at every plant – find an example of each dispersal mechanism.
    b. Feel free to use hand lenses, dissecting equipment & microscopes for you to observe the fruits and seeds more closely, but you will definitely be able to determine the dispersal method using just your eyes.
2. Draw a few representative fruit/seeds for each type.
    a. What about their form tells you how it's dispersed? Feel free to test how good they are at a certain dispersal method ☺
3. Think about the unit of dispersal - is the whole fruit dispersing, or just the seed? Do you see convergent forms even among these two different dispersal units?

Dispersal Syndrome Drawings
Wind:

Animal (via ingestion):

Animal (via carrying):

Water:

Bonus: megafaunal dispersal syndrome: Some of the plants you've looked at already most likely evolved before the last ice age when enormous animals ('megafauna') like wooly mammoths & giant sloths roamed the continent. Any guesses as to which ones?

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Materials and Resources:

Herbarium specimens (please review how to handle herbarium samples below)*:
Acer rubrum (red maple)
Asclepias syriaca (milkweed)
Taraxacum ceratophorum (dandelion)
Arctium lappa (thistle)

Arboretum samples**:
Quercus spp (oak)
Platanus x acerifolia
Symplocos paniculata
Ptelea trifoliata
Catalpa ovata
Chaenomeles japonica (Japanese quince)
Maclura pomifera (Osage orange)
Pterocarya x rehderiana (wingnut)
Callicarpa dichotoma

Other samples (photos, obtained from Etsy & Whole Foods):
Proboscidea spp ('devil's claw')
Trapa natans ('devil pod')
Bidens spp (beggar ticks)
*Cocos nucifera (*coconut)

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**We collected live material from the Arnold Arboretum of Harvard University in Jamaica Plain, home to 2,000 species of woody plants collected from around the world. It is a fantastic place to learn about plant diversity and we highly recommend visiting for a field trip! (more info here: https://www.arboretum.harvard.edu/education/childrens-education-programs/for-teachers/) Their collections can also be accessed online- if you search for any of the listed species in their plant image database, you'll find tons of images of leaves, flowers, and fruits! https://www.arboretum.harvard.edu/plants/image-search/

Station 3: Stomata structure and function

Stomata are the pores in the epidermis of the leaf (~30 um in size) that allow CO2 to diffuse in for photosynthesis. However, by opening to allow the CO2 in, they also allow water to evaporate out. This is a fundamental trade off they must optimize for – the more CO2 brought into the leaf, the more photosynthesis (this is not linear, but generally true), but the more water they lose, the more likely they will dehydrate and become damaged irreversibly. Because they are the gatekeepers to the inside of the plant, researchers, for over a century, have been interested in their structure and function.

1. Why not just have one stomata per leaf? Why not just have a cuticle that is permeable to gas diffusion?

Many things influence stomata structure and patterning. Different species have different patterns – monocots typically have stomata in straight lines, whereas oak trees typically have a semi-random patterning. Additionally, the environment the plant grows in can influence the patterning.

Climate change is a phenomenon that occurs naturally on large time scales, and more recently on short time scales due to anthropogenic driven changes in CO2. Currently, the CO2 concentration in our atmosphere is 405 ppm.

2. Do you think stomatal density would change as a result of different atmospheric CO2 concentrations? If so, why?

Short-term climate change

(Figure from Woodward et al. 1987)

3. Does this plot show the same relationship between CO2 concentration and stomatal density as you predicted?

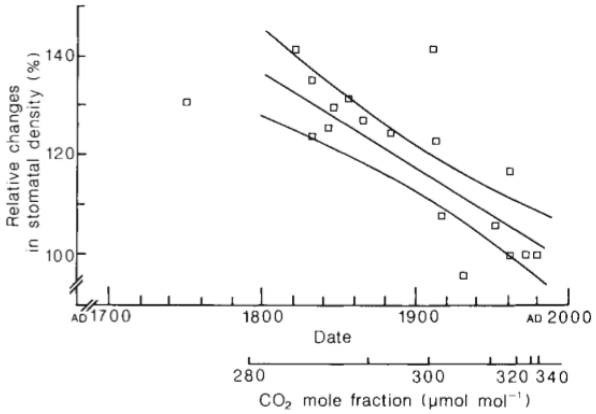


Fig. 1 Abaxial stomatal densities of herbarium stored leaves of *Acer pseudoplatanus*, *Carpinus betulus*, *Fagus sylvatica*, *Populus nigra*, *Quercus petraea*, *Q. robur*, *Rhamnus catharticus* and *Tilia cordata*. Leaves had been stored in the herbarium in the Department of Botany, University of Cambridge. Only leaves on reproductive shoots were sampled, with the assumption that these leaves had developed in full irradiance. Five leaves of each species were sampled from different dates, back to AD 1750, and from collections made in the midlands of England. Stomatal densities varied between species by a factor of about two, however the changes in stomatal densities relative to the recent collections (1970 to 1981) were similar for all species. Reconstructed changes in atmospheric CO_2 based on ice-core studies[1] are also included. The linear regression line, with 95% confidence limits, shows a 40% reduction in the ratio of stomatal densities over a period of 200 years, $r = -0.828$.

Long-term climate change

(Figure from Beerling and Chaloner, 1994)

Table 1
Provenance, stratigraphy and dates of *Salix herbacea* material examined

	Provenance	Estimated age	Stratigraphy	Corresponding CO_2 concentration (ppmv)
(1)	Toll Creagach, Invernesshire, Scotland	Present	Holocene	350
(2)	Morrone, Deeside, Scotland	10,000	Holocene	290
(3)	Utsira, S. Norway	10,500	Devensian late-glacial stadial	270
(4)	Ballybetagh, Ireland	10,600 ± 60	Devensian late-glacial stadial	260
(5)	Neasham, NE England	10,851 ± 630	Devensian late-glacial stadial	240
(6)	Morrone, Deeside, Scotland	11,087	Devensian late-glacial interstadial	235
(7)	Ashleam Bay, Ireland	11,170 ± 120	Devensian late-glacial interstadial	235
(8)	Morrone, Deeside, Scotland	11,500	Devensian late-glacial interstadial	235
(9)	Kerry, Ireland	11,950 ± 200	Devensian late-glacial interstadial	250
(10)	Morrone, Deeside, Scotland	12,300	Devensian late-glacial interstadial	260
(11)	Beetley, Norfolk, England	16,500	Devensian full-glacial	190
(12)	Broome Terrace, Norfolk, England	>140 ka	Wolstonian full-glacial	190

Radiocarbon dates are uncalibrated and presented ±sd were appropriate. Values of CO_2 concentration were derived from the Vostok ice core, Antarctica (Barnola et al., 1987). Full details of each macrofossil sample are given by Beerling et al. (1993).

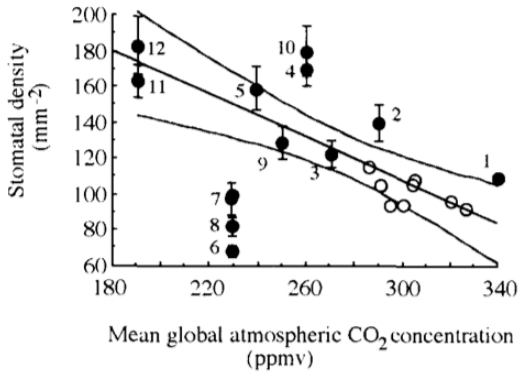


Fig. 2. The response of stomatal density to long-term changes in atmospheric CO_2 concentration and climate. The figure was derived from fossil *Salix herbacea* leaves spanning a glacial cycle. Numbered points correspond to samples listed in Table 1, open circles represent herbarium material dated 1845–1971. The line was fitted by linear regression, excluding points 6, 7 and 8 and is shown together with the 95% confidence limits of the mean. (Source: Beerling et al., 1993.)

4. Based on the information in Table 1, how far back in time does the data presented in Figure 2 go? What types of plant samples do they use to get this information? Is it all from living plants?

Examining stomatal density and size

Method:

A. Paint clear nail polish on the leaf, let it dry. (Some have already been painted.)
B. Peel it off with tape and put it on a microscope slide.
C. Observe under compound microscopes.

5. Are the stomata on the top (adaxial) or the bottom (abaxial) side of the leaf? Functionally, why do you think they exhibit this adaxial:abaxial ratio?

6. Do they appear open or closed? Why?

Station 4: Xylem structure and function

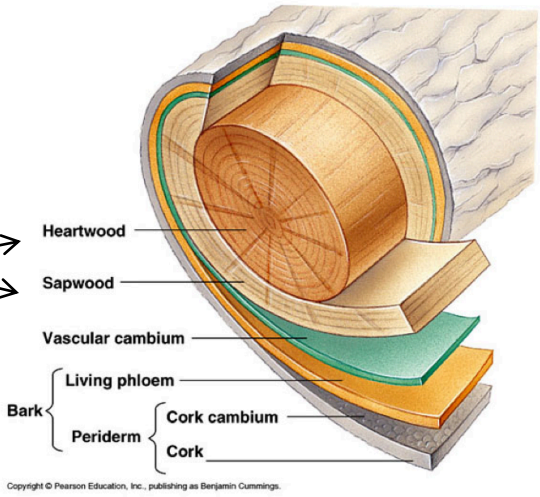
While their stomata are open and allowing water to evaporate out of the leaf, plants must constantly be replenishing the water in their leaves to maintain functioning. They do this by transporting water from the soil to the leaf through many xylem pipes known as **tracheids** or **vessels**. Water comes into the xylem and then flows between pipes until it leaves the leaf through the stomata. Recent research has shown that if ~60% of the flow through these pipes is lost (if air enters the system, **cavitation** occurs, which blocks the xylem tube - this typically happens when the plant is punctured or has experienced excessive drought or freeze-thaw cycles), the tree has a higher chance of dying. Therefore, the structure of these tubes is very important for thinking about a safety/efficiency tradeoff: big enough to supply enough water to the leaves so they can maintain functioning (it is important to note that the Hagen-Pouiseulle equation of volumetric flow describes that flow through a tube is directly proportional to the radius of the tube to the fourth, a larger diameter can go a long way!), and small enough (or with enough safety measures) to prevent or slow the spread of air throughout the system. "Safety" in this context refers to the ability of the plant to prevent loss of function (cavitation) when the system is stressed.

1. Why can't you just have one large pipe in the plant? Why can't you just have a million small pipes in the plant?

Gymnosperms (typically conifers and evergreens i.e. pines, spruces, firs) and angiosperms (typically deciduous plants i.e. maples, oaks, poplars) have independently evolved different strategies for dealing with this trade off.

Vascular cambium: A lateral meristem giving rise to xylem and phloem elements as well as parenchyma

Phloem: The sugar transport pathway



Periderm: Tissue that contributes to the outer bark of stems and roods during secondary growth of woody plants, replacing the epidermis. Also forms over wounds and abscission layers after the shedding of plant parts.

<u>Observing xylem conduits</u>

A. Cut a small transverse slice of 1 gymnosperm and angiosperm stem (the slice should be a pancake shape, as thin as you can make it!).
B. Place the cut on a microscope slide (no water or coverslip needed).
C. Observe the xylem conduits under a compound scope.

2. Can you identify a key difference between gymnosperm and angiosperm xylem conduits?

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Questions regarding functional efficiency
(Figure from Sperry et al. 2006)

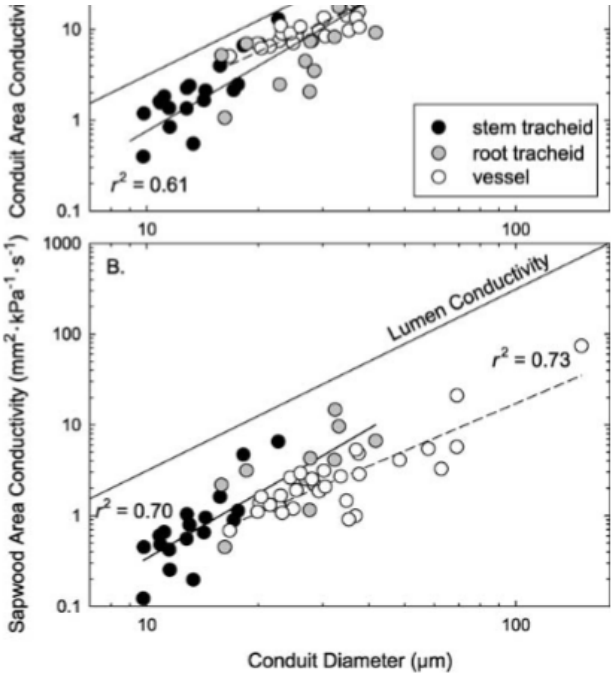
The xylem conduits in gymnosperms are known as tracheids, and the xylem conduits in angiosperms are known as vessels.

3. Based on this figure, which one (gymnosperms or angiosperms) is more conductive to water flow?

4. How does higher conductivity potentially influence photosynthesis?

5. Why does the group of species that has conduits with higher conductivity not out compete the other group of species on a global scale?

Fig. 3. Scaling relationships between hydraulic conductivity and conduit diameter. (A) Conduit area conductivity (conductivity per lumen area) and conduit diameter in conifer tracheids and angiosperm vessels. Data points are mean values for different species or organs; vessel data from stems only. Regression slopes (reduced major axis) were 2.38 in conifers and 1.85 in angiosperms and not different ($P > 0.05$) from the second-power scaling predicted for lumen conductivity from the Hagen–Poiseuille equation (solid diagonal). (B) Sapwood area conductivity (conductivity per sapwood area) vs. conduit diameter for same conifer and angiosperm wood samples as in (A).



Questions regarding functional safety

(Figure adapted from Gleason et al. 2016)

6. How does size relate to safety? If a stem is punctured, would a species with larger conduits lose more or less functioning than a species with smaller conduits due to a cavitated conduit?

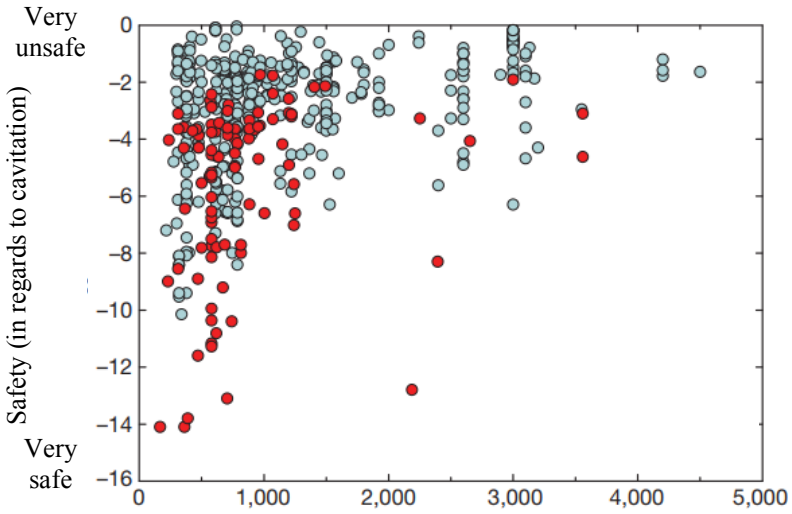


Figure 3 | Embolism resistance as a function of mean annual precipitation for 384 angiosperm and 96 gymnosperm species. Each point represents one species. A generalized model indicated that embolism resistance (Ψ_{50}) was significantly related ($P < 0.00001$) to mean annual precipitation (MAP) for angiosperms and gymnosperms (see Methods for details), with decreasing resistance to embolism corresponding to increasing rainfall. The full data set is available in Supplementary Table 1.

7. Which color represents gymnosperms, and which color represents angiosperms? (In other words, which group of species is safer?)

8. What is one reason that gymnosperms are typically found in harsher environments (the boreal forest and at tree-lines)?