**COURSTHIP ASSAYS INTERCROSS F2**

**Measuring courtship behavior before and after experience**

**Note:** 24 hours prior to assays, move virgin males into individual 2.5 gallon tanks with gravel on the bottom and a 5x2 grid on the front. Record all meta data (population, date born, family) and assign male an ID number. Move individual tanks into whisper room and let males acclimate overnight.

**DAY 1: 8AM**

1. Assign virgin females to males based on population (pair same populations together).
2. Set up cameras in whisper room to film the front of the tank
3. Begin recording on cameras. Show label to the camera that includes ID, date, and code for Before
4. Add the female to the appropriate tank. Leave whisper room and close door.
5. Record behavior for 12 minutes. Allow males to remain with females for 1 hour.
6. Return and remove females and from tanks.
7. Allow males to remain in whisper room until the next morning.

**Day 2: 8AM**

1. Repeat Day 1, using a different virgin female from the same population.
2. Remove both males and females from the tanks. Move back into original individual tank for later processing.
3. Fully clean assay tank, replacing gravel and water, to set up for next batch of males.