# Cohort Setup for Thermal Assays

## Hannah Chu 2018-06-25

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### 1 Model

- Drosophila melanogaster
- Mated
- 10 cohorts
- 520 individuals
  - 260 male
  - -260 female

#### 2 Workflow

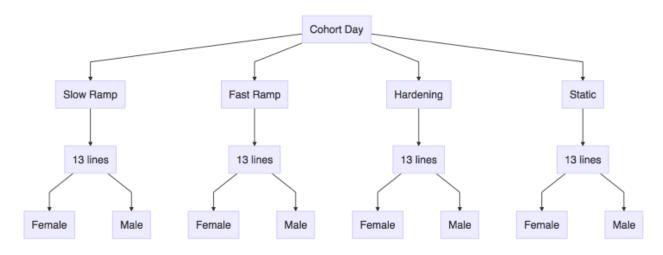


Figure 1: Workflow

- One cohort per day
- 4 different treatments per day for all 13 lines
- $\bullet$  50/50 male and female based on cohort, strain, and treatment
- Position, sex, and treatment all randomized

```
library(DiagrammeR)
mermaid("
  graph TD
  A[Cohort Days]--> B[Slow Ramp]
  A --> C[Fast Ramp]
  A --> D[Hardening]
  A --> E[Static]
  B --> F[13 lines]
  C --> G[13 lines]
  D --> H[13 lines]
  E --> I[13 lines]
  F --> J[Female]
  G --> K[Female]
  H --> L[Female]
  I --> M[Female]
  F --> N[Male]
  G --> O[Male]
  H --> P[Male]
 I --> Q[Male]")
```

### 3 Cohort Setup

Keep 3 rearing vials and 1 "fresh" vial for replicates

- 1 old vial (backup)
- 1 middle vial (backup)
- 1 new vial (backup)
- 1 exp. vial
  - dump the adults and wait for newly eclosed adults
  - flip newly eclosed adults into fresh vial consecutive days
  - keep fresh vials for 5 days before using for tests

#### 4 Measure

- 1. Knockdown times
  - ullet based on thermal assay protocol here
- 2. Survivorship
  - after flies knockdown, move them into  $5\mathrm{mL}$  plastic tubes with food
  - monitor every day for individual fly death