EXPLORING GENETICS OF ADAPTATION AND SPECIATION BY COMPARING *MIMULUS* WILDFLOWER SPECIES

Lab 10

INTRODUCTION

This week in lab we will take a genetic approach towards understanding how **adaptations** and **reproductive isolation** evolve. We will be working with two familiar *Mimulus* wildflower species, *M. lewisii* and *M. cardinalis*, that are adapted to different pollinators and are reproductively isolated from each other in nature by both **pre-zygotic** and **post-zygotic** barriers. This reproductive isolation means that they are considered to be different species according to the **Biological Species Concept**. Our goals in this lab are to use classical genetic analyses to try to figure out the genetic basis of these adaptations and reproductive isolating barriers. Obviously we cannot go back in time to the common ancestor of these two species and then follow each incipient species forward in time as they begin to diverge from each other by mutation, natural selection, and random genetic drift. But if we could do such time travel, we might be able to directly observe each mutation that has contributed to the evolution of the adaptive traits as it arose and was increased in frequency due to natural selection caused by specific ecological pressures. We would therefore have a complete picture of whether evolution occurred **gradually** with many intermediate steps, as Darwin believed, or whether just a small number of mutations of large effect occurred. We would also discover whether the reproductive isolation between the two modern species evolved as an **incidental by-product of adaptive divergence** in allopatry, or whether perhaps the pre-zygotic isolation evolved at least in part by the process of **reinforcement**.

Obviously we can't answer all of those interesting questions by time travel, but we can begin to make headway by "genetically dissecting" the traits that differ between the two *Mimulus* species because, luckily for us, we can cross the two species and observe how the traits are inherited in the hybrid progeny. Any mutations that contribute to the trait differences between the species, ***and therefore that accumulated in the two lineages since their divergence from their common ancestor***, will segregate as Mendelian factors in an advanced hybrid generation such as an F_2 population. If only one mutation contributed to a specific trait difference between the species, then it will **segregate** as a single genetic locus with something like a 3:1 or a 1:2:1 ratio in an F_2. On the other hand, if numerous mutations at different loci, each with a small effect, contributed to the difference in a trait, then we might expect to see a continuous "bell-shaped" distribution of phenotypes in the F_2 with very few if any F_2s having parental trait values. And if different traits evolved due to different mutations on different chromosomes, then the loci should **assort independently** from each other and the traits should not be correlated among F_2 progeny. In this way we can look at many of the traits that are involved in adaptation to different pollinators (and therefore pre-zygotic isolation), such as flower size, shape, color, and nectar production, as well as traits such as pollen viability that may contribute to postzygotic isolation, and determine whether few or many genetic "steps" were involved in the origin of these species. (Ideally we would map each and every evolutionarily important mutation with Quantitative Trait Locus (QTL) mapping methods, but such an enormous project is beyond the scope of the current lab!)

Most, but not all, of the ecologically and evolutionarily important traits that we'll be looking at in *M. lewisii* and *M. cardinalis* and their F_2 hybrids are floral traits related to bumblebee vs. hummingbird pollination. As you may recall, we spent some time in the very first lab for this class learning about these "**pollination syndromes**." Here you can review a couple of paragraphs and figures from your first lab handout:

One useful way to understand the diversity of floral forms is to group the flowers according to the kinds of visitors they attract by identifying sets of floral adaptations corresponding to different modes of pollination. These suites of adaptations are referred to as **pollination syndromes**. For this part of the lab you will compare pairs of species in the same genus that are pollinated primarily by **bees** or by **hummingbirds.** Bees are by far the most common and important group of animal pollinators, and many plant species depend on them. Hummingbird pollination is more specialized, and most hummingbird-pollinated flowers are thought to have evolved from bee-pollinated ancestors. However, there is a distinctive biogeographical pattern to the distribution of hummingbird-adapted flowers. They are found naturally only in North and South America because hummingbirds also occur only on these New World continents.

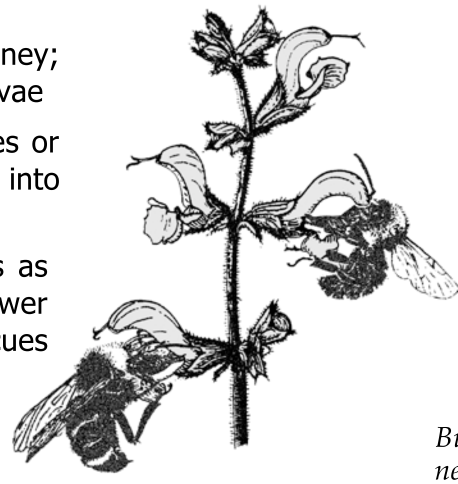
<u>Hummingbirds</u>

- use nectar as flight fuel, and as warm-blooded vertebrates need large, energy-rich nectar rewards
- probe for nectar in floral tubes or spurs with stout bills and long tongues; ***hover*** while feeding and can rotate body and wings to feed with bill pointing straight up.
  (flower-visiting birds in Australia and the Old World, such as honey creepers and sunbirds, do not hover and must perch to feed.)
- olfactory sense poorly developed;
- color vision similar to humans and able to discriminate red wavelengths;
- intelligent in finding openings to flowers and learning to associate distinctive floral color (especially red) and shape with nectar rewards.

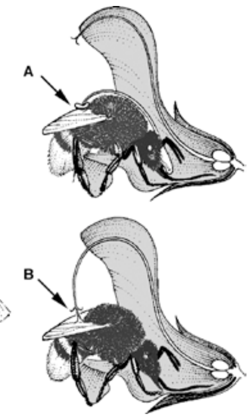
Hummingbird hovering in front of trumpet creeper flower

Bees

- use nectar as flight fuel and to make honey; *collect pollen* as protein source to raise larvae
- use tongues to reach nectar in short tubes or spurs, especially by crawling part way into flower (See illustration.)
- adept at using color markings on flowers as guides to nectar; also sensitive to flower shape, especially bilateral symmetry, as cues for finding nectar
- visual sensitivity shifted to short (blue) wavelengths; less ability to discriminate long (red) wavelengths; often attracted to blue flowers;
- olfactory sense well developed
- do not feed while hovering, need landing platform or place to grab onto floral parts



Bumblebees entering mint flowers to find nectar



Bumblebee probing for nectar inside a meadow clary flower. (Note the precise placement of the anthers (A) and stigma (B) in the same location on top of bee's abdomen.)

This week in lab we will look at two of the species you examined in that very first lab for this course - - the pale pinkish-blue flowered, bumblebee pollinated *M. lewisii* and the bright red flowered, hummingbird pollinated *M. cardinalis*. These two species are native to western North America. *M. lewisii* is found at high elevations, in the Sierras and Cascade Mountains, for example, whereas *M. cardinalis* is found at lower elevations. Mostly these two species are allopatric, but in a few places like Yosemite National Park their ranges partly overlap. Nonetheless, even at these sympatric sites where the two species growi near each other, hybrids have only rarely been observed in nature. In the greenhouse it is easy to hybridize them however, and F_1 hybrids are vigorous. There is some postzygotic isolation, however, because these F_1s produce fewer seeds than the parents, and about $^2/_3$ of their pollen grains fail to develop properly and so are inviable. But if you self-pollinate enough F_1 flowers you can get enough seeds to produce a large F_2 population like the one you will study today.

Exercise 1: Observation and comparison of floral traits in *M. cardinalis* and *M. lewisii*

Your TA will show you the two flowering parental species that we've brought in from the greenhouse - - there should be lots of flowers of each type. Everyone should go look at the plants and collect one fresh, recently opened flower of each species by carefully snipping it off the main stem of the plant. Take your two flowers back to your seat, and look at them carefully without tearing them apart.

1. First look at them "head-on" to see what the bee or hummingbird would see when they approach the flower and decide to visit it or not. What visible flower traits might attract the pollinators? Sniff the flowers - - are they fragrant? Make a **list** of all floral traits that might be involved in **pollinator attraction**. Make a note of how they differ between the plant species, and **how you might measure these traits in the parents and hybrids (quantitatively or qualitatively).**

2. Now look at the flowers from the side view and other views, and think about how the pollinator would enter the flower. How does the flower "fit" to the head or body of the pollinator? Look carefully at the location of the pollen-receiving stigma (it may be open or it may have closed by touch, like a Venus flytrap!) and the pollen containing anthers - - how might the placement of these sexual parts facilitate efficient pollen deposition and removal by the pollinator? Make a **list** of floral traits that might be involved in **pollination efficiency.** Make a note of how they differ between the plant species, and **how you might measure these traits in the parents and hybrids (quantitatively or qualitatively).**

3. Why do you think the pollinator would want to visit the flower in the first place - - what might be its reward, and do you think the preferred rewards may be different for bees and hummingbirds? Make a **list** of potential **pollinator rewards**. Make a note of how they differ between the plant species, and **how you might measure these traits in the parents and hybrids (quantitatively or qualitatively).**

After you have listed your traits and how you might measure them, discuss your ideas **first** with your lab partner, **and then with the lab as a whole**. Do you need to dissect the flower to measure your traits??? How are you going to measure flower color???

Some pollinator traits are not very obvious visually, and these include fragrance and nectar production. In addition, traits related to post-zygotic isolation like pollen viability are not obvious visible traits. In the class discussion, be sure to discuss how you might measure these important traits.

Your TA will help you come up with a plan to measure traits related to pollinator attraction, pollination efficiency, reward, and post-zygotic isolation.

Once your lab has a plan of action, and your table knows what to do, measure the traits for 1 flower/pair of students of each species, and record your data on the lab spreadsheet.

Exercise 2: Making predictions about the inheritance of traits involved in adaptations to pollinators and reproductive isolation

Now that you have looked carefully and quantitatively at the traits in the parental species, begin to think about how the trait differences will be inherited in the F_2 hybrid offspring. Measure the traits for at least 1 F_1 flower/pair of students and record your data on the lab spreadsheet.

Continue your observations with the F_2s. As you probably know already, many of the plants in the lab are F_2s from a cross between *M. lewisii* and *M. cardinalis* F_1 hybrids. Hopefully there will be large numbers of these F_2s that are flowering – the display of variation should be incredible, after your focus on the uniform parentals and F_1s! Working with your lab partner as before, measure your traits on one flower from each of 1-2 different F_2. Be sure that all the groups in your section choose flowers from different plants. Enter your data on the lab spreadsheet.

Make a list below of all of the traits the lab as a whole has decided to measure.

For each trait, look at the parental, F_1, and F_2 values, and write down a prediction about how the trait differences might be inherited. Be sure to include each of these factors in your prediction:

1. Are the differences for a trait due to one locus (or perhaps two) of large effect or many loci of small effect?
2. Based on the appearance of the F_1, does the trait show dominance or is the F_1 intermediate, implying additivity or incomplete dominance?
3. Do you think any of the different traits are due to variation at the same loci or did you observe independent assortment (or perhaps some linkage)?
4. Think especially hard about pollen viability. The F_1 has low fertility but the two parents have high fertility. What level of fertility did you observe in the F_2s? Do you think this is due to 1 or more loci with heterozygote disadvantage, perhaps chromosome inversions, or instead due to two or more interacting loci (Dobzhansky-Muller incompatibilities)?

Pollinator Attraction Traits:

Pollination Efficiency Traits:

Pollinator Reward Traits:

Post-zygotic Isolation Trait:

Now have a brief discussion of your predictions with your lab table, and then your lab instructor will help lead a discussion with the lab as a whole!

Exercise 3: Genetic Dissection of Traits by Examining F_2 Hybrids

Now put your predictions to the test! Your TA will pass out the data collected by all lab sessions in a previous semester. The lab was organized differently then, so these data are the result of the measurements of over 1500 flowers from over 500 different plants – a huge sample size! Use these data to answer the following genetic and evolutionary questions:

1. Which, if any, of the measured traits involved in pollination show simple 1- or 2-locus Mendelian inheritance? Which traits seem to be true quantitative, or complex traits, with many loci contributing to their variation in the F_2? How can you tell?

2. Do any pollination traits seem to be inherited together, suggesting either tight linkage or the same gene(s) contributing to the species differences in two or more traits?

3. Based on your answers above, what is your guess as to how many genes are involved in the differences in pollinator adaptations between the species? Was evolution gradual and incremental, or not? This answer might depend on which traits you think are most important to pollinator adaptation!

4. Now think about the fact that the F_1's were partially sterile, with only $^1/_3$ as many viable pollen grains as the parents. Based on the data from the F_2s, how do you think this post-zygotic isolating trait is inherited? Do you think it involved factors like chromosomal inversions that have heterozygote disadvantage, or does it seem to involve interactions between two or more loci? How can you tell, or can you? What do the two genetic mechanisms imply about the possible role of natural selection and random genetic drift in causing these loci to evolve?

5. Taking the results as a whole, what do you think is a reasonable ecological scenario for how these species evolved from a common ancestor that was most likely bumblebee pollinated and probably looked a lot like modern *M. lewisii*? Which genetic changes might have first been favored by natural selection, and which ones might have occurred later? Do you think reinforcement was involved in the origin of these species? What are the reasons for your answers? **And most importantly, can you think about experiments that you could do now to test these ideas???????**

6. Finally, compare the data collected by Duke Bio 202L students to published data. Your TA will project the QTL results for many of these traits. Do the number of loci for each trait that you predicted from the class data match the number of QTLs found?