Experimental evolution protocol

Last edited: Taylor — updated 10/23/2025

Cage setup plan

- Total cages: 6
    - 3 XO cages
    - 3 XY cages
- Setup schedule:
    - Week 1: 1 XO cage and 1 XY cage
    - Week 2: 1 XO cage and 1 XY cage
    - Week 3: 1 XO cage and 1 XY cage

Flies per cage

- Total flies per cage: 750
    - 350 males (from XO or XY stock; same male background for all cages except XO vs XY difference)
    - 400 females (20 females from each of 20 isofemale lines; isofemale lines are a different background than XO/XY males)

Materials & Equipment

- 20 isofemale lines
- XO males and XY males (same background; only differ by presence of Y chromosome)
- 6 fly cages
- 30 mL vials (virgin collection and holding flies before cage setup)
- Malt food
- 6 oz bottles for malt food (16 per cage on setup, 4 per cage per week to swap out)
- Cotton rolls
- CO_2 pad
- Permanent markers
- Incubator

- Freezer (-20°C)
- 2 mL tubes

Protocol: Cage setup

1. Collect virgin females from each of the 20 isofemale lines.
    - 20 virgin females per iso line per cage = 400 females total.
2. Collect 350 males from XO or XY stock (depending on cage type).
3. Add 16 malt food bottles to the cage.
    - Score food and add yeast to each bottle
4. Place 400 females and 350 males into the cage.
5. Label cage with XO or XY, replicate number, and start date.
6. This marks Week 1 for that cage.

Weekly maintenance

- Remove the first row of 4 food bottles.
- Push remaining bottles forward.
- Add cotton rolls to any bottles without cotton.
- Add 4 new malt food bottles at the back.

P1–P2 sperm competition assay (every 10 generations) and 6-week sampling

P1–P2 sperm competition assay (every 6 weeks):

~Look at sperm competition protocol~

6-week sampling and sperm competition schedule:

- Every **6 weeks** from each cage, collect **~100 males and ~100 females** (total ~200 flies) as the routine population sample; freeze and store as fossil-record samples.
- Additionally, at each 6-week timepoint collect an extra **~50 males** from each cage to be used specifically for the P1–P2 sperm competition assays run that week (these males should not be frozen prior to the assay).

- If possible, stagger collections slightly across days to avoid over-handling a single cage on the same day.
- After collection, run the P1–P2 assays using the collected extra 50 males (aim for at least 10–30 trials per cage depending on throughput; prioritize balanced sample sizes across XO and XY replicates).

Quality control

- Label all vials, bottles, and cages.
- Record start date, cage type (XO or XY), and isofemale composition.
- Monitor temperature and humidity daily.
- Keep sample inventory for all frozen flies by date and cage.
- Periodically check for contamination between cages