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Title: High-Throughput Assays of Critical Thermal Limits in Insects

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Author Questionnaire

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

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

Videographer: Screen captures provided; do not film

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length

Number of Shots: **46**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Nicholas M. Teets**: Thermal tolerance is a critical component of species distributions and responses to climate change. We provide methods for rapidly assessing heat and cold tolerance in small insects **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Nicholas M. Teets**: These methods for measuring cold tolerance via the critical thermal minimum and heat tolerance via the heat knockdown time are high throughput, semi-automated, and require minimal equipment **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Introduction of Demonstrator on Camera

1.3. **Nicholas M. Teets**: Demonstrating the procedure will be David Awde, a Postdoc from my laboratory **[1][2]**.

1.3.1. INTERVIEW: Author saying the above

1.3.2. Named demonstrator(s) looks up from workbench or desk or microscope and acknowledges camera

Protocol

2. Critical Thermal Minima (CT_{min}) Assay Setup

2.1. To set up a critical thermal minima assay, power on a temperature-controlled fluid bath **[1]** and press the play button to run a program raising and maintaining the temperature of the bath to 25 degrees Celsius **[2]**.

2.1.1. WIDE: Talent powering on bath

2.1.2. Talent starting program

2.2. Wait 5-10 minutes to allow the fluid bath and column 5 to reach 25 degrees Celsius **[1]** before replacing the plug at the top of the column with a 5.08-centimeter-diameter funnel **[2]**.

2.2.1. Talent checking watch or setting timer, with bath and column visible in frame

2.2.2. Talent replacing plug with funnel

2.3. Tap approximately 40 flies from their food vial into the column through the funnel **[1]** and quickly replace the funnel with the plug, taking care not to let any flies escape **[2]**.

2.3.1. Talent tapping flies into column *Videographer: Important step*

2.3.2. Talent replacing funnel with plug *Videographer: Important step*

2.4. Then allow the files to settle, occasionally tapping the bottom plug to encourage the flies to climb **[1]**.

2.4.1. Flies settling/Talent tapping bottom plug

3. Critical Thermal Minima (CT_{min}) Assay

3.1. After 5 minutes, press the start button on the fluid bath again to begin the critical thermal minima ramping program **[1-TXT]**.

3.1.1. WIDE: Talent pressing start button **TEXT: 25 °C: 5 min -> 25 °C to 10 °C at 0.5 °C/min -> 10 °C: 2 min ->10 °C to -10 °C at 0.25 °C/min**

3.2. Click open the thermocouple recording software on the computer **[1]** and click **Record** to begin recording the temperature inside the column every second for the duration of the assay **[2]**.

3.2.1. Talent opening software, with monitor visible in frame

3.2.2. SCREEN: 1.3.7: 00:24-00:37

3.3. Ensure that each temperature recording includes a time stamp specific to the second, so that temperature data can later be merged with data from the *Drosophila* Funnel Monitor **[1]**.

3.3.1. SCREEN: 1.3.7: 00:37 *Video Editor: please emphasize Time stamps*

3.4. Add 5 milliliters of 90% ethanol to a 15-milliliter conical centrifuge tube **[1]** and place the tube in a rack below the column **[2]**.

3.4.1. Talent adding ethanol to tube, with ethanol container visible in frame

3.4.2. Talent placing tube into rack

3.5. Occasionally tap the bottom plug of the column to entice any flies on the bottom to climb **[1]**. Most of the flies will be on a perch or near the top of the column by 15 degrees Celsius **[2]**.

3.5.1. Plug being tapped

3.5.2. Shot of flies on perch and/or near top of column

3.6. At 15 degrees Celsius, place a 75-millimeter outer diameter glass funnel into the *Drosophila* Funnel Monitor **[1]**.

3.6.1. Talent placing funnel into DFM

3.7. Remove the bottom plug **[1]** and collect any flies still on the plug in the ethanol **[2]**.

3.7.1. Plug being removed

3.7.2. Flies being collected in ethanol

3.8. Adjust the retort ring, funnel monitor, and funnel so that they are under the column **[1]**.

3.8.1. Talent adjusting ring, DFM, and/or funnel under column

3.9. **[1] [2]**.

~~3.9.1. Shot of flies collected from plug~~

~~3.9.2. Talent recording fly number~~

3.10. Make sure that the lip of the funnel completely seals the bottom of the column **[1]** and insert the bottom of the funnel into the 15-millliter collection tube **[2]**.

3.10.1. Seal being checked *Videographer: Important step*

3.10.2. Funnel being inserted into tube *Videographer: Important step*

3.11. Open the *Drosophila* Funnel Monitor software **[1]**. The software will immediately start recording the time and date at which flies reach their critical thermal minima **[2]**.

3.11.1. Talent opening software, with monitor visible in frame

3.11.2. SCREEN: 1.3.13: 00:06-00:10

3.12. Flies that reach their critical thermal minima will lose their neuromuscular function and fall from their perches through the *Drosophila* Funnel Monitor **[1]**.

3.12.1. Fl(ies) failing from perch/into DFM

3.13. To monitor whether all the of flies have reached their critical thermal minima as the temperature decreases, check the top plug and perches to see if any flies are still perched **[1]**.

3.13.1. Talent checking top plug

3.14. When all the flies have reached their critical thermal minima, move the *Drosophila* Funnel Monitor and funnel away from the column opening **[1]**.

3.14.1. Talent moving DFM/funnel away from column

3.15. On rare occasions, flies may reach their critical thermal minima but remain stuck in the column **[1]**. Open the top plug to remove these flies **[2-TXT]**.

3.15.1. Shot of fl(ies) stuck to column

3.15.2. Talent opening plug/removing fl(ies) **TEXT: CT_{min} of flies adhered to column unknown**

4. High-Throughput Heat Knockdown (KD) Assay Setup

4.1. To set up a high-throughput heat knockdown assay, use an aspirator and septum lid to load individual carbon dioxide-sedated flies into each well of a modified, 96-well, no-bottom plate **[1]** and seal the place with a clear, tight-fitting lid **[2]**.

4.1.1. WIDE: Talent placing flies into wells *Videographer: Important step*

4.1.2. Talent covering plate *Videographer: Important step*

4.2. Then place the plate over a supply of food **[1]** and allow the flies to acclimatize to the 96-well plate for at least 48 hours **[2]**.

4.2.1. Talent placing plate

4.2.2. Shot of flies in plate

5. High-Throughput Heat Knockdown (KD) Assay

5.1. On the day of the assay, set the incubator to 37.5 degrees Celsius **[1]**.

5.1.1. WIDE: Talent setting incubator

5.2. After 30 minutes, place the fly culture plate into the incubator with the bottom of the plate against the white paper on the bottom of the tray **[1]**.

5.2.1. Talent placing plate onto paper

5.3. Note the orientation of the well column and row names on the tray and in the frame of the webcam **[1]** and place colored tape along the sides and edges of the plate to help verify the orientation **[2]**.

5.3.1. Talent checking webcam OR Shot of tray through webcam viewfinder

5.3.2. Tape being placed OR Shot of tape on sides and edges

5.4. When the plate is in position, close the incubator door **[1]** and click **Record** in the video recording software **[2]**.

5.4.1. Talent closing door

5.4.2. Talent clicking Record, with monitor visible in frame

5.5. After two hours check the recording to see whether all of the flies have reached their final resting spot and stopped moving **[1]**.

5.5.1. SCREEN: 2.2.5.

5.6. When all of the flies have stopped moving, click **Stop [1]** and dispose of the flies **[2]**.

5.6.1. Talent clicking stop

5.6.2. Talent discarding flies

5.7. Open the video file and record the knockdown time of each fly in each well. The most consistent measure of the heat knockdown time between the trials and observations is the time at which a fly reaches its final resting spot **[1]**.

5.7.1. SCREEN: 2.2.8. *Video Editor: approximately with "time at which … spot" please emphasize fly in 4th column, 4th from bottom well as it finishes moving and stops*

5.8. Then track the video in reverse, focusing on a single well, and noting the time at which the fly first moves off its final resting spot **[1-TXT]**.

5.8.1. SCREEN: 2.2.9. *Video Editor: please speed up and emphasize time at bottom of frame* **TEXT: Repeat tracking for each well**

5.9. **David N. Awde**: Determining the final resting time of each fly can be difficult. To ensure an accurate analysis, it is important to take your time and to observe each well individually **[1]**.

5.9.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.3., 3.12., 4.1.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
n/a

Results

6. Results: Representative Lower and Upper Thermal Limits of Select Lines from the *Drosophila* Genetic Reference Panel (DGRP)

6.1. In this representative analysis, females from the DGRP (D-G-R-P) Line 913 had significantly lower mean critical thermal minima temperatures **[1]** than females from the DGRP Line 714 **[2]**.

6.1.1. LAB MEDIA: Figure 5A *Video Editor: please emphasize 913 data box*
6.1.2. LAB MEDIA: Figure 5A *Video Editor: please emphasize 714 data box*

6.2. In addition, the heat knockdown time at 37.5 degrees Celsius differed significantly between females from the 73 and 461 DGRP lines **[1]**.

6.2.1. LAB MEDIA: Figure 5B *Video Editor: please sequentially emphasize 73 and 461 data boxes*

Conclusion

7. Conclusion Interview Statements

7.1. **David N. Awde**: With minor modifications, this method for measuring the heat knockdown time could be used to measure other thermal tolerance traits, such as chill coma recovery time or critical thermal maximum **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

7.2. **David N. Awde**: By significantly reducing the hands-on time for investigators, these methods have allowed the exploration of genetic variations in thermal tolerance traits at a scale that was not previously possible **[1]**.

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*