Objectives:

- Understand the **Biological Species Concept** its strengths and its weaknesses.
- Understand the types of information biologists use when delineating species, and the strengths and weaknesses of these different sources of information.
- Understand the terms **cryptic species** and **sexual dimorphism**, and be able to give examples of each.
- Understand the difference between **anagenesis** and **cladogenesis**.
- Understand the terms **adaptive radiation, niche** and **key innovation**.
- Understand the difference between **prezygotic** and **postzygotic** reproductive barriers.
- Be familiar with the diversity of **Darwin’s Finches** and understand the relationship between diet and beak form in these birds.
- Understand the mechanics of producing bird songs, and know the components of song structure.
- Understand the relationship between beak form and song structure in Darwin’s Finches, and know what is meant by **vocal deviation**.
- Understand the importance of song structure in mate recognition in Darwin’s Finches and understand the impact that changes in beak form may have in the maintenance of distinct species of Darwin’s Finches.
- Be comfortable with using Excel to generate a graph.

**Important Note!!** – Throughout this Lab you will be asked to open up documents on your computer. All of these may be found online (see the course website, then go to Lab Information).

**Introduction:**

In a previous lab, the focus was on changes in allele frequencies within a population. This is evolution, but it represents **anagenesis** (or evolution within a single branch of an evolutionary tree). This week we will turn our focus towards **cladogenesis** (or the branching of an evolutionary tree to produce new clades – e.g. species).

First, we will discuss what a species is. Defining what species are is a task that seems simple at first glance, but is more
complex once you take a closer look at it. Then we will take a look at the forces that can drive speciation and the mechanisms by which species remain separate from one-another. We will be taking a close look at a group of organisms that is famous for improving our understanding of how evolution works – Darwin’s Finches.

Species:

You may not think defining what a species is would be a difficult task. After all, we can all recognize that a wolf is different from a chimpanzee, which is different from a bullfrog, which is different from a pangolin, which is different from a sunflower, which is different from a toadstool, etc., but, as you will see, it is not always a straightforward process. Consider the brown bear (Ursus arctos) and the polar bear (Ursus maritimus), which do not interbreed in nature (no natural hybrids are known), but can interbreed in captivity and leave fertile offspring. How about the horse and the donkey, which produce an infertile (sterile) mule when bred. Biologists have proposed dozens of species concepts to deal with these and other cases.

The Biological Species Concept (championed by Ernst Mayr) is probably the most commonly used definition of species among biologists. At its heart is the idea that members of the same species can and do mate and produce viable offspring, which they do not do (in nature) with members of a different species. Sometimes, however, separate species can hybridize, especially in captivity (e.g. mules, or Napoleon Dynamite’s favorite animal – the liger, a cross between a lion and a tiger). There are other problems with this concept as well – e.g., not all organisms reproduce sexually (think of bacteria). There have been many alternative ideas put forth as to how to define species (a few are discussed in your textbook). In practice, however, most systematists (people who study the classification and evolutionary relationships of organisms) rely on the same tools to delineate species. They typically group together organisms that share traits not found in other organisms. Those traits can be morphological (physical features), molecular (e.g. DNA), and/or behavioral. This does not mean that there is always 100% agreement, and some species can be difficult for researchers to recognize – as you will see in the following exercise.

Exercise 1: Caminalcules

Members of the genus Caminalcule are imaginary creatures that were named after their creator Joseph H. Camin. You will be given a packet that contains cards that give relevant information for 50 individual caminalcules.
A. Flip the cards to the side that contains ONLY the picture and sort them into species:

► How many groups of species did you end up with? ________

B. Now flip the cards to the side that contains ALL the information (picture, DNA sequence and Sex) and reassess your species groups:

► How many groups of species did you end up with now? ________

► What process did you use to determine which group each individual belonged to? Which information did you use first? Did you have to use more than one type of information to make your decisions? Were there any groups that caused you any difficulty or changed after you had all the information instead of just the picture? If so, why were they tricky?

► Did you find any dimorphic species (in which males and females look very different)? How do such species affect your ability to classify organisms into species?

► Did you find any sibling species (cryptic species) that look the same but are really distinct? How did you recognize them? How do such species affect your ability to classify organisms into species?
Speciation:

The process of speciation is what has lead to the abundant biodiversity present on earth today. Speciation can occur when a population becomes subdivided into smaller groups because of a geographic barrier (Allopatric speciation). These subdivided groups tend to undergo different changes, and may become dissimilar enough to be considered separate species. Speciation can also occur within a population by polyploidy (addition or multiplication of entire genomes), a process that is somewhat common in plants, often making them incompatible with rest of the individuals in a population. Less commonly (and often controversially), sympatric speciation may occur if there is habitat differentiation or sexual selection, but many such speciation cases are disputed.

Any speciation event requires the establishment of reproductive isolation, any mechanism that prevents the interbreeding of populations belonging to different species. Reproductive isolating mechanisms can take the form of different habitat preferences (so that breeders of the two species never meet), different reproductive seasons (ditto), mechanical incompatibility of genitalia, hybrid sterility, and differences in developmental rates that prevent hybrid embryos from surviving and growing. In many birds (and frogs and insects), mating calls and behavioral mating rituals commonly differ between related species and serve as reproductive isolating mechanisms.

Certain factors can sometimes lead to abundant and rapid (in the geological sense) speciation known as adaptive radiations where speciation occurs as the result of new niches opening up. A niche describes how an organism interacts with its environment (e.g. what it eats, what eats it, when does it reproduce, where does it live etc.). Some of the factors that can cause new niches to become available are mass extinctions, which open up the niches of extinct species; key innovations, which allow organisms to do something completely new (e.g. change from being aquatic to terrestrial, or develop flight); or the development of new island chains, which provide new and differing habitats that can be exploited. One famous example of an island chain and the speciation that has occurred on them is the Galapagos Islands; the very islands that aided Darwin’s thinking about the process of evolution and the home of Darwin’s finches, which are the subject of the next exercise.

No matter how the division of an ancestral population into separate species gets its start, the question remains as to how these divisions are reinforced. In sexually reproducing organisms (the focus of this lab), divisions are reinforced by reproductive barriers. These barriers fall into two major categories premating and postmating. Premating barriers are factors that prevent members of different species from mating with each other in the first place. For instance, we know that it is possible for lions (Panthera leo) and tigers (Panthera tigris) to produce offspring
(called ligers), but lions and tigers have virtually no overlap in their geographic distributions, making it highly unlikely that they would copulate in the wild. Postmating barriers are those that occur after mating has occurred. For instance the offspring that are produced may be sterile or otherwise have reduced viability (they may be less vigorous or have reduced fertility). Again, in the case of the ligers – even when matings do occur between captive lions and tigers, the ligers that are produced have reduced viability. For a more thorough explanation of reproductive barriers, please see your textbook. In the following exercise you will investigate how reproductive barriers may actually have arisen in the famous Darwin’s Finches of the Galapagos Islands.

**Exercise 2 – Reproductive Barriers in Darwin’s Finches:**

**Beaks, Food and Evolution:**

Darwin’s Finches (members of the genera Geospiza, Certhidea, Platyspiza, Cactospiza, and Camarhynchus) represent an adaptive radiation. A single common ancestor came to inhabit the Galapagos Islands and speciation occurred as populations adapted to the different food sources found on each island. Darwin’s Finches provided a classic example of microevolution (evolution occurring within a population over a span of generations). Peter and Rosemary Grant measured beak depth in the medium ground finch (Geospiza fortis) over a span of nearly a decade and kept records of weather conditions over that same span of time. During normal conditions, a wide range of seed sizes is available for these birds to eat; however, in dry years – only the larger seeds tend to be available.

► On your computer, go to the class website page at http://abacus.bates.edu/acad/depts/biobook/SpeciLab.htm (also available as a link from the Lab Info page)

► From this page, download and examine the figure labeled “Microevolution in Beak Depth” (BeakDpth.pdf). What happened to the average beak depth in *G. fortis* during dry years? What do you think accounts for this change in beak depth?
This example of microevolution in beak dimensions gives us a glimpse at how natural selection can drive changes in morphology. Darwin’s Finches are also an excellent example of macroevolutionary changes because they show a great deal of variation in beak proportions that corresponds to the type of food they eat.

► On your computer, look at the figure labeled “Adaptive Radiation in Darwin’s Finches” or "Phylogeny of Darwin's Finches" (PhyFinch.pdf). How would you describe the differences between the beaks of insect eaters and the beaks of seed-eaters? Are seed eaters and insect eaters clustered together in the same groups, or are they scattered all over the tree?

Singing in Darwin's Finches:

Male songbirds sing for two main purposes – to establish and defend their territories, and to attract females. The song that a bird sings to attract a female is a critical component of mate recognition. In other words, the song that a male bird sings is one of the main features that females use to recognize members of their own species.
Bird songs are comprised of several components, **notes, syllables** (a group of notes) and **trills** (a rapid succession of syllables; see upper right). Birds produce sound by having air from the lungs pass through an inflated region at the base of the trachea called the **syrinx** (see bottom right), causing its tissues to vibrate. While the source of the sound is in the syrinx, the quality of the sound is manipulated by other features of the avian vocal apparatus. The trachea, larynx and beak all act to modify the sound that is produced by the syrinx. They all contribute to “cleaning up” the sound, by acting as a resonance filter, to produce a pure, whistlelike sound. Birds produce songs with many different notes (high pitched notes are caused by high frequency vibrations and low pitched notes are caused by low frequency vibrations) that change in rapid succession. To maintain the pure whistlelike quality of all of the notes within the song, birds must actively (and rapidly) change the configuration of the vocal tract so that it maintains its resonance filtering function. One of the main ways a bird does this is to change the gape of its beak (how wide the beak is opened) throughout the song. If the beak is opened very wide, then the length of the vocal tract (resonance chamber) is shortened – favoring the production of high pitched sounds. If the beak is nearly closed, then the length of the vocal tract (resonance chamber) is lengthened – favoring the production of low pitched sounds (see Figure on following page).
Think of the higher pitched sound that a clarinet plays vs. a bassoon, or the sound of a trumpet vs. a tuba. In each case the instrument producing the higher pitched sound has a shorter resonance chamber than the instrument producing the lower pitched sound (see figure on right).

As you learned earlier, beak morphology in Darwin’s Finches is strongly influenced by the type of food they eat. You should have noticed that seed eaters have large, deep bills that are powerfully built to crush hard seeds, while insect eaters have small pincer-like bills for grasping insects. Because of biomechanical tradeoffs between power and speed, it is predicted that that larger, more powerful bills cannot be opened and closed as rapidly as smaller, less powerful bills.
Given all of this information about the role of the beak in sound production and the predicted influence of beak size on the ability of the finch to rapidly open and close their bills, Jeffrey Podos (an Ornithologist and Evolutionary Biologist at UMass Amherst) made some predictions about the effects of bill morphology on song construction in birds, which are summarized in the following table.

<table>
<thead>
<tr>
<th>Predicted Effects of Beak Morphology on Song Construction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Large Beaks</strong></td>
</tr>
<tr>
<td>- Because of its larger size the overall range of frequencies produced in the song would be lower; both the minimum and the maximum frequencies (i.e. the songs would be lower pitched).</td>
</tr>
<tr>
<td>- Along with over lower frequency of the entire song, the range of frequencies within a trill would be narrower.</td>
</tr>
<tr>
<td>- Because of the tradeoff between power and speed the trill rate should be slower.</td>
</tr>
<tr>
<td><strong>Small Beaks</strong></td>
</tr>
<tr>
<td>- Because of its smaller size a bird with a smaller bill should be able to produce songs with higher frequencies.</td>
</tr>
<tr>
<td>- They should be able to produce a wider range of frequencies within a trill.</td>
</tr>
<tr>
<td>- They should be able to open and close their beaks more rapidly producing a faster trill rate.</td>
</tr>
</tbody>
</table>
Look at the data below. Based on the information regarding beak morphology and song construction given above, which of these birds is likely to produce the highest pitched notes in their songs?

<table>
<thead>
<tr>
<th>Species</th>
<th>Bill Depth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certhidea olivacea</td>
<td>4</td>
</tr>
<tr>
<td>Geospiza fuliginosa</td>
<td>7</td>
</tr>
<tr>
<td>Camarhynchus parvulus</td>
<td>7</td>
</tr>
<tr>
<td>Cactospiza pallida</td>
<td>8.75</td>
</tr>
<tr>
<td>Geospiza scandarius</td>
<td>8.9</td>
</tr>
<tr>
<td>Camarhynchus psittacula</td>
<td>9.75</td>
</tr>
<tr>
<td>Geospiza fortils</td>
<td>12</td>
</tr>
<tr>
<td>Geospiza magnirostris</td>
<td>16</td>
</tr>
</tbody>
</table>

How might you go about testing the prediction that smaller beaks lead to higher pitched notes being included in a finch’s song?
Listen to the songs of these eight finch species that you can download from the class website at [http://abacus.bates.edu/acad/depts/biobook/SpeciLab.htm](http://abacus.bates.edu/acad/depts/biobook/SpeciLab.htm). Can you test your predictions just by listening to these songs?

As you have probably surmised, it would not be possible to quantify the differences among bird songs simply by listening to them. Scientists interested in studying bird songs do so by analyzing spectrograms (see above right), which allow for the characterization of bird songs. Spectrograms give important information about bird songs such as, minimum and maximum frequency, the frequency of individual notes, frequency range within a trill and trill rate. The figure on the bottom right shows spectrograms for each of the 8 species of Darwin’s Finches whose songs you listened to. You will take a closer look at these spectrograms, and do some basic analysis using the program “Raven Lite.”
Spectrogram Analysis Using Raven Lite:

1) If you have not yet already done so, download the RavenLite program on your computer by going to this website: https://store.birds.cornell.edu/ProductDetails.asp?ProductCode=RAVENLITE

2) Open the program RavenLite on your computer.

3) Under “File” choose “Open Sound Files”. Copy each of the finch song sound files from Http://abacus.bates.edu/acad/depts/biobook/SpeciLab.htm (right column) onto your computer, then open them with RavenLite. You will need to repeat this process for each song.

4) Choose one of the songs to start with and maximize its window so that it fills your screen. Both the Waveform (top) and Spectrogram (bottom) will be displayed. Hide the Waveform so that only the Spectrogram is visible by pressing the “Hide Waveform” button.
5) You will notice that spectrogram contains dark lines that represent sounds produced by the birds, and that these dark lines are surrounded by gray “noise.” You can reduce the noise and make the spectrogram easier to read by increasing the contrast to between 80 & 100.

6) Press the play button to listen to the song.

7) Float your cursor over portion of the song you believe to be sung at the highest frequency (note that the y axis of the spectrogram measures frequency in kHz). At the bottom left hand corner you will see that the program gives you details about that specific part of the spectrogram (Time, Frequency, and Power). Record the maximum frequency in the table that follows these instructions.
8) Repeat steps 3 – 5 for each of the 8 finch songs.

9) Have fun with this program. This program allows you to do some fun things such as take a closer look at any one section of the song, change the playback speed of the song, as well as other things. As time allows you should feel free to play around with this program.

<table>
<thead>
<tr>
<th>Finch Species</th>
<th>Bill Depth (mm)</th>
<th>Maximum Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certhidea olivacea</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Camarhynchus parvulus</td>
<td>7</td>
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<td>16</td>
<td></td>
</tr>
</tbody>
</table>

**Analysis of Bill Depth and Frequency Data:**

Using the data in the above table generate a Best-fit line graph (using Excel) that plots maximum frequency vs. bill depth. Be sure to label your axes, provide a title, and include the $R^2$ value in your graph. (Tips on how to use the graphing function in Excel are given in a separate handout that you can download from the left column of the SpeciLab web page that you have been using. This handout also explains how to interpret the $R^2$ values.) When you have completed your graph, be sure to show it to your professor.

- Describe the relationship that is depicted in your graph between bill depth and maximum frequency.

- Do the data support the predicted relationship between beak size and the maximum frequency produced in the finch’s song?
How strong is this support (based on the $R^2$ value)?

**Vocal Deviation:**

To further quantify differences in songs, Jeffrey Podos devised a metric called **vocal deviation**. A large value for vocal deviation indicates that the bird has either slower trill rate and/or a narrower frequency range within a trill.

What would your hypothesis be with regards to the relationship between bill size and vocal deviation? (Be sure to run your hypothesis by your professor.)

Open up the “Vocal Deviation Data” file on your computer (VocalDev.doc). Use the data in this table to generate a graph (using Excel) that creates a best-fit line graph for vocal deviation vs. bill depth. Be sure to label your axes, provide a title and include the $R^2$ value in your graph. When you have completed your graph, be sure to show it to your professor.

Describe the relationship that is depicted in your graph between bill depth and vocal deviation.
Do the data support your hypothesis? ______________

How strong is this support (based on the R² value)?

What implications do these findings have with regards to speciation in Darwin’s Finches? (Once you have answered this question – share your thoughts with your instructor; then look at the "Geospiza fortis vocal deviations" file (GfVocDev.tif) that you downloaded from the Speciation Lab Files webpage.

This story that you have just put together regarding diet, beak size, song production and speciation in Darwin’s Finches is a very cool story. So cool, in fact, that it was published in one of the science community’s most prestigious journals – Nature (Podos, 2001). However, the Podos (2001) study was not without limitations. Podos was unable to fully account for the influence of body size, which varies among these finch species and can influence song characteristics. There is no such thing as a flawless study, which is why multiple studies are done – each building on the findings of previous studies (or sometimes refuting them) and improving our understanding of biological systems. As it turns out, the same brilliant mind behind the Nature paper joined forces with another investigator to take a closer look at a single species of finch (Geospiza fortis) that exhibits a bimodal distribution for beak depth (Huber and Podos, 2006). A bimodal distribution means that there are two different modes within a data set (the mode is the most frequently occurring value in a data set). In this case it means that there are two different modes for bill depth within G. fortis, one being a few millimeters smaller than the other. Presumably this is because subsets of this species have specialized on slightly different seed types that favor different bill dimensions. The investigators wanted to know if the differences in bill depth found within this single species of finch would be enough to
affect song characteristics, and this time they were able to take body size into account.

► Open the “Geospiza fortis Vocal Deviations” file on your computer (GfVocDev.tif) and examine the relationships between the three different bill dimensions and vocal deviation. Describe this relationship below.

► Do these relationships mirror those that you discovered between bill depth and vocal deviation in the 8 different species of Darwin’s Finches?

► What do you think these results might mean for the future of G. fortis?
Literature Cited:


Species & Speciation – HW Instructions

For this lab HW you need to work with your lab group and write an Abstract and a Results section for the experiment you conducted on Reproductive Barriers in Galapagos Finches. These should be written as though you were including them in a research paper that will be published in a scientific journal. Refer to your Writing Guide ("A Student Handbook for Writing in Biology") for guidelines as to how to write these critically important sections of a research paper. You will need to include data (with graphs and statistics) on beak depth, frequency, and vocal deviations. Pay close attention to when tables and/or figures should be included, and how they should be formatted! Please put all tables and/or figures at the end of your report (do not embed them in your text!).

Your report should have clear headings and be double-spaced, using 12 pt. Times New Roman font. It should have a descriptive title and the full name of each group member who contributed to this assignment should appear at the top of your document (but omit the name of anyone who did not contribute). Please have all members of the group proofread the document, then submit one document (either on paper or electronically) for the entire group.