Social Interactions and Aggression in Drosohila melanogaster

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1 Background

Why do animals fight?

From tiny insects to large mammals, aggressive behavior is a universal strategy for competing over limited resources such as food, territory, and mates. Understanding aggression allows researchers to investigate how animals make decisions during social interactions and how neural circuits, genes, internal state, and environmental conditions influence behavior.

Drosophila melanogaster provides a powerful model system for studying aggression because it displays a rich and highly stereotyped repertoire of social behaviors that can be observed, quantified, and linked to underlying biological mechanisms. Under appropriate experimental conditions, male flies compete over a centralized food resource and exhibit a predictable sequence of escalating aggressive behaviors.

Aggressive interactions often begin with lower-intensity actions and gradually progress toward more intense physical encounters. Researchers use ethograms, standardized catalogs of behavior, to identify, define, and quantify these actions. By assigning clear definitions to individual behaviors, aggression can be measured consistently across animals and experimental conditions.

In this module, you will record aggressive encounters between pairs of male flies and analyze behavior using behaviorScoreR. By scoring behavioral events and visualizing their timing, you will investigate how aggressive contests unfold over time, whether fights follow predictable rules of engagement, and which behaviors are associated with contest escalation and dominance.

1.1 Experimental Conditions

You will observe pairs of male flies under one or more conditions:

1. Control males competing over a food resource
2. Genetically manipulated males
3. Environmentally manipulated males
4. Additional instructor-provided experimental treatments

1.2 Behavior Categories

O (Orienting)

WF (Wing Flick)

F (Fencing)

L (Lunging)

RL (Reciprocal Lunging)

T (Tussling)

WK (Walking)

S (Standing)

2 Part 1: Behavioral Recording

Pairs of two-day socially isolated male flies are introduced into custom fighting chambers containing a centralized food resource.

After a brief acclimation period, a divider is removed and the flies are allowed to interact freely. Interactions are recorded from above using a camera mounted over the chamber.

Record approximately 20 minutes of behavior per fly pair.

Ensure:

- The entire arena is visible
- Both flies remain visible throughout the recording
- Lighting is consistent
- Camera position remains fixed

2.1 Scoring Technique:

Open the recording in behaviorScoreR.

Score each occurrence of a behavioral event using the assigned key.

Unlike grooming, aggressive behaviors are recorded as discrete events rather than time-sampled states.

For example:

- If a fly performs three lunges, record three lunges.
- If a fly performs one wing flick, record one wing flick.

2.2 Data Visualization for Students

Behavioral timelines can be represented as ethograms showing the sequence of aggressive events through time.

Time (s)	5	12	18	24	31
Behavior	WF	F	L	L	T

These timelines allow visualization of behavioral escalation during contests.

3 Part 2: Quantitative Analysis

3.1 Step 1: Count Behavioral Events

Calculate:

- Total Wing Flicks
- Total Fencing Events
- Total Lunges
- Total Reciprocal Lunges
- Total Tussling Events

Calculate event frequency:

Event Frequency = Number of Events ÷ Observation Time

3.2 Step 2: Graphical Representation

Create:

- Bar graph showing frequency of each behavior
- Timeline ethogram showing behavioral progression
- Cumulative aggression plot through time

Suggested colors:

- Orienting: Gray
- Wing Flick: Yellow
- Fencing: Green
- Lunging: Red
- Reciprocal Lunging: Dark Red
- Tussling: Black
- Walking: Blue
- Standing: White

3.3 Step 3: Escalation Analysis

Measure:

- Latency to first Wing Flick
- Latency to first Fence
- Latency to first Lunge
- Latency to first Tussle
- Total escalation events
- Maximum escalation level reached

Calculate:

Escalation Index =

(1 × WF) + (2 × F) + (3 × L) + (4 × RL) + (5 × T)

Higher values indicate more intense aggressive interactions.

After scoring the contest, calculate:

Metric	Value
Total Wing Flicks	_____
Total Fencing Events	_____
Total Lunges	_____
Total Reciprocal Lunges	_____
Total Tussles	_____
Escalation Index	_____

3.4 Step 4: Dominance Analysis

Measure:

- Which fly lunged first
- Which fly occupied the resource most frequently
- Which fly displaced the opponent
- Whether dominance was clearly established

Questions:

- Did the first fly to lunge become dominant?
- Did reciprocal aggression prevent dominance establishment?
- Were highly escalated contests associated with clearer winners?

4 Part 3: Behavioral Scoring

Record aggressive behaviors using behaviorScoreR.

4.1 Categories and Actions Associated

Category	Abbreviation	Example Action
Orienting	O	Facing opponent
Wing Flick	WF	Rapid wing movement
Fencing	F	Foreleg contact

Category	Abbreviation	Example Action
Lunging	L	Rapid downward strike
Reciprocal Lunging	RL	Alternating lunges by both flies
Tussling	T	Sustained physical engagement
Walking	WK	Locomotion
Standing	S	Immobile

Goal: Track the order, frequency, and timing of aggressive behaviors throughout the contest.

5 Part 4: Procedure Overview

5.1 A. Fly Preparation

1. Collect male flies shortly after eclosion.
2. House males individually for two days.
3. Maintain flies on standard food.
4. Use age-matched animals.

5.2 B. Aggression Assay

1. Place two males into the fighting chamber.
2. Allow acclimation.
3. Remove divider.
4. Begin recording.
5. Record approximately 20 minutes of behavior.

> Tip
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> Aggression is strongly influenced by age, social isolation, and resource availability. Maintain consistent conditions across all experimental groups.

5.3 C. Behavioral Scoring with behaviorScoreR

1. Open the video in behaviorScoreR.
2. Load the aggression ethogram.
3. Assign scoring keys if necessary.
4. Score behavioral events.
5. Export the behavioral log as a CSV file.
6. Analyze behavioral frequencies and escalation patterns.

6 Part 5: Interpretation Questions

- Do aggressive encounters follow predictable rules of engagement?
- Which behaviors typically occur first?
- Does aggression escalate in a stereotyped sequence?
- Does the first fly to lunge become dominant?
- Are some contests more likely to escalate than others?
- How might genes, neural circuits, or environmental conditions alter aggression?
- Can aggressive outcomes be predicted from early interactions?

7 Part 6: Data Recording Options

Option	Description	Tools
A	Manual event scoring	behaviorScoreR
B	Timeline ethogram construction	Excel / Google Sheets
C	Statistical analysis	CSV files, MATLAB, R, Python

> Tip
>
> Behavioral timelines often reveal escalation patterns that are difficult to identify using event counts alone.

8 Sample Data Recording Sheet: Aggression Event Log

Fly Pair ID: _____

Condition: _____

Genotype: _____

Time (s)	Behavior Code	Notes

Behavior Codes:

O (Orienting) · WF (Wing Flick) · F (Fencing) · L (Lunging) · RL (Reciprocal Lunging) · T (Tussling) · WK (Walking) · S (Standing)