Video Article

An Improved Method for Accurate and Rapid Measurement of Flight Performance in *Drosophila*

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Abstract

Drosophila has proven to be a useful model system for analysis of behavior, including flight. The initial flight tester involved dropping flies into an oil-coated graduated cylinder; landing height provided a measure of flight performance by assessing how far flies will fall before producing enough thrust to make contact with the wall of the cylinder. Here we describe an updated version of the flight tester with four major improvements. First, we added a "drop tube" to ensure that all flies enter the flight cylinder at a similar velocity between trials, eliminating variability between users. Second, we replaced the oil coating with removable plastic sheets coated in Tangle-Trap, an adhesive designed to capture live insects. Third, we use a longer cylinder to enable more accurate discrimination of flight ability. Fourth we use a digital camera and imaging software to automate the scoring of flight performance. These improvements allow for the rapid, quantitative assessment of flight behavior, useful for large datasets and large-scale genetic screens.

Video Link

The video component of this article can be found at http://www.jove.com/video/51223/

Introduction

Drosophila has long been used to study the genetic basis of behavior[1], and researchers have devised a number of ways to analyze various types of behavior[2-6]. Flies have been particularly useful in providing useful models of neuromuscular disorders[7]. A common assay used to study locomotor behavior is flight performance. The original flight tester is useful for identifying flight defective mutants and for quantitative assessment of flight ability[1], but it has several shortcomings that limit its application for high throughput screens: the use of oil-coated cylinders is messy and cumbersome, certain features such as the length of the cylinder and introduction of flies into the tube with variable force reduce quantitative accuracy; and it is difficult to recover live flies from the tester. To overcome these limitations, we have modified the flight tester to include a number of improvements. We added a "drop tube" to introduce flies to eliminate variability between experiments and users. We use removable acrylic sheets coated with an adhesive that allows for easier cleanup and recovery of individual flies. We have increased the length of the flight tube to improve quantitative accuracy and reliability. Finally, we use a digital camera and imaging software to calculate the landing heights of flies. We believe these improvements will be useful to any laboratory interested in conducting large-scale genetic screens for defects in flight performance.

Protocol

1. Assemble Flight Tester

1. Secure flight cylinder to Ring Stand 1 using chain clamps. (Leave approximately 3 cm underneath the cylinder for weigh dish.)

   (Note: The flight cylinder we use is 90 cm long with a diameter of 13.5 cm.)
2. Insert weigh dish with a thin layer of mineral oil underneath the flight cylinder.
3. Secure funnel to Ring Stand 2 using a ring clamp and claw clamp. Adjust the height of the funnel so that the bottom of the funnel is flush with the top of the flight cylinder. (Note: the tip diameter of the funnel must be less than the outer diameter of vials placed into the drop tubes so that the vials will not fall through.)
4. Insert drop tube into the top of the funnel and secure using a claw clamp.

   (Note: We use a drop tube that is 25 cm long. Dropping fly-containing vials from this height allows consistent ejection of all flies with uniform force. The inner diameter of the drop tube should be slightly larger than the outer diameter of the vial to allow the vial to drop freely.)
5. Cut polyacrylamide sheet(s) to the proper size. (Note: To aid in inserting and removing the sheet, the width should be slightly smaller than the inner circumference of the flight cylinder).

6. Apply a thin layer of Tangle-Trap to the sheet. Allow to sit for 1 hr before use. (Note: Leave enough room at the top and bottom of the sheet (approximately 3 cm) uncoated to grasp the sheet for insertion/removal.)
7. Insert the polyacrylamide sheet in the flight cylinder.
8. Assemble the camera track using pine support brackets. (Note: ensure that the bottom of the track can support the camera without blocking the lens. Refer to **Figure 1B.**)
9. Add stoppers and screw into place. (Note: place the stoppers in locations that will allow the camera to view the entire plastic sheet in panoramic mode.)

2. Run Experiment

1. Collect vials of flies to be tested. For best results, use no more than 20 flies/vial.
2. Gently tap flies to bottom of vial, unplug, then insert into drop tube and release vial.

   (Note: The vial falls down the drop tube until it hits the narrow funnel opening. When the vial hits the funnel, the flies are ejected into the flight cylinder.)
3. Lift the drop tube to remove the empty vial.

   (Note: Multiple vials of flies of the same test group can be assayed on a single polyacrylamide sheet. We find that up to 200 flies (10 vials of 20 flies each) can be tested and imaged readily on a single sheet.
4. Remove the plastic sheet and place it on a flat white surface.

   (Note: white poster board may be used if bench tops are dark colored.)
5. Assemble the camera track over the plastic sheet. The camera should be sufficiently high above the sheet to have both the top and bottom of the sheet in the field of view.
6. Slide the camera along the track while holding the "capture" button to acquire a panoramic image.
7. The number of flies landing in the oil may be counted manually for each trial.
8. Repeat steps 2.2-2.7 for all conditions in a given experiment. The flies can be removed from the sheet between each trial. Alternatively, several sheets may be used, with a new sheet for each trial.

3. Data Collection

1. Open image files using ImageJ software.
2. Crop images if necessary to include only the landing surface area. (This is the area coated in Tangle-Trap.)
3. Convert images to 8-bit grayscale.
4. Create a "Threshold" to filter out the white background.

   (Image → Adjust → Threshold).
5. Set the parameters to identify each fly using the "analyze particles" menu.

   (Analyze → Analyze Particles) Define the parameters used to identify a particle. With our set up, we find that using an area of 5-90 pixels^2 and a circularity of 0.4-1.0 will accurately identify all samples.
6. Measure the location of each fly using the generated list of coordinates for each particle. The x-coordinate in pixels can be converted to centimeters to calculate the landing height.
7. Import table into a spreadsheet (such as Microsoft Excel).

Representative Results

Figure 1A shows a schematic of the updated flight tester assembly. **Figure 1B** illustrates the track design allow the camera to take a panoramic image without blocking the field of view. Representative results are shown in **Figure 2**, where the flight performance of *slowpoke* mutant flies, which have a known flight defect[8-10], are compared to wild-type *Canton-S* flies. Control flies consistently land near the top of the cylinder, with very little spread among individuals and an average landing height of 73±2.0 cm. In contrast, *slowpoke* flies display a much more varied landing spread, and land significantly lower, averaging 44±4.1 cm. All flies were 3 days old, raised at room temperature (23 °C).

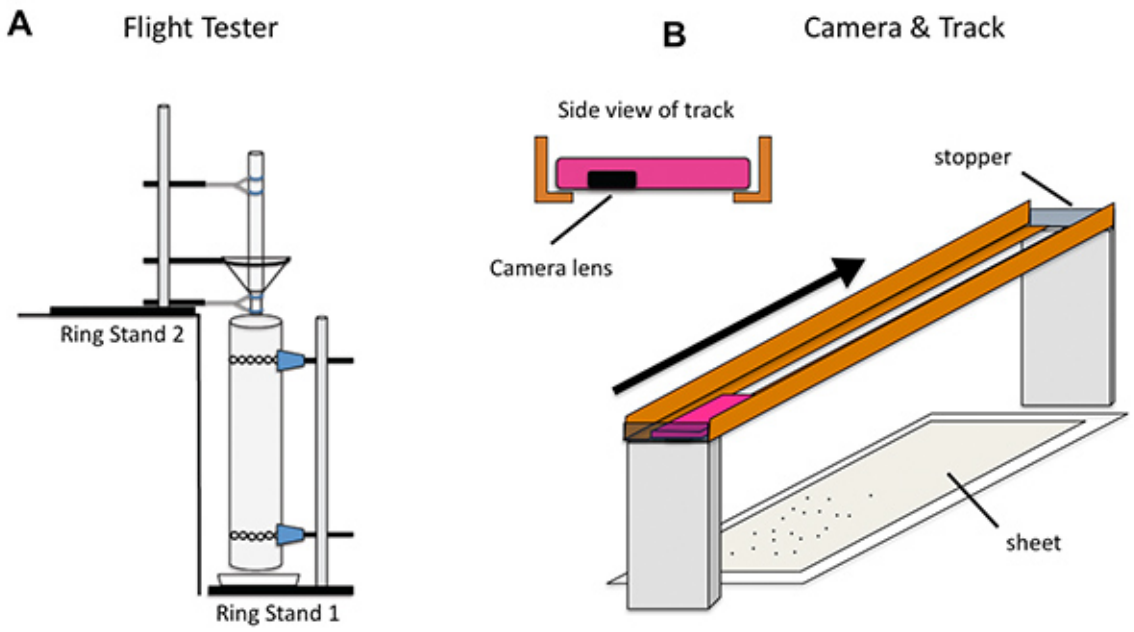

Figure 1. Diagrams of Flight Tester, Camera, and Track. (**A**) Illustration of the set up for the updated flight tester. Ring Stand 1 holds the 90 cm-high flight cylinder; Ring Stand 2 holds the funnel and the 10 cm-long "drop tube". (**B**) Diagram of camera and track used to produce a panoramic image. The camera should be supported by the track without obstructing the view from the lens. Click here to view larger image.

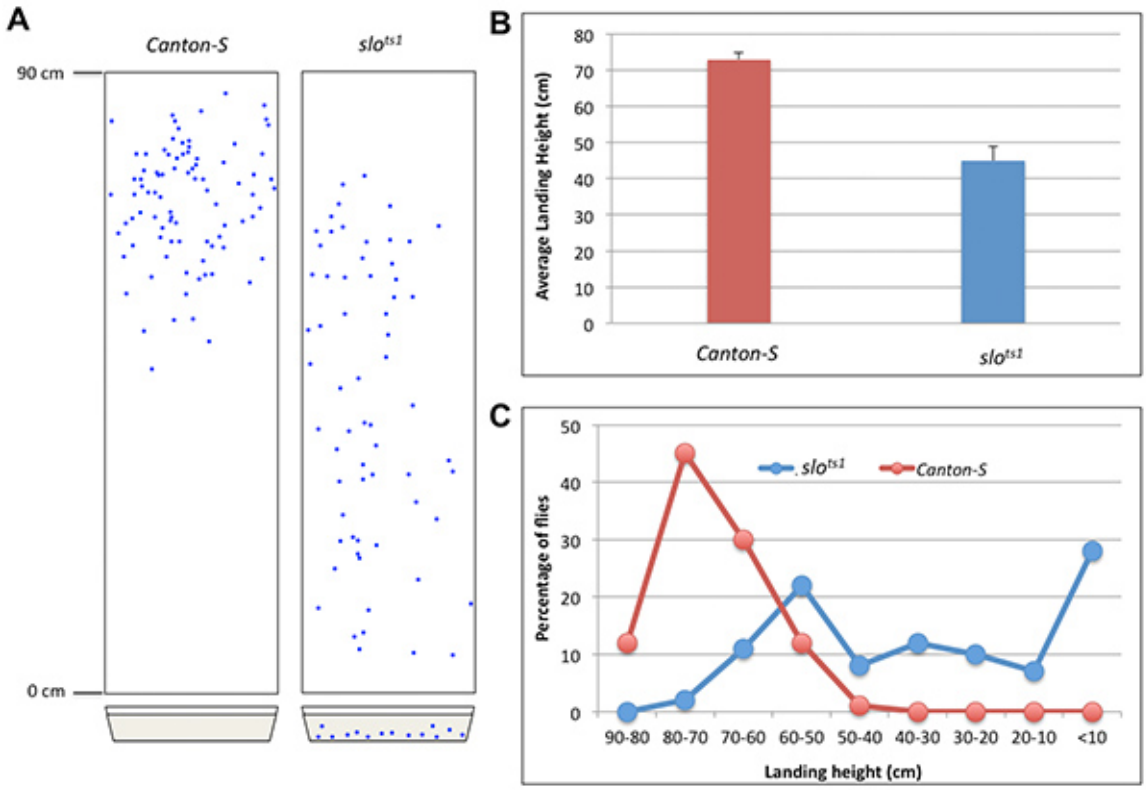

Figure 2. Representative results from a sample flight experiment. Comparison of flight ability of 3-day old *Canton-S* control flies to *slowpoke* mutants (slo^{ts1}). (**A**) Screen captures for *slowpoke* and control flies displaying the landing heights of individual flies. Each blue circle represents the location of an individual fly. These landing heights are used to calculate the average landing height (**B**) as well as overall distribution (**C**) for each genotype. Male and female flies were pooled together in each sample. Error bars represent the standard error of the mean. Click here to view larger image.

Discussion

Using the methods described here, we have been able to rapidly assess flight performance of a large number of *Drosophila* mutants, providing greater efficiency than previously possible. For our experiments, we routinely separate males and females and raise them at low density (less than 20 flies/vial) to limit aggression that could damage wings. Another important consideration is to control properly for differences in flight performance owing to differences in genetic background. We also find it helps to allow flies a minimum of 24 hr to recover from anesthetization with carbon dioxide prior to flight testing. Alternatively, flies may be anesthetized by exposure to cold temperature (4 °C) to allow for faster recovery without potentially influencing behavior.

The rate-limiting step in this protocol is removing the flies from the plastic sheet between trials. One method to increase efficiency is to use a large number of sheets simultaneously, setting aside the sheets as they are used and cleaning them all at once following data collection. Flies that fall to the bottom will need to be counted manually, as they will not be included on the sheet. Still, this is easy compared with manually calculating landing height. The need to reapply Tangle-Trap to each sheet will vary depending on how thick the coating is. In our experience, an individual sheet will last a month before a new coating is needed.

An additional advantage to using Tangle-Trap over mineral oil is the ability to recover live flies from the sheet. Since the flies simply stick to the surface of Tangle-Trap rather than becoming immersed, individual flies can be easily removed. "Flightless" flies that fall to the bottom can also be recovered by replacing the mineral oil tray with an empty flask.

We believe the automated measurement of flight behavior described here provides a number of advantages over previous methods, allowing for a higher level of throughput, reproducibility, and accuracy for genetic screens. Automated scoring has also been used to increase throughput for behavioral assays such as the RING assay[11]. Furthermore, the direct measurement of landing height provides a greater sensitivity than a simple pass/fail measurement (% fliers, *etc.*), allowing us to detect more subtle differences in flight performance.

The assay described here can be complemented by subsequent further assays that measure more complex aspects of flight behavior, including visual control of flight speed[12] and free-flight responses to motion[13]. While these tests are more time-intensive and not amenable to large-scale genetic screens, they may help to provide more information regarding a particular gene's function in a locomotor response.

Disclosures

The authors have no conflicts of interest to disclose.

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