Mimulus Speciation TA Notes

Lab 10

No lab assignment due this week

Lab assignment due next lab:

Phylogenetics pre-assignment turned in via Sakai and multiple choice so graded automatically.

Other important issues/announcements/etc:

1. Quiz OK?
2. PPT and Excel template are in the TA folder for this week.
3. We need help transferring plants this week:
    i. At the end of prep today (everyone if possible)
    ii. Wednesday afternoon
    iii. Friday afternoon at 4
4. Students may review their exams in lab this week
    - They may not take either questions or answers sheets with them
    - Make it very clear than any answer sheets that are "misgraded" will be compared to the original scanned images
    - Watch students to make sure they don't make changes or photograph anything
    - Regrade requests are due by Friday, April 11 (students should talk to Mohamed about requests before or after lecture)
5. Keep in mind that there are only two more labs after this one and that you'll have to have all grades (lab assignments, lab quizzes, and the second half of lab participation (40 possible points unless your lab was cancelled)) entered by the evening of Monday, April 21. If one or more of your sections was cancelled, be sure you've entered "snow day" in the comments for all the missed quizzes.

Objectives:

1. Identify different characters involved in reproductive isolation
2. Measure these traits in parental species and F_1 and F_2 hybrids with the goal of identifying how many genes determine each trait and therefore how many genes are involved in the divergence of these species

***Schedule*:**

1- Quiz

2- Exercise 1: each pair of students takes one *M. lewisii* and one *M. cardinalis* flower back to their seat to make observations. Cut flowers at the stem below the base of the flower. No actual measurements at this time, just overall observations of traits that differ between the 2 species. Then a discussion between students and then among whole class, whole class decides which traits to measure and makes predictions. See the table at the end of these notes for possible

traits and ones that *must* be measured. The take these measurements on 1 flower per pair of students of each species. Students should enter their data on spreadsheet that you're projecting.

3- Exercise 2: measurements of F_1s and F_2s. Each pair of students should measure 1 F_1 flower and 1-2 F_2 flowers and again enter their data on the projected spreadsheet. ***You should pick the F_2 flowers for your students. Make sure to only pick one flower per F_2 plant per section (so each F_2 measurement in the section is from a different plant).*** Once the data is collected, they should make the predictions asked for on pages 6-7 of the lab handout based on the section's data. Discuss their predictions as a class.

4- Exercise 3:

a. Assign traits to groups of students and pass out old giant data results (pollen viability should be assigned by itself)

b. Students answer the 2-3 questions for their traits (pollen viability group answers #4-5, other groups answer #1-3)

   For #4-5
   Underdominance (caused by inversions on chromosomes that mess up meiosis) is only bad in heterozygotes, so F_1s that are all heterozygotes will all have the minimum amount of pollen viability

   Dobzhansky-Muller incompatibilites – possible for some F_2s to have less viable pollen than F_1s, for example, if *M. lewisii* is AABB and *M. cardinalis* is aabb, the F_1s are AaBb, but some F_2s could be AAbb or aaBB which could be worse than AaBb

c. Class discussion about findings (but keep it kinda short)

d. Class discussion comparing class results to published results (see ppt)

Traits (*important)	**Units**	**Cat.**	**Measurement method instructions**
Corolla width*	mm	A	at widest point, viewed looking directly into flower and across petals while in natural position
Corolla aperture width	mm	A	across tube opening between the joints of upper & lateral petals
Corolla aperture height	mm	A	across tube opening from upper to lower petals at widest point
Lateral petal width	mm	A	at widest point
Upper petals reflexion*	1-5 scale	A	amount the 2 upper petals bend back from the midline, 1 = unreflexed (*lewisii*); 5 = fully reflexed (*cardinalis*)
Lateral petals reflexion*	1-5 scale	A	amount the 2 lateral petals bend back from the medial petal, 1 = unreflexed (*lewisii*); 5 = fully reflexed (*cardinalis*)
Nectar guides	1-5 scale	A	hairiness of the lower petals in the throat 1= brushy (*lewisii*); 3 = hairy; 5 = mostly smooth (*cardinalis*)
Color of lower petals*	1-6 scale	A	medial petal; match to color strip provided
Carotenoids*	yes/no	A	***back*** of upper petals, tips, not near the throat; yes = if any yellow is present; no = no yellow present
Nectar volume*	mm	R	gently pull the green base off of the petals, place a 10μl capillary tube into the hole in the base of the petals (look for a droplet of nectar to put the tube in), rub tube gently along the base of the petals, when no more nectar is sucked up, measure the height of the nectar in the capillary tube (making sure the nectar is even with the base of the capillary tube)
Pistil length*	mm	E	gently pull apart the sepals between the lateral and medial sepals (side away from pistil), measure from the tip of the stigma to where the pistil attaches to the sepal
Stamen length*	mm	E	gently pull apart the petals between the lateral and medial petals (side away from stamens), choose the longest stamen, measure from the tip of the anther to where the stamen attaches to the petal
Pollen viability*	%	PZ	note the plant ID number(s) on the slide, place the smallest drop possible of dye on the slide, hold as many anthers as possible from a single flower with forceps and mash them into the dye droplet, cover with a coverslip (discard anthers, rinse forceps in ethanol and wipe with Kimwipe) – 2 samples should fit on a single slide; under microscope use counters to score 100 pollen grains as viable (round, stained) or inviable (deformed, unstained) or count 100 pollen grains clicking for viable ones; ruptured grains shouldn't be counted
Students' choices??	??	??	if your students think of another trait to measure, be sure to note the measurement technique in the Google Doc

Categories: A = attraction, R = reward, E = efficiency, PZ = postzygotic

* Important traits that must be measured, unstarred traits can be measured if your students are interested in them

Instructions are given diagrammatically in ppt and sheets available in the lab rooms