Submission ID #: 61176
Scriptwriter Name: Susan
Project Page Link: https://www.jove.com/account/file-uploader?src=18658728

Title: Circadian Entrainment of Drosophila melanogaster

Authors and Affiliations:

Austin Dada[1], Minh Q. Nguyen[1], Shea Marie Peterson[1], Vy Ngo[1], Dayanne Viviana Cornelio Parra[1], Bwaar Omer[1], Ada Thapa[2], Sarah R. Rapp[1], Veronica Cloud[1], and Ryan D. Mohan[1]

[1]School of Biological and Chemical Sciences, University of Missouri – Kansas City, Kansas City, Missouri
[2]School of Public Health, University of Maryland, College Park, Maryland

Corresponding Author:
Ryan D. Mohan (MohanRD@umkc.edu)

Email Addresses for Co-authors:
Austin Dada (aodkg8@mail.umkc.edu)
Minh Q. Nguyen (mqn9q3@mail.umkc.edu)
Shea Marie Peterson (smsqy4@mail.umkc.edu)
Vy Ngo (vtny77@mail.umkc.edu)
Dayanne Viviana Cornelio Parra (dvcvvd@umkc.edu)
Bwaar Omer (bsonhh@mail.umkc.edu)
Ada Thapa (athapa1@terpmail.umd.edu)
Sarah R. Rapp (srrz63@mail.umkc.edu)

Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   no

   If **No**, JoVE will need to record the microscope images using our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Leica S6E light microscope

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **no**

3. **Filming location:** Will the filming need to take place in multiple locations? **no**

Introduction

1. Introductory Interview Statements

Videographer: Interviewee headshots are required. Take a headshot for each interviewee.

Authors: While filming the interview portion, our videographer will also photograph you for the **JoVE Dedicated Author Webpage**. Please look at this **example**. For questions about the author profile pages and pictures, please contact **author.liaison@jove.com**.

Authors: Please memorize the interview statements prior to your filming day.

1.1. **Austin Dada:** Synchronizing Drosophila to defined circadian times is the first step in studying events that either control, or respond to, the biological clock **[1]**.

1.1.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.

1.2. **Minh Q. Nguyen:** Synchronization of other models will follow the same basic approach **[1]**.

1.2.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.

1.3. **Dayanne Viviana Cornelio Parra:** There are many steps to this protocol and it may seem overwhelming to put them all together. Expect to try a few times before it feels natural. Keep temperatures stable and unwanted light out during entrainment steps **[1]**.

1.3.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.

Answers have been edited for length, clarity, and consistency with journal style guidelines.

Protocol

Please use this draft script to help you prepare for filming day.

- Filming should take no more than 10 minutes per step. If a step will take more than 10 minutes, prepare the product from that step in advance.

2. Fly Food Production

2.1. Prepare the fly food in a crockpot at 121 degrees Celsius, mixing as ingredients are added **[1-TXT]**. Cover the crockpot and bring the contents to a rolling boil, mixing the contents every 10 minutes **[2]**.

2.1.1. Talent adds ingredients and mixes while adding. **TEXT: See manuscript for ingredients.**

2.1.2. Talent lifts cover and mixes ingredients.

2.2. After 20 minutes at a rolling boil **[1]**, turn off the heat and add 83.6 milliliters of water to the crockpot. Leave the lid off as the food cools **[2]**.

2.2.1. Contents at roiling boil.

2.2.2. Talent turns off heat, adds water, and leaves the lid off.

2.3. While the food is cooling, stir it every 10 minutes to avoid allowing a film to form on the top **[1]**. Also, measure the temperature every 10 minutes with a glass thermometer **[2]**.

2.3.1. Talent stirs food.

2.3.2. Talent measures temperature.

2.4. Once the food has cooled to below 60 degrees Celsius, add Tegosept and propionic acid as described in the manuscript **[1]**. Mix well, and adjust the crockpot to maintain a temperature just a few degrees less than 60 degrees Celsius **[2]**.

2.4.1. Talent adds Tegosept and propionic acid to crockpot.

2.4.2. Talent mixes contents and adjusts crockpot temperature.

2.5. Use a peristaltic pump to distribute the fly food **[1]**. For narrow vials, pump 10 milliliters, and for 6-ounce square-bottom bottles, pump 60 milliliters **[2]**.

2.5.1. Talent runs tubing from crockpot to peristaltic pump.

2.5.2. Talent places other end of tubing in a bottle of flies.

3. Collection and Entrainment

3.1. Store wild-type flies at 25 degrees Celsius for 10 to 12 days, until approximately 200 pupae are attached to the insides of the bottle **[1]**.

3.1.1. CU: Bottle showing large numbers of pupae on the inside surfaces. *Videographer: This is one of the most important steps for viewers to see.*

3.2. To remove the adults from the bottle, tip them into a new bottle **[1]**, and use the dull end of a number zero paintbrush to push any remaining adults in **[2]**. Wipe the paintbrush with 70 percent ethanol before and after use **[3]**.

3.2.1. Talent tips bottle into new bottle.
3.2.2. Talent uses paintbrush to push remaining adults into new bottle.
3.2.3. Talent wipes paintbrush with ethanol.

3.3. Incubate the pupae at 25 degrees Celsius to allow the next generation to emerge **[1]**. After 3 days, the flies will be 0 to 3 days old **[2]**.

3.3.1. Talent places bottles in incubator.
3.3.2. CU: Bottle of 0- to 3-day-old flies.

3.4. **Austin Dada:** Remember that temperature, light, and even your choice of food, will influence entrainment. Be sure to keep them each controlled during your experiment. **[1].**

3.4.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.

3.5. At the designated time points for collection, use a carbon dioxide anesthesia pad to immobilize the flies **[1-TXT]**, and collect 100 males and 100 females **[2]**.

3.5.1. Talent uses carbon dioxide anesthesia pad. **TEXT: Time points: ZT1, ZT7, ZT13, ZT19. (See Table 1.)** *Videographer: This is one of the most important steps for viewers to see. Video editor, please keep this text onscreen for the next shot.*
3.5.2. Talent places flies under microscope.

3.6. Female flies are much larger than males. The flies can also be differentiated by examining genitalia **[1]**. Males have dark rounded genitalia **[2]**, whereas females have lighter, more pointed genitalia **[3]**.

3.6.1. SCOPE: View of flies through microscope, with both male and female flies.
3.6.2. SCOPE: Male fly genitalia.
3.6.3. SCOPE: Female fly genitalia.

3.7. Place males and females in separate vials, with 100 females per vial and 50 males per vial. Male social interactions lead to many deaths when there are 100 individuals in a vial **[1]**.

3.7.1. Talent transfers flies to vials.

3.8. To enable Circadian entrainment to occur, place the vials in light-regulated incubators for 3 to 5 days **[1]**.

3.8.1. Talent places vials of flies in light-regulated incubators.

3.9. To produce flies for future entrainment, place 25 of the remaining females and 5 to 7 of the remaining males in a new bottle **[1]**. Incubate the bottle at 25 degrees Celsius **[2]**.

3.9.1. Talent transfers flies into new bottles.
3.9.2. Talent places bottle in incubator.

4. Fixation and Storage for Immunofluorescence

4.1. For every 100 flies that will be collected, prepare a narrow vial by adding 4.8 milliliters of fixative **[1-TXT]**. Place the narrow vials in a bucket of ice, and take the bucket, paper towels, foil, and tape to the incubator **[2]**.

4.1.1. Talent adding fixative to each narrow vial. **TEXT: Fixative: 4% formaldehyde in 1x PBS + 0.05% Triton**
4.1.2. Talent places the narrow vials in ice, and assembles materials to take to incubator.

4.2. To collect the flies, place a funnel in the narrow vial **[1-TXT]**. Then, remove the cap from the vial of flies and quickly invert it into the funnel. Tap gently to help guide the flies into the vial of fixative **[2]**. When collecting males, combine two vials of 50 flies into one narrow vial **[3]**. Wrap the vials for ZT13 and ZT19 in foil **[4]**.

4.2.1. Talent places funnel in narrow vial. **TEXT: Perform ZT13 and ZT19 collections in the dark, using a red light.** *Video editor, please keep this text onscreen for the next three shots.*
4.2.2. Talent removes cap from vial of flies, inverts the vial into the funnel, and taps on the vial. *Videographer: This is one of the most important steps for viewers to see.*
4.2.3. Talent adds a second vial of 50 flies to a vial containing 50 flies.
4.2.4. Talent wraps ZT13 and ZT19 vials in foil.

4.3. After transferring the flies, tape the caps in place to avoid spillage **[1].** Place the vials on the nutating mixer at 165 RPM and 4 degrees Celsius, for 4 hours **[2]**. The flies are no longer light-sensitive, so the foil may be removed to verify that the flies are being submerged in the fixative **[3]**.

4.3.1. Talent tapes caps in place.
4.3.2. Talent places vials on nutating mixer. *Videographer: This is one of the most important steps for viewers to see.*
4.3.3. CU: Vials on mixer, showing the flies are being submerged in fixative.

4.4. After removing the vials from the nutating mixer, remove the formaldehyde **[1]**. Then, wash the flies three times with 3,000 microliters of 1x PBS, inverting the vial during each wash **[2]**.

4.4.1. Talent removes formaldehyde from vials.
4.4.2. Talent adds PBS and inverts vial. *Videographer: This is one of the most important steps for viewers to see.*

4.5. Store the vials at 4 degrees Celsius to await future immunofluorescence **[1]**.

4.5.1. Talent places vials in refrigerator.

Results

5. Results: Verification of Entrainment

5.1. Light and darkness were used to entrain flies to circadian cycles. Entrainment was verified by immunoblotting and immunofluorescence analysis of the period protein, a marker for circadian entrainment **[1]**.

5.1.1. LAB MEDIA: Figure 1.

5.2. Immunoblotting of whole cell extracts prepared from heads of entrained flies shows a canonical pattern of period protein mobility and intensity **[1]**. Immunofluorescence of entrained brains collected at ZT1 shows period proteins in a characteristic pattern **[2]**.

5.2.1. LAB MEDIA: Figure 1. *Video editor, please show only Figure 1A.*

5.2.2. LAB MEDIA: Figure 1. *Video editor, please show only Figure 1B.*

Conclusion

6. Conclusion Interview Statements

6.1. **Austin Dada:** This method is a jumping-off point for immunofluorescence, immunoblotting, immunoprecipitation, behavioral analysis, and any other technique you may want to use to compare how pathways change as the biological clock ticks **[1]**

6.1.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.

6.2. **Minh Q. Nguyen:** With the ability to entrain your model organism, you can ask if your favorite pathway changes over time, and whether defects in your favorite pathway can lead to disruption of the biological **[1].**

6.2.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.

6.3. **Dayanne Viviana Cornelio Parra:** A number of diseases are worsened when the biological clock is disrupted. Identifying the underlying mechanisms will help us create more effective medicines for people suffering from diseases such as Alzheimer's and Parkinson's disease. **[1].**

6.3.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.