Factors shaping flight of milkweed seeds and winged fruit (samaras)

BEFORE LAB

- **Read the Introduction and skim the lab exercises below.**

OBJECTIVES

1. Clarify issues of random sampling and sources of error.
2. Understand the importance of dispersal for plants, including tradeoffs between light versus heavy seeds for species that are dispersed by wind.
3. Understand the main principles of biological scaling and allometry.
4. Practice skills in data management (Excel) and graphing (Excel).

INTRODUCTION

Samples and Populations

Morphological variation (differences in shape among individuals) links ecological performance with evolution and adaptation.

It is usually impossible to measure all the members of a taxon. Can you imagine measuring body length for all blackbirds in North America (millions)? Or wing length on all silver maple seeds in North America (billions)? Biologists almost always have to measure a **sample** of specimens taken from the larger, complete **population** to which the sampled specimens belong. Statistics is really the mathematics of describing and testing hypotheses about populations, from samples.

This means that selection of the sample to be measured has a powerful impact on the resulting data. Which forest and which trees do we choose for collecting the maple fruits? What geographic region for the blackbirds? What time of year? What age or developmental classes? These and a hundred similar questions influence sampling decisions long before the first data point is measured and recorded. **Random sampling** has two components; that all individuals in the population of interest are equally likely to be sampled, and that individuals within a sample are independent of each other. Most samples violate the ideals of random sampling in many ways.

One last term to define is **test statistic**. A test statistic is a measure of how different your data are from the null-hypothesis prediction. If you are looking at the means of two samples that differ in some **categorical** way (species, color, location collected), the **null hypothesis** is that the means are equal. If you are looking at the relationship (slope) between two **continuous** variables, the null hypothesis is that there is no relationship, so the slope is zero. Examples of test statistics you might come across are t, for comparing two means or a single slope, F to compare one or more means or slopes, or more complex models, and χ^2 used to test frequencies of combinations of different categories (cured or not after taking medicine vs. placebo, genotypes in observed vs Hardy-Weinberg predicted frequencies).

FOCUS ON TODAY'S WORK: MORPHOMETRICS, FLIGHT, AND DISPERSAL OF WINGED FRUITS

Terrestrial plants are sessile, so they rely on seed dispersal via animals or wind or water flow to colonize new habitats and escape local extinction. For wind-dispersed plants there are likely several trade-offs between light weight and aerodynamic shape to disperse farther and heavier weight to store more food for the plant embryo.

The winged fruits of trees like maple, ash, and tulip poplar are called **samaras**. Everyone has seen these fruits dispersing through the air because they spin like little helicopters and are fun to watch. In the lingo of aerodynamics, they are called **autorotators** because they spin on their own without any internal rotating force being applied (like the engine on a helicopter). Most dispersing fruits and seeds do not spin. Why do the samaras do it? The answer is that they do it for the same reason real helicopters do it: to generate **lift** and stay aloft against the force of gravity. How do they do it? The wings of samaras have special shapes which create an upward force on the wing when air moves past it. The more lift that is created, the easier it is to stay aloft, the slower a samara falls (the slower the **descent rate**), and the farther it may travel on the wind before landing on the ground. All other things being equal, potential dispersal distance is positively correlated with the amount of lift generated by the wing and negatively correlated with weight.

Numerous morphological factors influence descent rate. *Your task in this lab is to develop hypotheses about the relationship between morphology and flight performance in maple samaras and measure and graph the relationship between those variables*. We will return to these data to test our hypotheses statistically in a later lab. As a warmup we will conduct an experiment on seed dispersal in dandelions as a function of height or release. Then, you will measure length and one other variable in each of two species of samara-producing maples, and we will examine

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Part 1: Height of release and dispersal distance in milkweed seeds.

Milkweed is a common plant that often grows in hayfields and other agricultural areas. It has a defensive sap that few herbivores can tolerate, but several specialists (Monarch butterflies *Danaus plexipus*, milkweed beetles, *Tetraopes tetrophthalmus*, and milkweed bugs, *Oncopeltus fasciatus*). It is also a subject of recent interest because the use of highly effective herbicides, enabled by insertion of herbicide tolerance genes into corn, soy, cotton, and canola among other crops, has reduced the amount of milkweed in the Midwest, probably contributing to sharp monarch butterfly declines (Flockhart *et al.* 2015).

I am interested in how seeds from milkweeds growing in active (mowed) hayfields vs uncut fields disperse. Milkweed seeds have feathery silk strands attached to help them disperse.

[*Asclepias syriaca*](https://en.wikipedia.org/wiki/Asclepias_syriaca) seed pods, upper image from August and lower from December, Photos Greg Hume; https://en.wikipedia.org/wiki/Asclepias#/media/File:Asclepias_syriaca_seed_pod.jpg

Milkweed can flower at greatly different heights depending on whether they grow in a field that has been mowed, or were allowed to grow un-disturbed, as you can see from the pictures below. Actually the picture on the right probably has some mowed/regrown milkweed towards the right rear.

Left: Regrown milkweed from mowed field, from Alcock et al. 2016.
Right: Mature common milkweed, photo by Phil Westra, Colorado State University, Bugwood.org

Recent attention has focused on how regrown, mowed milkweed is more attractive to monarchs laying eggs, and less attractive to predators of caterpillars (Alcock *et al*. 2016, Haan and Landis 2019). What might be an functional effect of this difference? It could have a large effect on spread and dispersal of seeds. Seeds that travel farther could avoid competition with parents and siblings, could find habitats much better (or worse) than where their parents grew. To test the effect of higher seedhead placement on dispersal, we will release at least 5 milkweed seeds from each of 5 heights in front of a fan and record the distance travelled for each one.

Why is it important to use a different seed for each measurement?

Each group of 3-4 students will create a file in Excel called dandeliondispersal or something more compact but clear, with two variables (columns); height (in cm) and distance traveled.

Below, record your notes on your methods – how did you choose the heights used? What fan and setting did you use? What distance was the fan from the release point? Over what kind of surface did you release the seeds? Include anything that might be useful to replicate your experiment

OK, go to it and record your data and save it in the Excel file, and make sure to record your group members for later analysis.

PART 2: OBSERVING SAMARA MORPHOLOGY AND FLIGHT

We now turn from milkweed seed dispersal to the properties of winged fruits that enhance seed dispersal. Each student team should collect a stopwatch, ruler, and dial calipers from the instructor. Then gather a small sample (6-10) of undamaged samaras from the maple (Norway, *Acer platanoides* and an unidentified species) fruit collections that are available in lab. Study the morphology of these samaras carefully. Now drop a samara (remember to split the fruit if it is still joined) and observe its descent. Continue flying samaras until you feel you have observed all the important features of samara flight. Note different phases of flight, if there are any. Try to account for notable differences in flight among samaras (are some samaras bigger? thinner? do some have bent wings?). Try various angles at which the samara is held before being dropped. Try timing the descent of a samara with your phone as a stopwatch. Discuss your observations as a group and do not overlook differences in what you see or think. Discuss until you are all satisfied that you are seeing the same things. For the actual experiment we will drop the samaras from the second floor terrace over the lobby.

PART 2: Define hypotheses and measure explanatory variables

We will examine samara flight as a function of two variables, and a third variable that integrates the two individual variables. The first one will be length of the samara. Wing length is usually closely tied to flight performance. Split your teams into pairs, and discuss how you are going to measure length. Have one pair pick 20 samaras from the pile, and number 20 envelopes so each can be identified individually. Each pair should measure the length of each samara, so you should have two variables in your excel file for this part, pair1length and pair2length.

Choose a second morphological variable that you will be able to measure in lab, and do your best to measure it in each of the samaras you already labeled and measured for length. As before it is best to measure each samara twice. Enter your measurements in Excel.

Finally, think of ways you could combine both morphological variables into a single variable to test their combined effect on flight performance. Discuss your method with your instructor, and use the formula function in Excel to create a new variable that integrates both variables into a single explanatory variable.

You will need to constrain your replicate selection in one other way. Since you are trying to predict flight time as a function of a selected morphological variable through regression, it is important that the morphological variable in question show some variation. If all replicates have the exact same value for the independent variable, then there is no possibility of predicting a second variable based on the first. So you need to choose your replicates so that they include a range of variation in the morphological variable you have chosen. *So the compromise will be to choose undamaged samaras as randomly as possible, but over a wide morphological range.*

Finally, pay attention to your experimental procedure, especially the dropping and timing of samaras. Considerable **experimental error** may be introduced by sloppy technique. This error increases the variation in the data and makes it more difficult to draw valid statistical conclusions. Write down the methods you use to measure both morphology and flight.

METHODS – MORPHOLOGY:

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METHODS – FLIGHT:

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OK, now go to the terrace and time those (20) samaras that you just measured!

NOTE: *Handle the samaras carefully. They are dry and brittle. If a samara is damaged during the experiment, replace it.*

When you have completed your measurements, enter your data into Excel. First, create histograms of the samara lengths measured by each pair in your group (Appendix on Excel Analysis tools). How similar are they? Create a new column that is the difference between the two measurements. What would you expect this distribution to look like if each measurement was done the same way?

Paste the histogram of the differences below:

Now graph a scatterplot of the relationship between samara length and flight time (**Instructions in appendix**). Does it follow the trend you expected? How much scatter (unpredictability) is there?

Now calculate a linear regression between the variables. Study the table of results, particularly the **p-value** for the regression line (<u>not</u> for the constant). The p-value is a measure of how unlikely your results would be if the null hypothesis were true. In this case, the null hypothesis is that the actual slope, the relationship between the two variables, is zero, or there is no relationship between the two variable. If $p > 0.05$, you should conclude that there is no significant relationship between the variables. If $p < 0.05$, they are significantly related. In p-value-speak, if your results were very unlikely if the null hypothesis were true (very small p) we reject the null hypothesis.

Now note the **squared correlation coefficient (R^2)**. This value represents the amount of variation in the y-variable that can be accounted for by variation in the x-variable. It is a measure of the goodness of fit of the regression line to the data points. R^2 ranges from 0.00 to 1.00. The higher the value, the better the fit and the greater the predictability of the regression line. An R^2 of 1.00 means that all points fall exactly on the regression line. R^2 values of 0.70 are greater are generally considered to be "good" correlations. Note that the higher the value of R^2, the greater the likelihood that the regression will be statistically significant (why?). But note also that it is possible to have a statistically significant regression but only a modest value of R^2, (even as low as 0.30 or so), especially if the sample size is large. *So you always want to report three primary results of a regression analysis: (1) the sample size, so readers know how many data points are involved, (2) the p-value for the regression, so readers know whether the regression is significant, and (3) the R^2, so readers know how strong the relationship is between the two variables.*

Now write down the sample size, the p-value for the regression, the R^2 value, your conclusion about your null hypothesis (reject or do not reject), and your conclusion about the relationship between the two variables.

SAMPLE SIZE (N):

p-VALUE:

R^2:

NULL HYPOTHESIS (reject or do not reject):

CONCLUSION: ____

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References:

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