Adv SRD Lab Notebook

Fly Housing Preparation	
Date: 10/8/2021	**Length of Research Period(s):** 2 hours

Goal(s)/Purpose:

To prepare the vials of medium that will house the Sepia-eyed flies for the experimental arm.

- Place the same amount of medium/water, and the same sized piece of mesh in each of the six vials. Essentially, I want to set up each of the vials as symmetrically as I can. This to ensure that there are no confounding variables that could affect the development/growth of the flies based on their housing situations. This is especially important to prevent, as the flies being housed in these vials will eventually be bred in my experimental arm.
- Label the vials "male 1", "male 2", "male 3", and "female 1", "female 2", and "female 3". And, I need to label one last vial "mixed". By having these specific vials, I will be able to have sex-specific prepared housing for the flies that will be divided up in the next entry.

Materials Required:

1. Drosophila Culture Vial Set 1 (7): Product #173076
   https://www.carolina.com/drosophila-fruit-fly-genetics/drosophila-culture-vial-set-1-36-vials-plugs-caps/173076.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
2. Vial Labels for Drosophila Crosses, Roll of 500 (1): Product #173190
   https://www.carolina.com/drosophila-fruit-fly-genetics/vial-labels-for-drosophila-crosses-roll-of-500/173190.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
3. Formula 4-24® Instant Drosophila Medium, Blue, 1 L (2): Product #173210
   https://www.carolina.com/flies/formula-4-24-instant-medium-blue-1-l/173210.pr?question=drosophila+medium+blue
4. Ultra Fine Point Black Sharpie (1): Product #B010BS53LG
   https://www.amazon.com/Sharpie-Permanent-Markers-Ultra-2-Pack/dp/B010BS53LG/ref=sr_1_15?dchild=1&keywords=ultra%2Bfine%2Bpoint%2Bblack%2Bsharpie&qid=1613938480&sr=8-15&th=1
5. Culture Mesh (1): Product #1713090
   https://www.carolina.com/flies/drosophila-culture-netting-pkg-100/173090
6. 100 mL beaker (3)
7. Ruler (1)
8. Scoopula (3)

Procedure:

Fly Feeding and Vial Preparation:

43. Repeat steps 9-20 for seven vials.
44. Locate the vial labels, and using a sharpie, write on one of them "Male 1". Place this label along the side of one of the seven prepared vials. Since I couldn't find the vial labels, I had to use tape for the entirety of this procedure.
    a. **Note: See image below for what the "Male 1" vial should look like after this step:

Figure 4

45. On another label, write, “Male 2”. Place this label along the side of another prepared vial. Same comment as step 44.
46. On another label, write, “Male 3”. Place this label along the side of another prepared vial. Same comment as step 44.
47.
48. On another label, write, “Mixed”. Place this label along the side of another prepared vial. Same comment as step 44.
49. Repeat steps 44-46 for the final three vials that have not been labeled. However, instead of marking the labels “Male 1”, “Male 2”, and “Male 3”, mark the labels “Female 1”, “Female 2”, and “Female 3”.
    a. **Note: Refer to image below for how all the vials should appear once all the steps of this section have been completed:

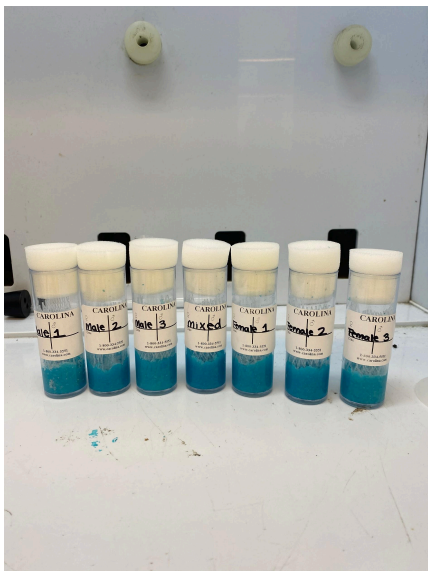
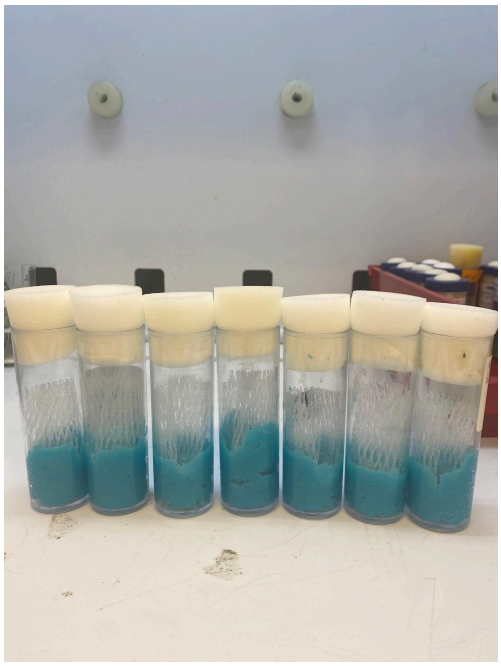
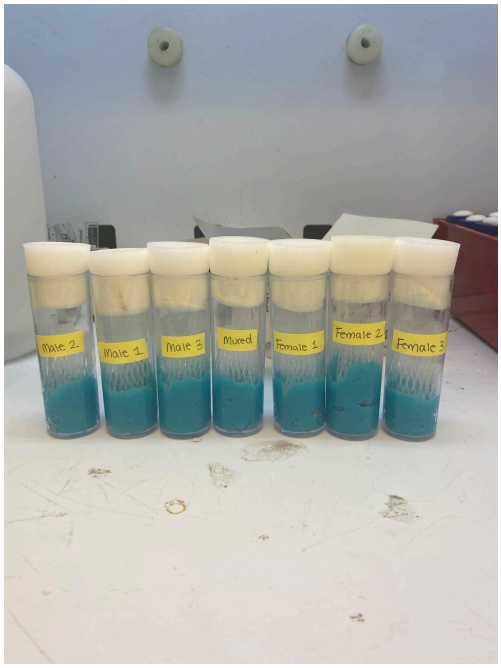


Figure 5

Data and Observations:





Procedure Reflection and Future Endeavors:

Overall, this section of my procedure went very smoothly. The only divergence I had from the original procedure was using tape

labels instead of official vial labels, which wasn't a problem at all. Unfortunately, many of the Sepia flies were still in larval stages, so I wasn't able to move on to the second or third entries of my lab notebook (as I had originally hoped to). I am glad that all the vials that are going to house the experimental flies are set up in a way that, I believe, will help limit confounding variables that could arise from variations in housing set-up. I used the extra time I had (as a result of the Sepia flies not being fully developed for crossing) to organize and gather materials for my bin--as you can see in the final image of my data & observations section. Now that all my materials are ready, once the Sepia flies emerge, I can start the anesthetization, gender differentiation, and fly compartimalization part of my procedure ASAP!

Anesthetization, Sex Differentiation, and Separation of the Experimental Subjects

Date: 10/13/2021	**Length of Research Period(s):** 1.5 hours

Goal(s)/Purpose:

To anesthetize and differentiate the flies by sex:

- Anesthetize the Sepia flies with the FlyNap to ensure that they are asleep while they are being divided. If they are awake while being divided, it would just be a mess. Since I don't know how long the anesthetization will last (and from doing this in the past they have been asleep for much longer than expected), I need to work quickly but cautiously to ensure that the division of the flies is completed before the flies wake up.
- Divide up the Sepia-eyed flies by sex. After the flyNap has been applied, I need to tap the flies out of the vial, and then once they are all out, using my eyes/a microscope (to ensure proper vision and to minimize confusion), I need to carefully examine their abdomens and section them into male and female sides of the plastic weigh dish. Then, I need to separate them into three groups of five males and three groups of five females, so that they are organized by sex for the breeding that will occur later in the experimental arm. It is important that the number of flies in each of the vials is constant so that the number of subjects is not varied and thus, is not a confounding variable.
- Put the gender differentiated flies into their corresponding vials that were created in the previous entry. This is to ensure that they are organized adequately, and are ready to be used in the following entry (the first part of the experimental arm).

Materials Required:

1. FlyNap Anesthetic Kit (1): Product #173010
   https://www.carolina.com/drosophila-fruit-fly-genetics/flynap-anesthetic-kit/173010.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
   MSDS of FlyNap: https://www.carolina.com/teacher-resources/Document/msds-flynap/tr-msds-flynap.tr
2. Drosophila Sorting Brush, Pack 12 (1): Product #173094
   https://www.carolina.com/drosophila-fruit-fly-genetics/drosophila-sorting-brush-pack-12/173094.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
3. Magnifying Glass (1): Product #77390B+50B
   https://www.amazon.com/Magnifying-Glass-10X-Magnifier-Soldering-Inspection/dp/B07WRK6F6W/ref=sr_1_1_sspa?dchild=1&keywords=Magnifying+Glasses&qid=1612491315&sr=8-1-spons&psc=1&smid=A20TFX64PGWL2G&spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUEyVkVNUUcyV0FFTlZFJmVuY3J5cHRlZElkPUEwNzA3NzM1M1ZaSEkwNEdHUDFOSSZlbmNyeXB0ZWRBZElkPUEwNTUyMTY2MUJERVg5Wlc1RExLTSZ3aWRnZXROYW1lPXNwX2F0ZiZhY3Rpb249Y2xpY2tSZWRpcmVjdCZkb05vdExvZ0NsaWNrPXRydWU=
4. Plastic Weigh Dish (1): Product #702332
   https://m.carolina.com/lab-balances-scales/weighing-boats-plastic-3-516-x-34-in-pack-500/702332.pr?question=
5. Drosophila Stand (1): Product #173030
   https://m.carolina.com/flies/drosophila-stand-pack-of-3/173030.pr?question=fly+stand
6. Flinn Scientific Dissection Microscope (1): Product #59-1815
7. Drosophila, Living, sepia, Chromosome 3 Mutant, Vial of 25-30 (1-2): Product #172575

https://www.carolina.com/flies/drosophila-living-sepia-chromosome-3-mutant/172575.pr?question=sepia

Procedure:

Anesthesia, Gender Differentiation, and Separation:

43. The following steps of this section should be done in a ventilated area, preferably in a hood.
    a. **Note: The hood's sash should be lowered until there is a ~10 inch gap to work through.
    b. Plug in/Turn on the hood.
44. Repeat steps 22-32, but for step 26, grab a vial of the Sepia~~Ebony~~-eyed Drosophila. I did this for two vials of the Sepia-eyed Drosophila because there were not enough flies in one of the vials.
45. Over a plastic weigh dish, hold the Sepia~~Ebony~~-eyed Drosophila vial upside down, and gently remove the foam cap so all the flies fall onto the dish. Tap the sides and bottom of the vial to ensure the remaining flies also fall onto the dish. This may require force. Did this for both vials.
    a. **Note: If some flies are still present after tapping the vial, gently remove them using a sorting brush.
46. Re-plug the Sepia~~Ebony~~-eyed Drosophila vial with its foam cap, and put it back in its residing storage spot. Did this for both vials.
47. Plug in the dissection microscope and put it to setting 2x.
48. Place the plastic weigh dish onto the microscope stage. Turn on the microscope's light.
49. By sight, separate the flies on the plastic weigh dish into male and female sections in different corners. Do so using the sorting brush. Once they have been divided by gender, verify they have been correctly divided by gender using the microscope. The note below explains how to identify the male vs female flies.
    **Note:
    a. In order to differentiate the male and female flies is it helpful to look at their abdomens. Male flies have a darker, more bristled, pointed abdomen, while females have a cream-colored, peach-shaped one. If necessary, a magnifying glass can be used to get an even clearer image. See the image below of the abdomens of fruit flies for reference. The male is on the left of the image, and the female is on the right:

Figure 6
(The Arrogant Scientist 2012)

   b. The flies will most likely stay sedated for ~15-20+ minutes. While it is extremely important to separate the flies accurately, if they begin to move around again, it is more important to contain them. So work as quickly and as cautiously as possible.
50. Remove the foam cap from the vial labeled "Male 1", and place the vial horizontally on the workspace.
51. Using the sorting brush, carefully guide a group of five male flies to one of the corners of the plastic weigh dish that is not occupied by other flies.
   a. **Important: The next step (58) will require a partner to hold the "Male 1" vial in place. Done without a partner
52. Using the sorting brush, guide the five flies one by one into the opening of the "Male 1" vial.
   a. **Note: Make sure that the gender-separated sections remain separated while transferring the flies into the vial.
53. While the vial is still sideways, cautiously insert the foam cap back into the vial. Make sure that no flies are crushed and that the fit is secure.
   a. **Note: The vial should be kept on its side until the flies are no longer sedated. So, do not place the vial upright in its residing storage spot.
54. Repeat steps 56-59 for the vial marked "Male 2".
55. Repeat steps 56-59 for the vial marked "Male 3".
56. Repeat steps 56-59 for the vial marked "Female 1", but instead of guiding a group of five male flies to one of the corners of the plastic weigh dish that is not occupied by other flies for step 57, guide a group of five female flies.
57. Repeat Steps 56-59 for the vial marked "Female 2", but instead of guiding a group of five male flies to one of the corners of the plastic weigh dish that is not occupied by other flies for step 57, guide a group of five female flies.
58. Repeat Steps 56-59 for the vial marked "Female 3", but instead of guiding a group of five male flies to one of the corners of the plastic weigh dish that is not occupied by other flies for step 57, guide a group of five female flies.
59. Now, there should be extra male flies and/or extra female flies remaining on the plastic weigh dish.
   a. **Note: If there are not extra flies, that is fine, and steps 66-68 may be skipped.
60. Remove the foam cap from the vial labeled "Mixed", and place it sideways on the workspace.
   a. **Important: The next step (67) will require a partner to hold the "Mixed" vial in place. Same comment as step 51a.
61. Using the sorting brush, guide the extra male and female flies into the opening of the vial.
62. While the vial is still sideways, insert the foam cap back into the vial. Make sure the fit is secure, and no flies are crushed.
   a. **Note: The vial should be kept on its side until the flies are no longer sedated. So, do not place the vial upright in its residing storage spot.
63. The anestesia wand may be disposed of.
   a. **Important: Unplug/turn off the hood. The hood's sash may be raised again

****Important:** Keep an eye on the flies. The Male and Female 1, 2, and 3 vials, and the "Mixed" vial may lay sideways in their residing storage spot until all the flies inside the vials are no longer sedated. Once all the flies are crawling/flying around again, the vials may return to their upright positioning in their residing storage spot.

****STOP:** If time is running out, wait until next session to start the Experimental Arm. Clean and return all miscellaneous materials to their designated areas.

Data and Observations:

Procedure Reflection and Future Endeavors:

I was finally able to anesthetize and divide the Sepia flies! Having not done this for roughly four months, my procedure was a helpful reacclimation, and, overall, reproducing it was a quick and straightforward process. The only divergences I made to this

procedure include, first, changing all the times I say “Ebony” to “Sepia” (as I am no longer following my original plan of crossing Wild-type drosophila with Ebony-eyed drosophila). Next, as indicated on steps 44-46, I anesthetized two vials of Sepia Drosophila instead of one. This is because one Sepia vial did not have sufficient flies for my crosses. Finally, I did not use a partner as recommended on steps 51 & 60--this is because I felt confident and concentrated enough to guide the flies on my own. Now that the Sepia male and female drosophila are in their specific vials, they will be ready to be crossed with the males once the *WT* males are added to the Male 1, 2, and 3 vials!

Setting up the Experimental Crosses	
Date: 10/13/21	**Length of Research Period(s):** 1.5 hours

Goal(s)/Purpose:

To Cross and Observe the experimental arms.

- Add a male Wild-type Drosophila to the male Sepia-eyed Drosophila vials.
- Put the flies from the corresponding female vials into the corresponding male vials (Female 1 into Male 1). This will ensure that the males and females interbreed so we can eventually track their offspring in later steps. This will also produce our Experimental 1, 2, and 3 vials.
- Write new vials labels for each of the experimental crosses, and put them on top of the original vial labels. This is to ensure that the replicate cohorts are organized and won’t be confused.
- Check in on the development of the F1 generation.

Materials Required:

8. Drosophila, Living, Wild Type, Vial of 25-30 (1): Product #172100
   https://www.carolina.com/drosophila-fruit-fly-genetics/drosophila-living-wild-type/172100.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
9. FlyNap Anesthetic Kit (1): Product #173010
   https://www.carolina.com/drosophila-fruit-fly-genetics/flynap-anesthetic-kit/173010.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
   MSDS of FlyNap: https://www.carolina.com/teacher-resources/Document/msds-flynap/tr-msds-flynap.tr
10. Drosophila Sorting Brush, Pack 12 (1): Product #173094
    https://www.carolina.com/drosophila-fruit-fly-genetics/drosophila-sorting-brush-pack-12/173094.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
11. Magnifying Glass (1): Product #77390B+50B
    https://www.amazon.com/Magnifying-Glass-10X-Magnifier-Soldering-Inspection/dp/B07WRK6F6W/ref=sr_1_1_sspa?dchild=1&keywords=Magnifying+Glasses&qid=1612491315&sr=8-1-spons&psc=1&smid=A20TFX64PGWL2G&spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUEyVkVNUUcyV0FFTlZFJmVuY3J5cHRlZElkPUEwNzA3NzM1M1ZaSEkwNEdHUDFOSSZlbmNyeXB0ZWRBZElkPUEwNTUyMTY2MUJERVg5Wlc1RExLTSZ3aWRnZXROYW1lPXNwX2F0ZiZhY3Rpb249Y2xpY2tSZWRpcmVjdCZkb05vdExvZ0NsaWNrPXRydWU=
12. Plastic Weigh Dish (1): Product #702332
    https://m.carolina.com/lab-balances-scales/weighing-boats-plastic-3-516-x-34-in-pack-500/702332.pr?question=
13. Drosophila Stand (1): Product #173030
    https://m.carolina.com/flies/drosophila-stand-pack-of-3/173030.pr?question=fly+stand
14. Flinn Scientific Dissection Microscope (1): Product #59-1815

Procedure:

Experimental Arm: Crossing a Wild-type Drosophila with a population of Sepia~~Ebony~~-eyed Drosophila

Crossing of the Flies:

70. The following steps of this section should be done in a ventilated area, preferably in a hood.
    a. **Note: The hood's sash should be lowered until there is a ~10 inch gap to work through.
71. Repeat steps 22-32, but for step 26, grab a vial of the Wild-type Drosophila.
72. Over a plastic weigh dish, hold the Wild-type Drosophila vial upside down, and gently remove the foam cap so all the flies fall onto the dish. Tap the sides and bottom of the vial to ensure the remaining flies also fall onto the dish. This may require force.
    a. **Note: If some flies are still present in the vial, gently remove them using a sorting brush.
73. Re-plug the Wild-type Drosophila vial with its foam cap, and put it to the side.
74. Plug in the dissection microscope and put it to setting 2x.
75. Place the plastic weigh dish onto the microscope stage. Turn on the microscope light.
76. By sight, identity one male. Do so using the sorting brush. Verify it is a male using the microscope.
    **Note:
    a. In order to differentiate the male and female flies, refer to step 55a and Figure 6.
    b. Keep in mind the flies will most likely stay sedated for ~15-20+ minutes. So work as quickly and as cautiously as possible.
77. Get out the "Male 1" vial and place it sideways on the work area. The vial should remain sideways until said otherwise.
    a. **Important: The next step (78) will require a partner to hold the "Male 1" vial in place. Same comment as step 51a.
78. Carefully unplug the "Male 1" vial ensuring no flies escape. Then, using the sorting brush, quickly but cautiously transfer the previously identified Wild-type male into the "Male 1" vial. Once the transfer is complete, re-plug the vial with its foam cap, ensuring no flies get crushed.
    a. **Important: Do not place the vial upright until all the flies inside the vial are no longer sedated.
79. Repeat steps 76-78, but instead of putting a Wild-type male in the "Male 1" vial, put it in the "Male 2" vial.
80. Repeat steps 76-78, but instead of putting a Wild-type male in the "Male 1" vial, put it in the "Male 3" vial.
81. Now, there should be a single Wild-type male Drosophila in the male 1, 2, and 3 vials.
82. Get out the previously used (from step 73) Wild-type Drosophila vial.
83. Unplug the vial, and place it sideways on the working area.
    a. **Important: The next step (84) will require a partner to hold the vial in place. Same comment as step 51a.
84. Using the sorting brush, guide the excess flies that are remaining on the plastic weigh dish, into the Wild-type Drosophila vial.
85. Once all the flies have been transferred, replug the vial with its corresponding foam cap.
    a. **Note: Ensure that the Wild-type Drosophila vial containing the excess flies remains sideways until all the flies inside the vial are no longer sedated.
86. Locate the vial labels, and write on one of them "Experimental Cross #1." Since I couldn't find the vial labels, I had to use tape for the entirety of this procedure.
87. Get out the previously prepared "Male 1" vial (containing five Sepia~~Ebony~~-eyed males and one Wild-type male), and the previously prepared "Female 1" vial (containing five Sepia~~Ebony~~-eyed females).
    a. **Note: the "#1" signifies that these organisms will make up the first experimental biological replicate-cohort of this study.
88. For the following steps (89-95), complete the steps that correspond to the state of the flies in the Male and Female vials being worked with. Whichever step does not correspond to the state of the flies, ignore it. For

example, if all the flies in the Male and Female vials being worked with are awake, follow steps 89-90. If not all the flies are awake, and some are still sedated, follow steps 91-95.

89. Transfer the “Female 1” vial into the “Male 1” vial; Like so:
    a. Hold the two vials upright and tap their bottoms against a tabletop or other hard surface simultaneously. This is to knock the flies to the bottom of the vials. If after the first attempt the flies are not knocked to the bottom of the vials, tap them on the hard surface again until the flies have lowered.
    b. Once the flies are near the bottom of the vial, quickly pull the foam caps off of both the vials, and tap once more.
    c. Place the opening of the “Female 1” vial on top of the opening of the “Male 1” vial, so that the mouths of the vials are flush.
    d. Tap the bottom of the “Female 1” vial until all the flies have fallen/entered the “Male 1” vial. Then, quickly and securely plug the “Male 1” vial, which now should house all the flies.
    e. Place the “Experimental Cross #1” vial label on top of the “Male 1” vial label. Same comment as step 81.
90. Ensuring that there are no leftover flies, the “Female 1” vial may be put to the side and/or discarded. Proceed to step 96.
91. Place the “Male 1” vial and the“Female 1” vial like the image below.

Figure 7

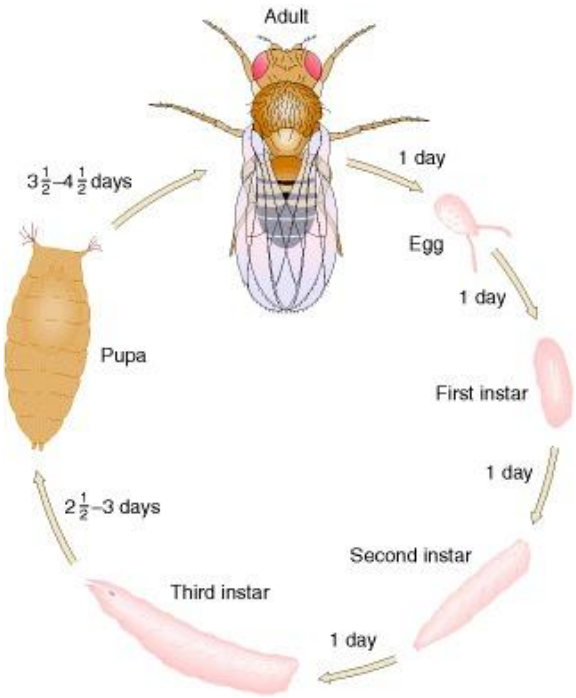
**Important: Step 93 will require a partner to hold the “Male 1” vial in place. In addition, ensure the vials remain sideways on the workspace for the duration of the transfer. Same comment as step 51a.

92. Unplug the foam caps of the “Male 1” vial and the “Female 1” vial.
93. Using the sorting brush, cautiously guide the flies from the “Female 1” vial into the “Male 1” vial.

94. Once all flies have been transferred, plug the "Male 1" vial with its foam cap, ensuring no flies are crushed.
95. Place the "Experimental Cross #1" vial label on top of the "Male 1" vial label. Same comment as step 81.
96. Repeat step 86, but instead of writing "#1", write "#2".
97. Repeat step 87, but instead get out the "Male 2" and "Female 2" vials.
98. Repeat steps 88-95, but instead for the "Male 2" vial, the "Female 2" vial, and the "Experimental Cross #2" vial label. Same comment as step 81.
99. Repeat step 86, but instead of writing "#1", write "#3".
100. Repeat step 87, but instead get out the "Male 3" and "Female 3" vials.
101. Repeat steps 88-95, but instead for the "Male 3" vial, the "Female 3" vial, and the "Experimental Cross #3" vial label. Same comment as step 81.
102. Unplug/turn off the hood. The hood's sash may be raised again.

****STOP:**

a. At this point, all three experimental crosses should be set up. Clean and return all miscellaneous materials to their designated areas. If all/some of the flies inside the vials are still sedated, the Experimental Cross 1, 2, and 3 vials, should lay sideways in their residing storage spot. If all the flies inside the vials are awake, the vials may be in their upright positioning.
b. Record any observations that were made/pictures that were taken during this section in the "Experimental Cross Observations" table.
c. In order to do the next section of this procedure, it is necessary to wait about 7-10 days for larvae to appear in the experimental cross vials. Sometimes it may take more time, and sometimes it may take less time. It is important to check on the vials everyday. Once larvae have appeared, the lab may be resumed.
d. Refer to the life cycle image below (Figure 8) to help the monitoring of the developmental stages of the Drosophila.



(Griffiths et al., 2000)
Figure 8

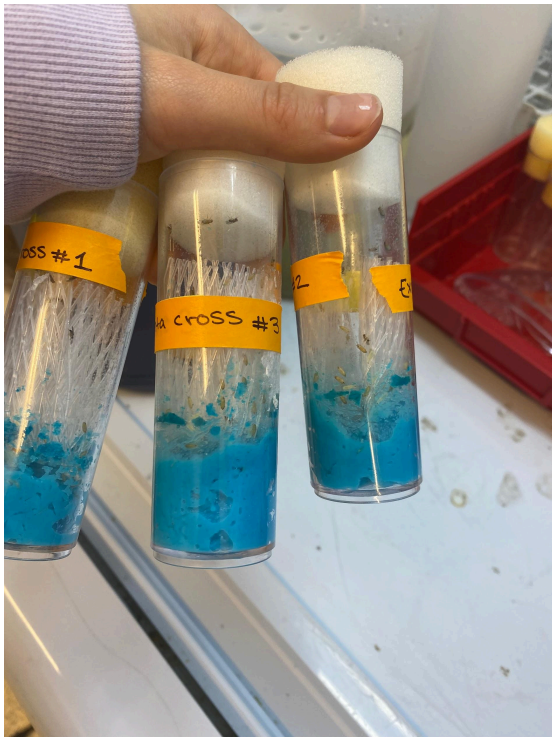
Data and Observations:

Check in day 10/15/21: All flies are no longer sedated, and appear to be thriving!

Check in day 10/19/21: No larvae yet but all the flies are present and they all seem to be moving around and doing well.

Check-in day 10/21/21: Some larvae are beginning to to appear… In a couple of days the flies will be ready to be sedated.

Check-in day 10/22/21: Lot's of larvae are present! The P1 generation are ready to be sedated and killed.



Procedure Reflection and Future Endeavors:

The experimental cross vials have been successfully set-up! Luckily, it was a short and simple process to anesthetize and pick out three male wt. Similar to my second entry's procedural edits, I once again edited all the parts of the procedure that said ebony to sepia, and I did not need to use a partner for the recommended partner steps. In addition, though, once again, similar to my first entry, I could not find official vial labels, so I instead had to use tape labels. In the "Data and Observations" section of this entry, I decided to include qualitative data for the days I have checked-in on the experimental cross vials, so that I can have photographical/anecdotal evidence of the experimental subjects and development of their larvae. Thus, in the meantime, while I wait on the appearance of larvae in the experimental cross vials, I will be taking pictures and reflecting on how the flies are doing.

Preparing Vials, Clearing out Overcrowded Vials, Discarding Vials	
Date: 10/22/21 & 12/7/21	**Length of Research Period(s): 2 hours & 75 minutes**

Goal(s)/Purpose:

To clear/divide out the overcrowded Sepia-eyed/Wild-type Drosophila vials.

- Prepare new vials with medium and mesh. This will be done to ensure that the divided out flies have a new home once removed from their original vial.
- Anesthetize the flies with the FlyNap to ensure that they are asleep while they are being transferred. If they are awake while being transferred, it would be difficult to contain them.
- Place the divided out flies in their newly prepared vials. This will help limit overcrowding in the vials housing the flies.

Materials Required:

1. Drosophila Culture Vial Set 1 (3): Product #173076
   https://www.carolina.com/drosophila-fruit-fly-genetics/drosophila-culture-vial-set-1-36-vials-plugs-caps/173076.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
2. Vial Labels for Drosophila Crosses, Roll of 500 (1): Product #173190
   https://www.carolina.com/drosophila-fruit-fly-genetics/vial-labels-for-drosophila-crosses-roll-of-500/173190.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
3. Formula 4-24® Instant Drosophila Medium, Blue, 1 L (2): Product #173210
   https://www.carolina.com/flies/formula-4-24-instant-medium-blue-1-l/173210.pr?question=drosophila+medium+blue
4. Ultra Fine Point Black Sharpie (1): Product #B010BS53LG
   https://www.amazon.com/Sharpie-Permanent-Markers-Ultra-2-Pack/dp/B010BS53LG/ref=sr_1_15?dchild=1&keywords=ultra%2Bfine%2Bpoint%2Bblack%2Bsharpie&qid=1613938480&sr=8-15&th=1
5. Culture Mesh (1): Product #1713090
   https://www.carolina.com/flies/drosophila-culture-netting-pkg-100/173090
6. 100 mL beaker (3)
7. Ruler (1)
8. Scoopula (3)
9. Drosophila, Living, Wild Type, Vial of 25-30 (1): Product #172100
   https://www.carolina.com/drosophila-fruit-fly-genetics/drosophila-living-wild-type/172100.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
10. FlyNap Anesthetic Kit (1): Product #173010
    https://www.carolina.com/drosophila-fruit-fly-genetics/flynap-anesthetic-kit/173010.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included

MSDS of FlyNap: https://www.carolina.com/teacher-resources/Document/msds-flynap/tr-msds-flynap.tr

11. Drosophila Sorting Brush, Pack 12 (1): Product #173094
https://www.carolina.com/drosophila-fruit-fly-genetics/drosophila-sorting-brush-pack-12/173094.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included

Procedure:

8. If at any point during the experiments the original Sepia~~Ebony~~-eyed or Wild-type Drosophila vials appear to be overcrowded with flies, such that there is not a lot of movable space for the flies, refer to the steps below:
    9. Get out the Instant Drosophila Medium.
    10. Untwist the medal clasp of the bag carefully; try to ensure that no contents spill out.
    11. Get out a scoopula, and scoop some medium out of the bag into a fresh vial.
    12. Using a ruler, measure and ensure there is .5 an inch of medium in the vial.
        a. **Note: If there is excess medium, using the scoopula, transfer the extra medium back into the bag.
    13. Turn on a faucet and fill a 100 mL beaker with approximately 20 mL of water.
    14. Hold a ruler beside the vial and cautiously pour water from the beaker into the vial. Do this until the volume of the mixture in the vial reaches about one inch.
        a. **Note: If the volume of mixture within the vial exceeds this amount, carefully pour the mixture into the sink until the necessary amount is present.
    15. Using a scoopula, mix the medium and water mixture in the vial until the mixture has a slushy tendency.
        a. **Note: If after thoroughly mixing the mixture it contains a liquid tendency instead of a slushy tendency, in very small increments, add more of the medium into the mixture, until it contains a slushy tendency. See the image below for reference of how the vial should appear after this step:

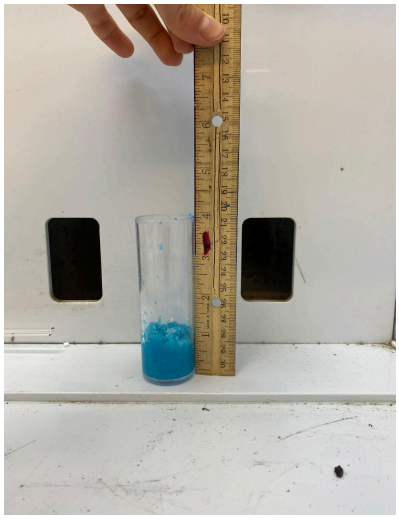


Figure 1

    16. Let the mixture sit for one minute.

17. Using the scoopula, scrape the extra mixture from the sides of the vial to the bottom on the vial; it is important to ensure that the totality of the mixture is as flatly piled on the bottom of the vial as possible.
18. Take a piece of culture mesh and stretch it out. Then, fold it vertically once and horizontally once.
19. Put the folded mesh into the vial.
    a. **Note: See image below for reference of how the mesh should appear in the vial:

Figure 2

20. Plug the vial with its corresponding foam cap.
21. The following steps (22-42) should be done in a ventilated area, preferably in a hood.
    a. **Note: The hood's sash should be lowered until there is a ~10 inch gap to work through.
22. Get out the FlyNap liquid container included in the FlyNap Kit.
    a. **Caution: Before using this substance, review the MSDS of this substance linked in the "Materials" section. Make sure to be extra attentive to sections 7, 8, and 13 of the PDF.
23. Plug in/Turn on the hood.
24. Unscrew the FlyNap liquid container, and using an anesthesia wand included in the FlyNap Anesthesia Kit, insert it into the FlyNap liquid container.
25. Securely screw the cap of the FlyNap liquid container back on.
26. Grab a vial of the Sepia~~Ebony~~-eyed Drosophila or the Wild-type Drosophila (whichever one is the one that needs clearing).
27. Place the vial of the Sepia~~Ebony~~-eyed Drosophila/Wild-type Drosophila sideways on the work area.
28. Using a finger, slightly push back the foam cap of the Sepia~~Ebony~~-eyed/Wild-type Drosophila vial, and insert the anesthesia wand soaked with the FlyNap into the vial.
29. Place the Sepia~~Ebony~~-eyed Drosophila/Wild-type Drosophila vial on a Drosophila stand.

    **Note:

    a. See image below for a visual representation. The reason the vial is placed this way on the stand is to ensure the anesthetized flies fall onto the foam cap instead of the medium.
    b. The flies should remain on the Drosophila stand until directed otherwise.

Figure 3

30. Leave the wand inserted in the vial for 3-4 minutes, or until all of the flies stop moving.
    a. **Note: Do not leave the anesthetized wand in for longer than 3-4 minutes, as the flies will die if exposed to too much FlyNap.
31. Carefully remove the anesthesia wand from the vial and try not to remove any flies with it.
32. Place the anesthesia wand on a nearby paper towel.
33. Over a plastic weigh dish, hold the vial upside down and gently remove the foam cap so roughly half the flies from the vial fall onto the dish. If necessary, tap the sides and bottom of the vial to ensure the sufficient amount of flies fall out. If too many flies fall out, using a sorting brush, guide some back into the vial while the vial is positioned sideways.
34. Re-plug the original Sepia~~Ebony~~-eyed Drosophila vial/Wild-type Drosophila vial with its foam cap while it is sideways. Ensuring it remains sideways, place the vial back in its residing storage spot.
    a. **Note: It is important to ensure that the vials being used to house sedated flies remain sideways until the flies are no longer sedated (this is when they are crawling and flying around again), or else the sedated flies could fall into the medium and drown.
35. Grab the previously prepared vial created in steps 9-20 and unplug it. Place it sideways on the work area.
    a. **Important: The next step (36) will require a partner to hold the vial in place.
36. Using the sorting brush, guide the flies from the plastic weigh dish into the opening of the vial.
37. While the vial is still sideways, replug it with its foam cap. Ensure that no flies get crushed and that the fit is secure.
38. Place the vial in its residing storage spot (maintaining its sideways positioning).
    a. **Note: The vial is to be kept sideways in its residing storage spot until all the flies inside the vial are no longer sedated; this will be when the flies are crawling/flying around again. At that point, the vial(s) may return to their upright positioning.
39. Get out the vial labels.

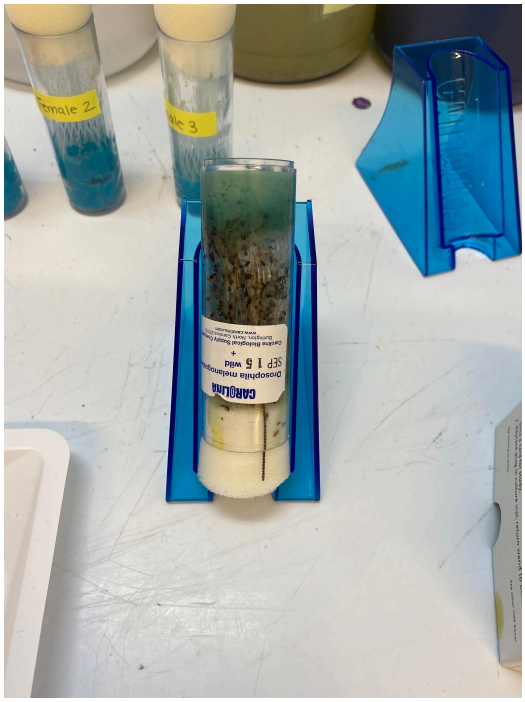
40. Write on one of them "Sepia~~Ebony~~-eyed Drosophila" or "Wild-type Drosophila" (depending on which vial was originally cleared).
41. Place this vial label along the side of the vial from step 38.
42. Unplug/turn off the hood. The hood's sash may be raised again.

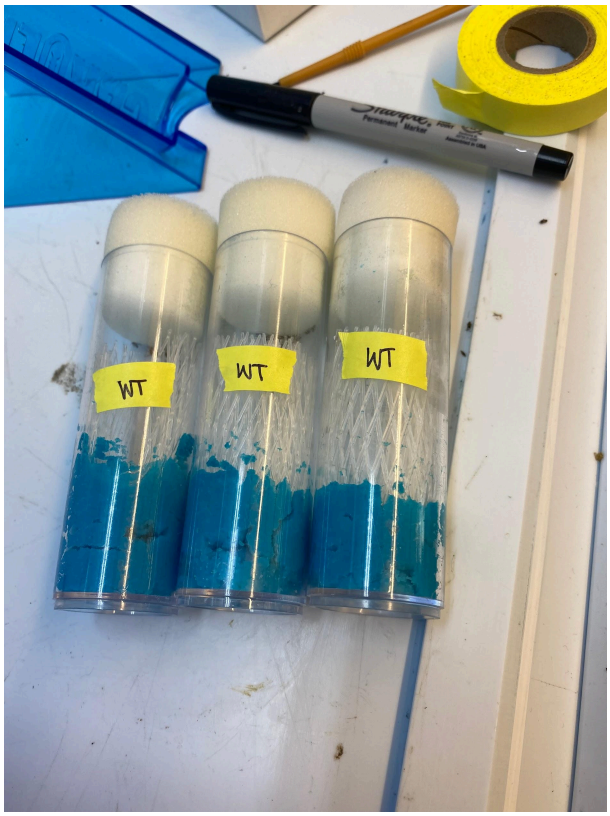
1. Fill a 1 L beaker with approximately 800 mL of water.
2. Add approximately 70 mL of bleach to the beaker.
3. Add around ~10 mL of dish soap to the beaker
4. Fill the culture vial with the water, bleach, and dish soap mixture.
5. Let it sit for 10 minutes.
6. Pour the contents of the vial into a trash bag.
7. Using a scoopula, scoop the mesh baffle and the remaining medium into the trash bag.
8. Wash the remaining contents out with water.

Greens steps 1-8 above are copied and pasted from Simon's Procedure

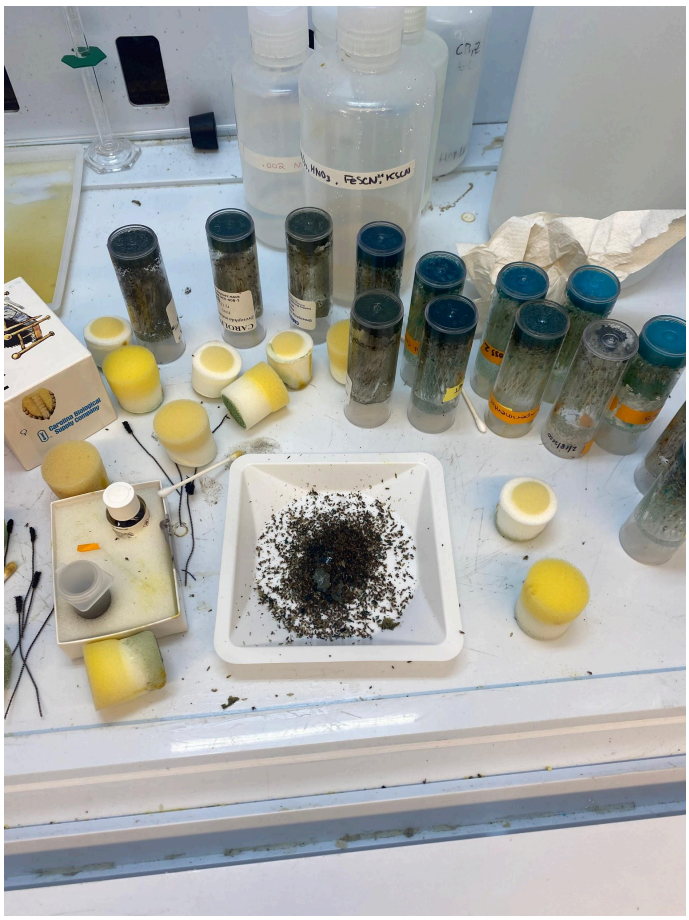
Data and Observations:

10/22/21:


female 2
male 3
CAROLINA
Drosophila melanogaster
SEP 15 wild


WT
WT
WT

12/7/21:



Procedure Reflection and Future Endeavors:

As indicated in the vial images/plastic weigh dish images included in the "data and observations" section of this entry, there were various vials that were overcrowded and/or depleted which were not fostering livable environments for the drosophila. Thus, on 10/22 and 12/7 I divided out and discarded of the flies/vials that needed to be dealt with. As represented in my procedure, the new vial creation, anesthetization of the flies, and division of the flies into fresh vials went great. However, I did not have steps for vial distinguishing in my original procedure, so, in green text at the end of my this procedure, I've referenced a section of Simon's procedure which addresses what needs to be done to extinguish the depleted vials—which are vital to follow to extinguish all the old experimental vials. Since these steps were lacking in my original procedure, and are generally vital to vial upkeep, these will be essential steps to incorporate into my updated procedure. Also, in this procedure, like the others, I edited all the parts that said ebony to instead say sepia.

Extermination of the P-Generations	
Date: 10/22/21 & 11/9/21	**Length of Research Period(s): 2 hours & 1 hour**
Goal(s)/Purpose:	

To exterminate the P-generation

- First I will need to anesthetize the P generation flies. This ensures that they won't be awake when I exterminate them.
- I will then put them in the morgue so they die. I am killing these flies (the parental generation) so that I don't get them confused with the F1 generation once they develop.

Materials Required:

1. FlyNap Anesthetic Kit (1): Product #173010
   https://www.carolina.com/drosophila-fruit-fly-genetics/flynap-anesthetic-kit/173010.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included

   MSDS of FlyNap: https://www.carolina.com/teacher-resources/Document/msds-flynap/tr-msds-flynap.tr
2. Plastic Weigh Dish (1): Product #702332

https://m.carolina.com/lab-balances-scales/weighing-boats-plastic-3-516-x-34-in-pack-500/702332.pr?question=

3. Ethanol (1): Product #861261
   https://m.carolina.com/specialty-chemicals-d-l/ethanol-70-laboratory-grade-500-ml/861261.pr?question=

MSDS:
https://www.carolina.com/teacher-resources/Document/msds-ethanol-denatured-95-percent/tr-msds-ethanol-denatured-95-percent.tr

Procedure:

Anesthetization and Extermination of the P generation:

91. The following steps should be done in a ventilated area, preferably in a hood.
    a. **Note: The hood's sash should be lowered until there is a ~10 inch gap to work through.
92. Repeat steps 22-25.
93. Grab the "Experimental Cross #1" vial.
94. Repeat steps 27-32, and 51-52, but instead of using the Sepia~~Ebony~~-eyed Drosophila vial/Wild-type Drosophila vial, use the "Experimental Cross #1" vial.
    a. **Note: There shouldn't be larvae on the plastic weigh dish, only P-generation (parental generation) flies. If there are larvae, using the sorting brush, place them back into the vial.
95. Take off the cap of the morgue included in the FlyNap kit.
96. Take the ethanol out of the flammable closet.
    a. **Caution: Before using this substance, review the MSDS of this substance linked in the "Materials" section. Make sure to be extra attentive to sections 6, 7, 8, and 13 of the pdf.
97. Carefully pour the ethanol up to the line of the morgue container.
98. Put the ethanol back in the flammable closet.
99. Fold the plastic weigh dish containing the flies from one corner of the dish to the opposite corner.
100. Pour the flies from the plastic weigh dish into the morgue.
101. Fasten the morgue with its cap.
102. Repeat steps 22-25.
103. Grab the "Experimental Cross #2" vial
104. Repeat steps 27-32, and 51-52, but instead of using the Sepia~~Ebony~~-eyed Drosophila vial/Wild-type Drosophila vial, use the "Experimental Cross #2" vial.
    a. **Note: There shouldn't be larvae on the plastic weigh dish, only P-generation (parental generation) flies. If there are larvae, using the sorting brush, place them back into the vial.
105. Repeat steps 107 and 111-113.

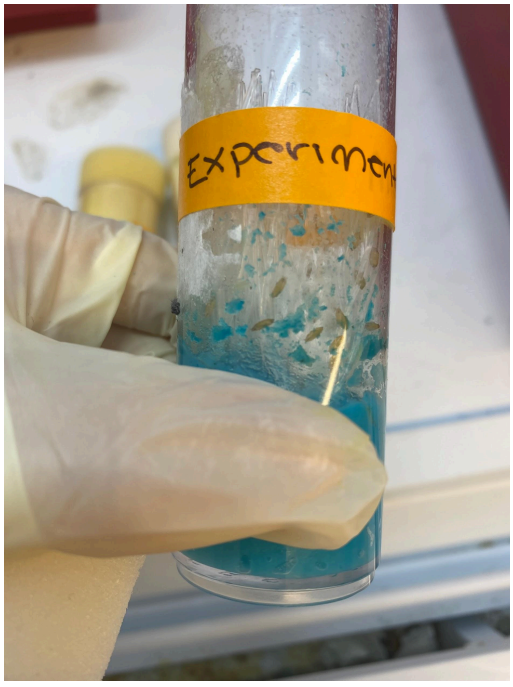
106. Repeat steps 22-25.
107. Grab the “Experimental Cross #3” vial
108. Repeat steps 27-32, and 51-52, but instead of using the Sepia~~Ebony~~-eyed Drosophila vial/Wild-type Drosophila vial, use the “Experimental Cross #3” vial.
    a. **Note: There shouldn’t be larvae on the plastic weigh dish, only P-generation (parental generation) flies. If there are larvae, using the sorting brush, place them back into the vial.
109. Repeat steps 107 and 111-112.
110. Pour the contents from inside of the morgue into a garbage.
111. Fasten the morgue with its cap and put it back in its spot in the FlyNap kit.
112. Unplug/turn off the hood. The hood’s sash may be raised again.

Data and Observations:

10/22/21: Killing off the P1 Generation


Cross #2


Cross #3



11/9/21: Killing off the P2 Generation

Procedure Reflection and Future Endeavors:

I'm very content with the outcome of this procedure. Killing off the P1 generation on October 22nd and the P2 generation on November 9th went seamlessly and quickly which I'm very satisfied with—this was all particularly thanks to steps 94-101. Now that this has been done, I'll be able to phenotype the F1 and F2 generations once the larvae develop into their adult forms. I'm looking forward to seeing the phenotypes of the F1 and F2 flies in my next entres. I'm curious to see who the P1 and P2 flies chose to reproduce with. Also, like the previous entries, I need to change all the parts of this preclude which say ebony to say sepia.

Phenotyping the F1 and F2 Flies	
Date: 10/29/21 & 11/12/21	**Length of Research Period(s): 75 minutes & 75 minutes**

Goal(s)/Purpose:

To record data on the eye color of the F1/F2 generations of the Experimental Crosses.

- First, I will need to anesthetize the F1/F2 generations. This is to ensure they aren't moving when I am observing/recording my data.
- Next, I will need to observe the eye colors of the female and male flies on the plastic weigh dishes. After this, I will record the phenotypic data of the flies.
- Finally, I will exterminate the F1 generation, to clear room for the F2 generation.

Materials Required:

1. FlyNap Anesthetic Kit (1): Product #173010
   https://www.carolina.com/drosophila-fruit-fly-genetics/flynap-anesthetic-kit/173010.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
   MSDS of FlyNap: https://www.carolina.com/teacher-resources/Document/msds-flynap/tr-msds-flynap.tr
2. Drosophila Sorting Brush, Pack 12 (1): Product #173094
   https://www.carolina.com/drosophila-fruit-fly-genetics/drosophila-sorting-brush-pack-12/173094.pr?utm_source=internal&utm_medium=link&utm_campaign=171995_included
3. Magnifying Glass (1): Product #77390B+50B
   https://www.amazon.com/Magnifying-Glass-10X-Magnifier-Soldering-Inspection/dp/B07WRK6F6W/ref=sr_1_1_sspa?dchild=1&keywords=Magnifying+Glasses&qid=1612491315&sr=8-1-spons&psc=1&smid=A20TFX64PGWL2G&spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUEyVkVNUUcyV0FFTlZFJmVuY3J5cHRlZElkPUEwNzA3NzM1M1ZaSEkwNEdHUDFOSSZlbmNyeXB0ZWRBZElkPUEwNTUyMTY2MUJERVg5Wlc1RExLTSZ3aWRnZXROYW1lPXNwX2F0ZiZhY3Rpb249Y2xpY2tSZWRpcmVjdCZkb05vdExvZ0NsaWNrPXRydWU=
4. Plastic Weigh Dish (1): Product #702332
   https://m.carolina.com/lab-balances-scales/weighing-boats-plastic-3-516-x-34-in-pack-500/702332.pr?question=
5. Drosophila Stand (1): Product #173030
   https://m.carolina.com/flies/drosophila-stand-pack-of-3/173030.pr?question=fly+stand
6. Flinn Scientific Dissection Microscope (1): Product #59-1815

Procedure:

Edited Procedure for the F1 Generation (10/29/21):

Vial Preparation:

1. Refer to steps 9-20. Repeat three times (for three vials).
2. Get out the vial labels.
3. Write on one of them, "F2 Experimental Cross 1". Place it on one of the newly prepared vials.
4. Write on another vial label, "F2 Experimental Cross 2". Place it on one of the other newly prepared vials.
5. Write on another vial label, "F2 Experimental Cross 3". Place it on the final newly prepared vial.

Data Observation and Recording of the F1 Generation:

125. The following steps should be done in a ventilated area, preferably in a hood.
    a. **Note: The hood's sash should be lowered until there is a ~10 inch gap to work through.

126. Repeat steps 104-106, however, ignore the "Note" of step 106.
127. Repeat step 55.
128. ~~Once the flies have been correctly divided by gender,~~ using the magnifying glass, observe the eye colors of the female and male flies on the plastic weigh dish. Then, record the phenotypic data of the flies in a notebook ~~in their corresponding section in the "Experimental Arm: F1 Phenotypic Frequency" data table. Follow these steps:~~
    a. ~~First, record the number of Wild-type eye colored females over the total number of males and females in the vial. Then, record the number of Ebony-eyed females over the total number of males and females in the vial.~~
    b. ~~Next, calculate the percentage of the Wild-type and Ebony-eyed female Drosophilas in the vial by dividing each fraction, and then multiply each quotient by 100 (i.e. Divide the number of Wild-type Drosophila females counted, by the total number of flies in the vial counted, and then multiply that by 100).~~
    c. ~~Repeat a-b for the males.~~
~~129. Once all data has been collected, repeat steps 107-113.~~
130. Once all the data has been collected, using the sorting brush, guide the F1 flies from the plastic weigh dish into the newly created "F2 Experimental Cross 2" vial. Ensure the vial remains sideways.
131. Repeat steps 114-116, however, ignore the "Note" of step 116.
~~132. Repeat step 55.~~
133. Repeat step 128.
134. Once all the data has been collected, using the sorting brush, guide the F1 flies from the plastic weigh dish into the newly created "F2 Experimental Cross 2" vial. Ensure the vial remains sideways.
~~135. Once all data has been collected, repeat steps 107 and 111-113.~~
~~136. Repeat steps 118-120, however, ignore the "Note" of step 120.~~
~~137. Repeat step 55.~~
138. Repeat step 128.
139. Once all the data has been collected, using the sorting brush, guide the F1 flies from the plastic weigh dish into the newly created "F2 Experimental Cross 3" vial. Ensure the vial remains sideways.
~~140. Once all data has been collected, repeat steps 107 and 111-112.~~
~~141. Pour the contents from inside of the morgue into a garbage.~~
~~142. Fasten the morgue with its cap and put it back in its spot in the FlyNap kit.~~
143. Discard of the Experimental Cross #1, #2, and #3 vials using the vial extinguishing modes previously outlined. (Will provide specific steps once this stuff is incorporated into the procedure).
144. Unplug/turn off the hood. The hood's sash may be raised again.

Edited Procedure for the F2 Generation (11/12/21):

Data Observation and Recording of the F1 Generation:

125. The following steps should be done in a ventilated area, preferably in a hood.
    b. **Note: The hood's sash should be lowered until there is a ~10 inch gap to work through.
126. Repeat steps 104-106, however, ignore the "Note" of step 106.
127. Repeat step 55.
128. ~~Once the flies have been correctly divided by gender,~~ using the magnifying glass, observe the eye colors of the female and male flies on the plastic weigh dish. Then, record the phenotypic data of the flies in a notebook

~~in their corresponding section in the "Experimental Arm: F2 Phenotypic Frequency" data table. Follow these steps:~~

~~d. First, record the number of Wild-type eye colored females over the total number of males and females in the vial. Then, record the number of Ebony-eyed females over the total number of males and females in the vial.~~

~~e. Next, calculate the percentage of the Wild-type and Ebony-eyed female Drosophilas in the vial by dividing each fraction, and then multiply each quotient by 100 (i.e. Divide the number of Wild-type Drosophila females counted, by the total number of flies in the vial counted, and then multiply that by 100).~~

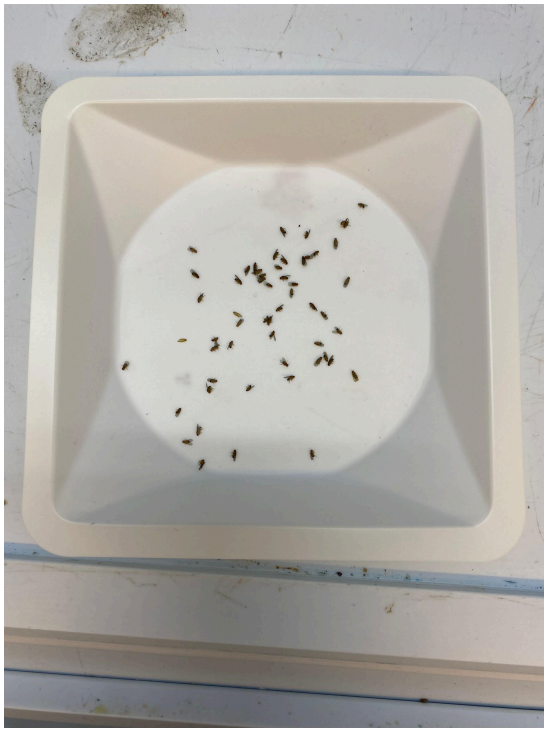
~~f. Repeat a-b for the males.~~

129. Once all data has been collected, repeat steps 107-113.
130. Repeat steps 114-116, however, ignore the "Note" of step 116.
~~131. Repeat step 55.~~
132. Repeat step 128.
133. Once all data has been collected, repeat steps 107 and 111-113.
134. Repeat steps 118-120, however, ignore the "Note" of step 120.
135. Repeat step 55.
136. Repeat step 128.
137. Once all data has been collected, repeat steps 107 and 111-112.
138. Pour the contents from inside of the morgue into a garbage.
139. Fasten the morgue with its cap and put it back in its spot in the FlyNap kit.
140. Discard of the F2 Experimental Cross 1, 2, and 3 vials using the vial extinguishing modes previously outlined. (Will provide specific steps once this stuff is incorporated into the procedure).
141. Unplug/turn off the hood. The hood's sash may be raised again.

Data and Observations:

10/29/21 Data and Observations:

Experimental cross #1: sepia: 18, wt: 13
Experimental cross 2: sepia: 29, wt: 23
Experimental cross 2: sepia: 12, wt: 47




Cross 2


FLYNAP

11/12/21 Data:

F2 Experimental cross 1: sepia: 32 wt: 7
F2 Experimental cross 2: sepia: 30 wt: 10
F2 Experimental cross 3: sepia: 7 wt: 11

Procedure Reflection and Future Endeavors:

10/29/21 - F1 generation
Since I originally wrote this procedure with a different purpose (only phenotyping one generation), my project has since changed quite a bit. However, it is this part of the procedure which deviates most from my updated project. Firstly, I had to add the steps about making three new vials because I am no longer killing off the F1 generation. I am going to use the F1 generation as the P2 generation for the next F2 generation to track their inheritance of eye color. Thus, I have to conserve the F1 flies after I phenotype them: not kill them off! These additions are visible in the first five "Vial Preparation" steps I added in green. Next, as indicated on step 128, I am no longer dividing and phenotyping the flies by sex, as it isn't necessary—I'm only dividing them by type (*se* vs *WT*) and, also on this step, I've cut the details on recording the fly phenotype data in the original data table I had. This is because my original—"Experimental Arm: F1 Phenotypic Frequency"—data table no longer accounts for the scope of data my updated procedure is accounting for. Thus, I've tentatively recorded my data in the "data and observations" section of this lab notebook. Next, steps 130, 134, and 139 were added to account for the transferring of the F1 flies into their new vials (as the P2 generation), hence why I am no longer killing them off and creating a new vial name. Finally, step 143 is also a new addition that will account for the extinguishing of the Experimental Cross 1, 2, and 3 vials, following the incorporation of the vial extinguishing modes from Simon's procedure from the lab notebook from 10/22/21 and 12/7/21. I will provide the new reference steps to these steps once my full procedure is updated. In all, my future endeavors include setting up a meeting with Dr. Gentile and Mr. Kwok in order to statistically understand and analyze my data, updating all my procedural reference steps, and making a new data table to be able to compartmentalize my F1 phenotypic information adequately.

11/12/21 - F2 generation
For my F2 generation, my original procedure deviated less than it did for my F1 generation, as I didn't need to preserve the F2 flies for another round of crosses. Hence why I kept steps 129-130 and 132-139. Next, as written above, and as indicated on step 128, I am no longer dividing and phenotyping the flies by sex, as it isn't necessary—I'm only dividing them by type (*se* vs *WT*) and, also on this step, I've cut the details on recording the F2 fly phenotype data in the original data table I had. Finally, step 143 is also a new addition that will account for the extinguishing of the F2 Experimental Cross 1, 2, and 3 vials, following the incorporation of the vial extinguishing modes from Simon's procedure from the lab notebook from 10/22/21 and 12/7/21. I will provide the new reference steps to these steps once my full procedure is updated. In all, my future endeavors include setting up a meeting with Dr. Gentile and Mr. Kwok in order to statistically understand and analyze my data, updating all my procedural reference steps, making a new data table to be able to compartmentalize my F1 phenotypic information adequately, and cleaning out and organizing my materials bin.