Sperm-Exhaustion Assay - Male Health Longevity (XO vs XY)

Goal

Measure how long individual males (XO vs XY) continue to sire offspring across repeated mating bouts separated by recovery periods.

Materials

- XO and XY males (emerged the same day; age to 7 days old before first use)
- Virgin females (same genetic background as each focal male; 3–5 days old at use)
- Standard malt vials (fresh)
- CO_2 pad and feather
- Sharpie
- Incubator (21 °C, 12:12 LD)

Experimental Design (per male)

- Start each focal male at **7 days post-eclosion**.
- Repeated “challenge” cycles: **48 h with 2 new virgins → 5 days solitary recovery**.
- Continue until predefined fertility-loss criteria are met.

Replicate: aim for **≥20 males per genotype** (more if you can).

Day-by-Day Schedule (per male)

Pre-Day 0: Collect males as they eclose; hold individually. Collect a surplus of virgin females daily.

Day 0 (Male age 7 d) – Bout 1 (48 h exposure)

1. Place **Male ID: M# (XO or XY)** with **2 virgin females** in a fresh vial (label “Bout 1”).

2. After **48 h (end of Day 2)**, **remove the male** to a **fresh, empty food vial** labeled "Recovery A."
   Leave the **two females** in their **Bout 1 vial** to lay eggs for a week (do not add the male back).

Days 3–7 – **Recovery (5 days alone)**

- Male remains alone in "Recovery A".

Day 7 – **Bout 2 (48 h exposure)**

1. Move the same male to a **new vial with 2 fresh virgins** ("Bout 2").
2. After **48 h (end of Day 9)**, move the male back to a **fresh recovery vial** ("Recovery B"), leaving the Bout 2 females in place to lay eggs for a week.

Days 10–14 – **Recovery (5 days alone)**

Day 14 – **Bout 3**, and so on…

- Repeat: **48 h with 2 new virgins → 5 d recovery**.
- Continue until two consecutive sterile bouts

Scoring Fertility & Counting Offspring

1. **Early fertility check:**
   - **3–5 days after removing females** from a given bout, **peek for larvae** (or later for pupae).
   - Record **Fertility = Y/N** for that bout.
2. **Final count:**
   - **10–14 days after female removal**, **count eclosed adults** from each bout vial.
   - Record **Total offspring** (split by sex if needed later).
   - This final count is what you should use for quantitative comparisons and the exhaustion call, since it's least noisy.

What You Record (per male, per bout)

- Male ID, genotype (XO/XY), date of male birth (for exact age)
- Bout #: start date/time; end date/time
- Female IDs (optional) or batch date/age
- Early check: larvae/pupae present? (Y/N)
- Final count: total eclosed offspring (by sex)