



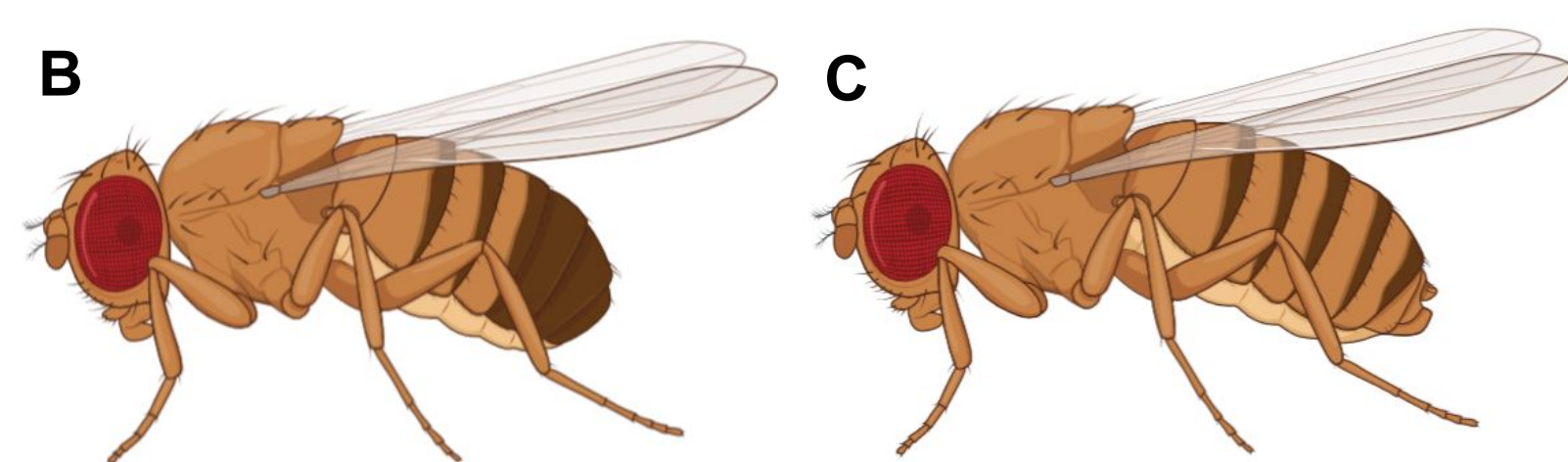
Introduction

- In this study we explore how starvation can cause different responses to appetitive tastants in *Drosophila melanogaster*.
- We also explore sex differences in feeding behavior using an assay known as the Proboscis Extension Reflex (PER).
- PER is a feeding-related behavior that occurs when a fly senses an appetitive stimulus, such as sucrose.

Methods



Figure 1. Anatomical Sex Differences.



Animal Sexing

- Males were identified based on presence of a sex comb (Figure 1a) and black ventral abdomen (Figure 1b).
- Females were identified based on white ventral abdomen (Figure 1c).

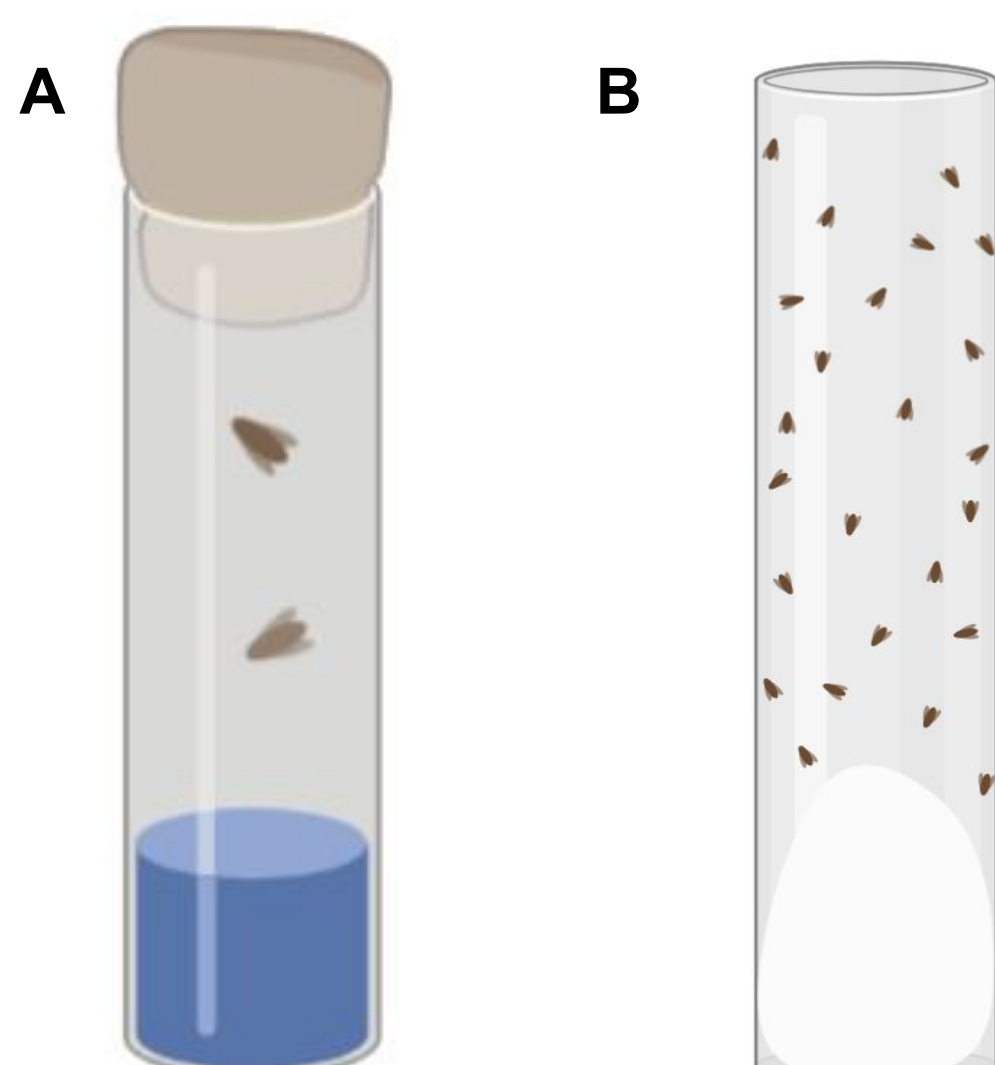


Figure 2. Vial Set-up.

Animal Treatment

- Control flies were kept in vials with food prepared with Formula 4-24 ® Instant *Drosophila* medium (Carolina Biological) (Figure 2a).
- Wet-starved flies were kept in vials containing filter paper soaked with 1 mL water (Figure 2b).

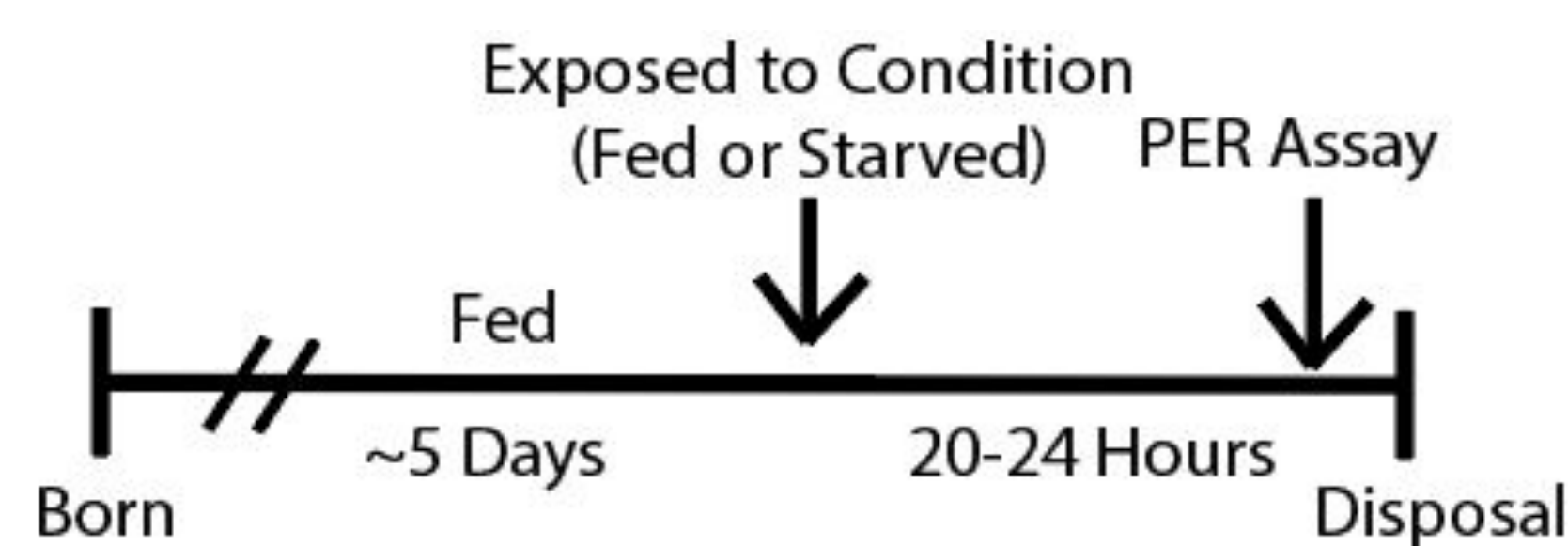


Figure 3. Treatment Timelines.

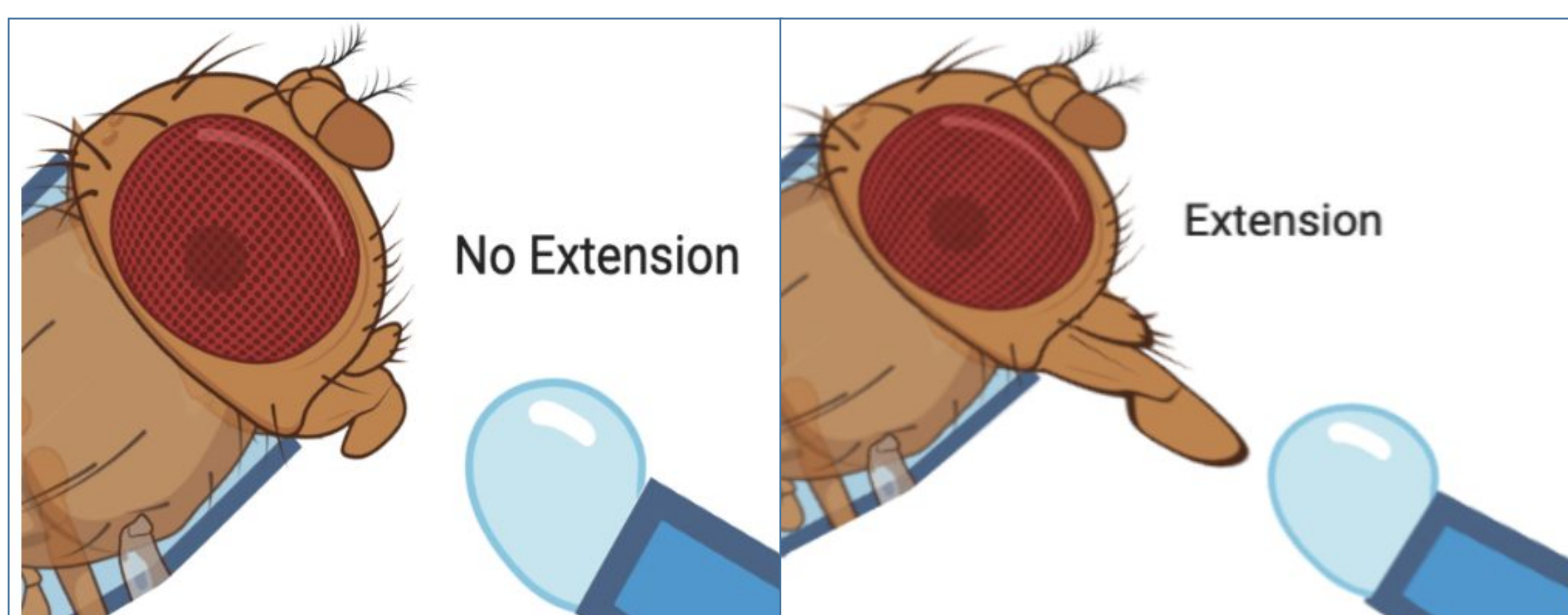


Figure 4. Sample PER Responses.

PER Assay

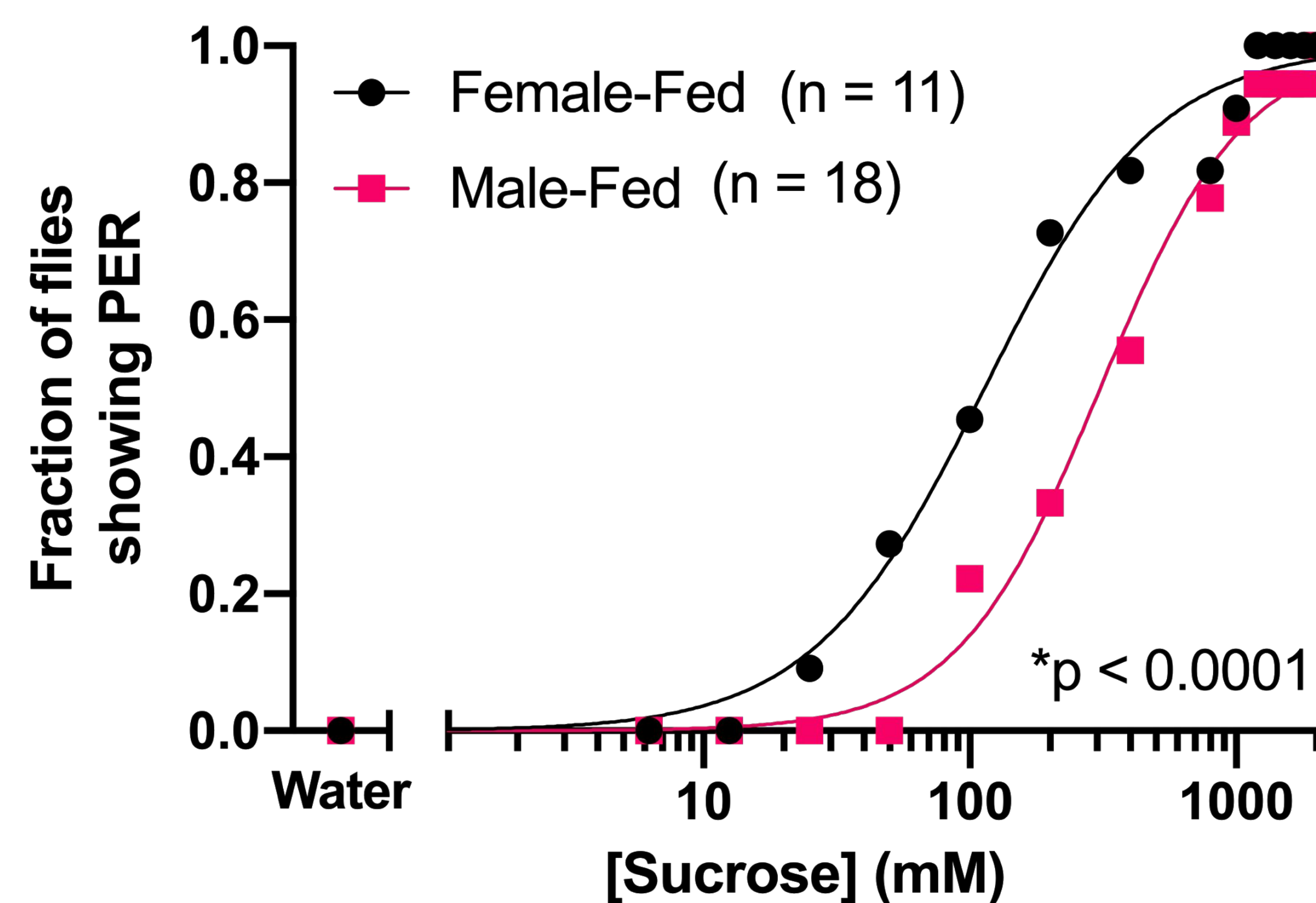
- A fly aspirator¹ was used to capture and immobilize a fly in a pipet tip.
- A micropipette was used to present a droplet of solution to the fly.
- Solutions were presented in order of increasing sucrose concentration, starting with 0 mM (water) and ending with 2000 mM.
- Only full extensions of the proboscis were counted (Figure 4).

Data Analysis

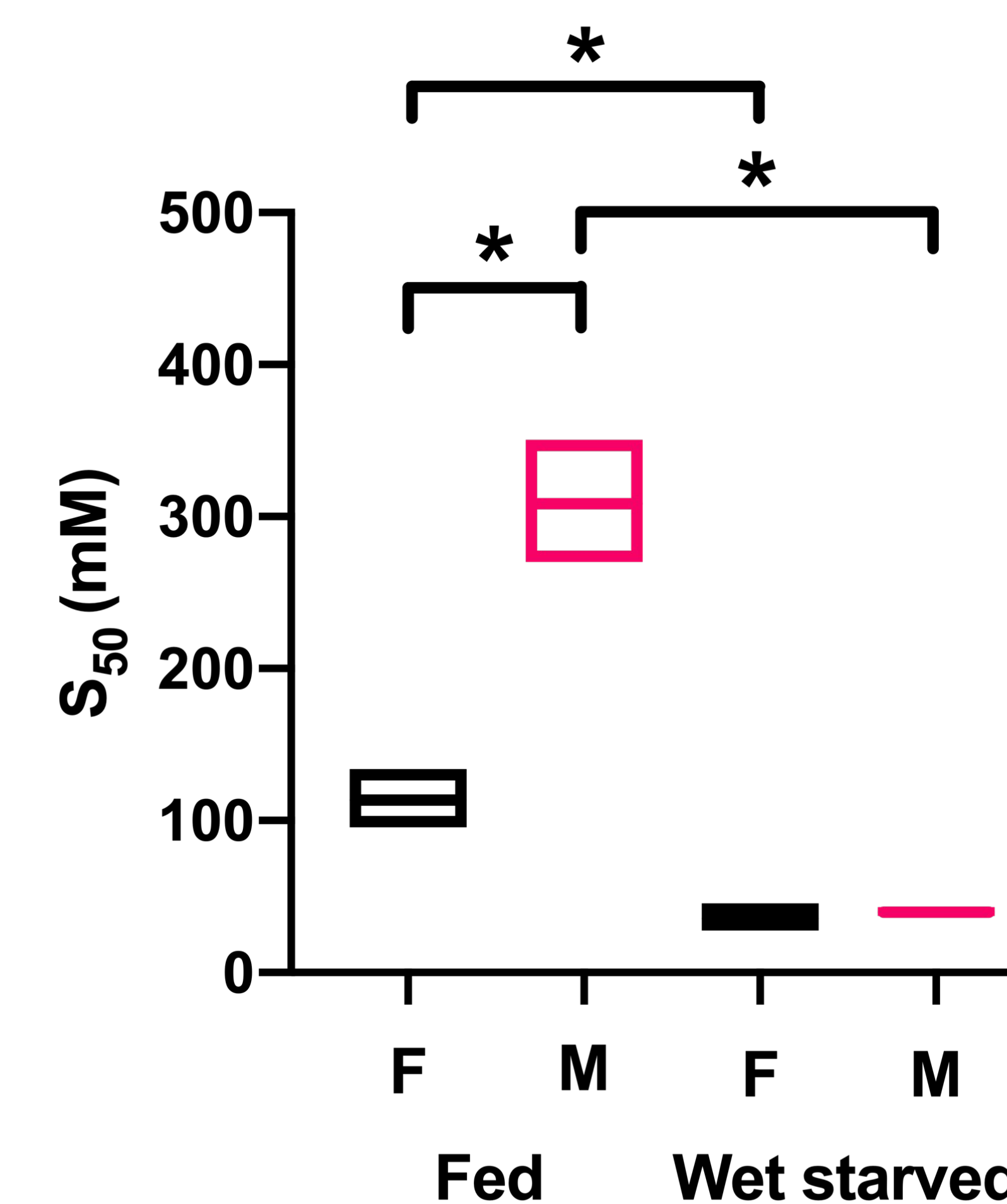
- For each set of data, the dose-response curves were fit and the S_{50} value (the sucrose concentration at which 50% of the flies in that group exhibited PER) was determined.

Results

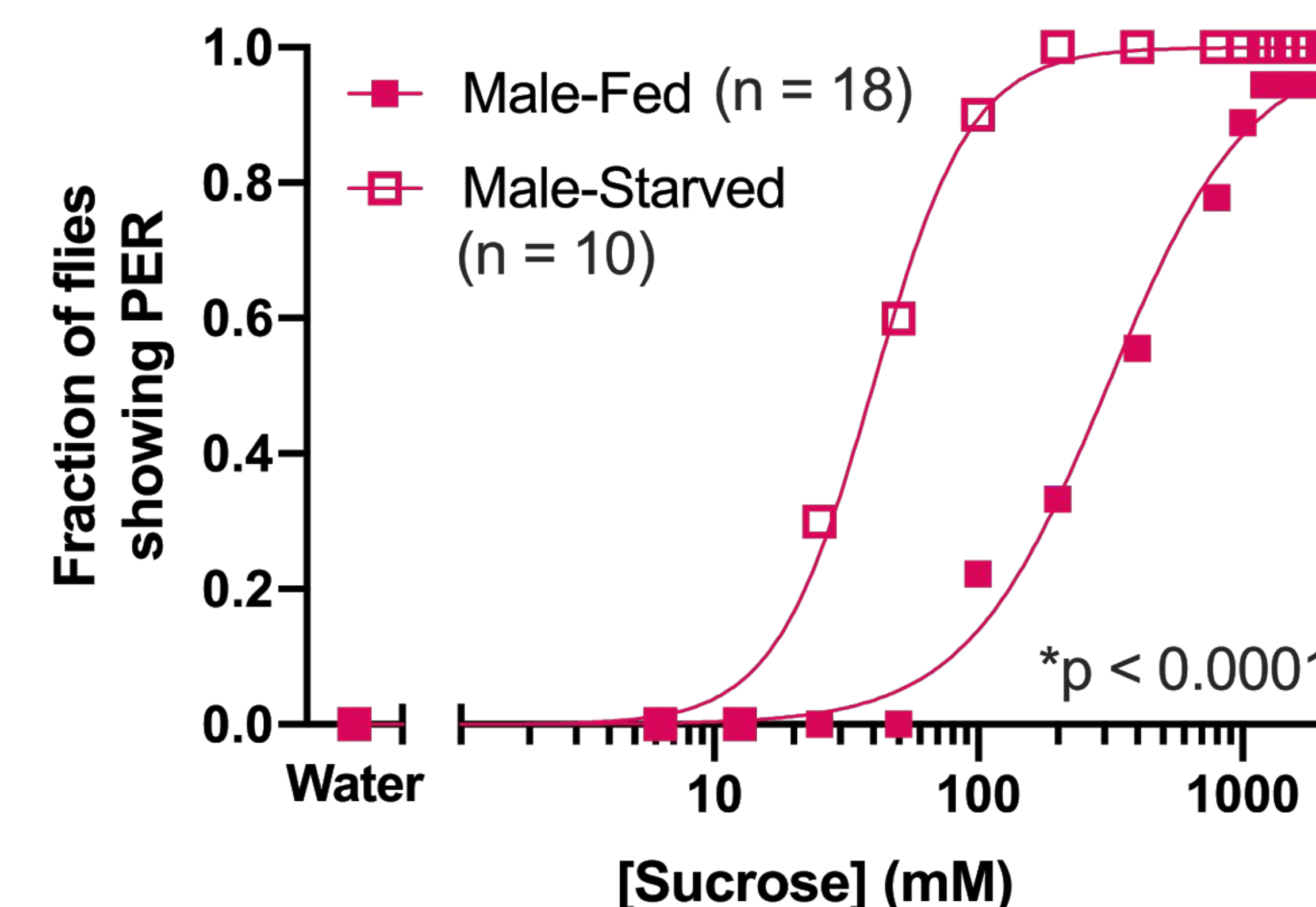
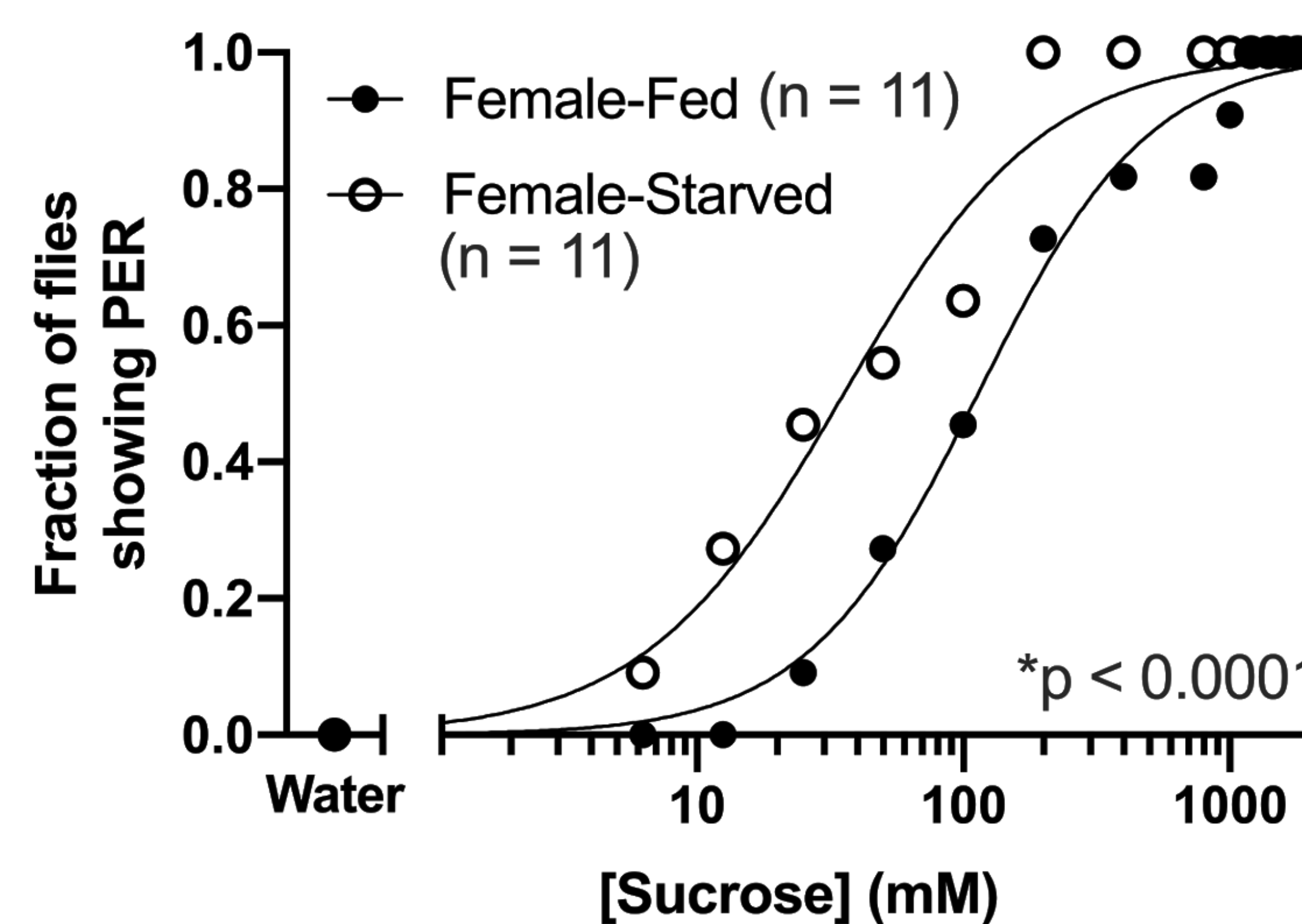
Fed Fly Sex Differences in Response to Sucrose



Comparison of S_{50} Values



Starvation Increases Sensitivity to Sucrose



Conclusions

- Both male and female flies exhibit increased sucrose sensitivity when starved compared to when they are fed.
- There was a significant difference in sucrose sensitivity between fed male and female flies, indicating a sex difference at baseline physiological conditions.
- These results indicate that sex should be considered as an independent variable in future fruit fly feeding behavior studies.
- Further testing should examine these sex differences at the receptor level, such as treating flies with L-DOPA or conducting electrophysiology studies to record from individual gustatory neurons.

References

1. Shiraiwa, T., & Carlson, J. R. (2007). Proboscis Extension Response (PER) Assay in *Drosophila*. *Journal of Visualized Experiments*, (3).
2. Inagaki, H. K., Anderson, D. J., Axel, R., Kitamoto, 5T., Barnea, G., Ishimoto, H., ... de-Leon, S. B.-T. (2012). Visualizing Neuromodulation In Vivo: TANGO-Mapping of Dopamine Signaling Reveals Appetite Control of Sugar Sensing. *Cell*, 148(3), 583–595.
3. Inagaki, H. K., Panse, K. M., & Anderson, D. J. (2014). Independent, Reciprocal Neuromodulatory Control of Sweet and Bitter Taste Sensitivity during Starvation in *Drosophila*. *Neuron*, 84(4), 806–820
4. Marella S, Mann K, Scott K (2012) Dopaminergic Modulation of Sucrose Acceptance Behavior in *Drosophila*. *Neuron* 73:941–950.