

## P1–P2 Sperm Competition Assay

### Number of trials:

- 20 in each direction (→ and reciprocal) = 40 total per assay.

### Flies used:

- Dark virgin females
  - Virgin males from the two variants (XO and XY, or 2 separate genotypes based on what the test is for)
  - All flies are aged to **8 days old** (day of eclosion = day 0)
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## Timeline

### Day 7

- Put each male (XO and XY) individually into its own vial.
- This allows the male scent/pheromones to condition the vial for 24 hours.

### Day 8 – First mating (P1)

1. Add an 8-day-old dark virgin female into the P1 male's vial.
2. Let them mate for **24 hours**.
3. After 24 hrs, remove and discard the male.
4. Leave the female alone in the same vial for **24 hours** (rest).

### Day 9 – P2 setup

5. Transfer the female into the P2 male's vial.
6. Allow mating for **24 hours**.
7. After 24 hrs, remove the female and place her into her **own malt food vial** that is:
  - Lightly scored
  - Has added yeast

### Days 10–16 – Egg laying

8. Leave the female in this vial for **7 days** to lay eggs.
9. After 7 days, remove the female.
10. Add a cotton roll to the vial to give larvae to pupate.

### Scoring offspring

- Once offspring start eclosing, count every **3–4 days**.

- Eye color tells you paternity:
  - **Red eyes** → **father was XY**
  - **Sepia/dark eyes** → **father was XO**
- You record for each female:

### Table Template

Female #	# dark males	# dark females	# red males	# red females
1				
2				
3				
...				
40				