

Cage experiment protocol — XY vs XO competition (3 replicates)

Summary

Maintain three replicate population cages (500 flies each, equal starting sex/genotype composition), let populations evolve/compete, and every 6 weeks sample 100 males per cage and freeze them. Later extract DNA from individual males (96-well plate format), PCR for a Y-specific marker (plus an autosomal control), and score XY vs XO frequency over time.

Materials

- 3 population cages
 - 125 each - XO males and females, XdY males and females
 - Malt fly food
 - CO₂ pad
 - 2 mL tubes (pre-labeled)
 - Funnel
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Cage setup (Day 0)

1. Label cages as Cage A, B, C (replicates).
 2. For each cage, introduce 500 adults total:
 - 125 XO males (♂)
 - 125 XO females (♀)
 - 125 XY males (♂)
 - 125 XY females (♀)
 3. Provide food: 16 total 50mL malt food in bottles scored with yeast per cage
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Maintenance (weekly)

- Replace the oldest row of food once per week
 - Remove old food to avoid mold/mites.
 - Record any unusual mortality, contamination, or environmental deviations.
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Sampling schedule

- Sample 100 adult males per cage **every 6 weeks** (i.e., 6, 12, 18 ... weeks). Record the date and cage.
 - Rationale: fixed, regular sampling captures frequency dynamics while minimizing disturbance.
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Sampling procedure (practical)

1. Prepare 3 × 100 pre-labeled 2 mL tubes per sampling day (label format recommended: `CageID_Date_Sample#` e.g., `A_2025-11-05_01`).
2. Place CO₂ pad next to cage (outside). Do not CO₂ the entire cage — use funnel method:
 - Place a clean funnel in the cage with the small end aimed at the open mouth of the 2 mL tube.
 - Place CO₂ near the funnel entrance/outside cage (the pad on outside is fine): this will anesthetize flies near the port and encourage them to move into the funnel
 - Collect 100 males per cage. If females enter, place them back in the cage and collect males only.
3. Immediately after collecting each batch of 100 males into the 2 mL tubes, freeze them ASAP

Notes:

- Aim to sample random males. Avoid bias toward flies that congregate near the cage port (if possible do a brief gentle agitation to randomize)

Example timeline (first 6 months)

- Day 0: establish cages
 - Week 6: sample 100 males/cage → freeze
 - Week 12: sample again
 - Week 18: sample again
 - Week 24: sample again
- (Extract DNA and run PCR in batches every 2–3 sampling events or as practicable.)