Effects of Dauer on C. elegans Behaviors: Assessing Impact of Various Induction Methods Across Mutants

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Background

C. elegans are microscopic nematodes commonly used as model organisms in neuroscience research because of their simple nervous system and predictable behaviors. C. elegans can undergo dauer, a diapause-like developmental stage that deviates from the standard life cycle. C. elegans go into dauer in response to environmental stressors during the L2 life stage, such as lack of food supply, uninhabitable temperatures, or overpopulation. This research is focused on the developmental distinctions between adult worms post-dauer, and ones that progressed straight through the life cycle.

Dauer is a stage in which the animal forms a hard cuticle around the body and "shuts off" to decrease energy expenditure as a means of maintaining itself, similar to hibernation (Fig 1). During dauer, an organism's development is essentially arrested until conditions are once again favorable, after which the worms will directly enter a post-dauer L4 adulthood stage.

Fig. 1

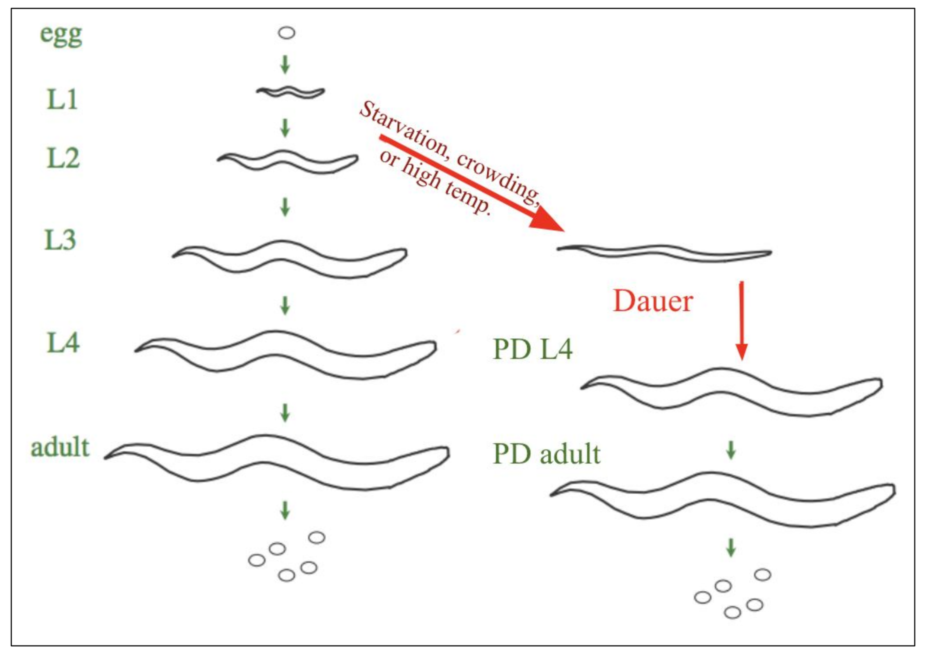

Figure 1: The typical C. elegans developmental cycle can lead to two different types of adults, based on whether or not they've undergone dauer.

Methodology

Two strains of C. elegans, N2 wild-type, and daf-2 mutants (which are more susceptible to undergoing dauer at high temperatures) were cultured on agar plates seeded with *E. coli* (provided by Hobert Laboratory). N2 worms were age-synchronized during both rounds of data collection, while the daf-2 strain were only synchronized during round 2. Synchronization followed a bleaching procedure as described by Cerón et al., 2012 and synchronized worms were subsequently separated into three groups: a control group kept at 20°C on seeded plates, an experimental group kept at 20°C on unseeded plates, and an experimental group kept at 27°C on seeded plates. Recovery involved chunking each condition on to seeded plates and storing at 20°C.

The recovered worms were examined for a series of three possible behavioral changes post-dauer. (1) Locomotion was assayed by tracking a worm's movement across 1 cm x 1 cm grid over one minute. (2) Response to physical stimuli was assayed with the 90° nose touch assay: placing a hair in front of a moving C. elegans and observing response upon contact (ten worms per plate). The expected response is the immediate initiation of backwards locomotion. (3) Feeding activity was assayed by recording pharyngeal pumping rate in pumps per minute for 5 worms per plate.

See supplemental methodology for additional information regarding culturing, age synchronization, dauer induction, and dauer recovery protocol.

Results

The results from each behavioral feeding assay were analyzed using appropriate statistical methods. The locomotion assays were analyzed using a student t-test, the response to stimuli assays were analyzed using a two-tailed proportion z-test, and the feeding behavior assays were analyzed using a paired t-test.

Fig. 2a

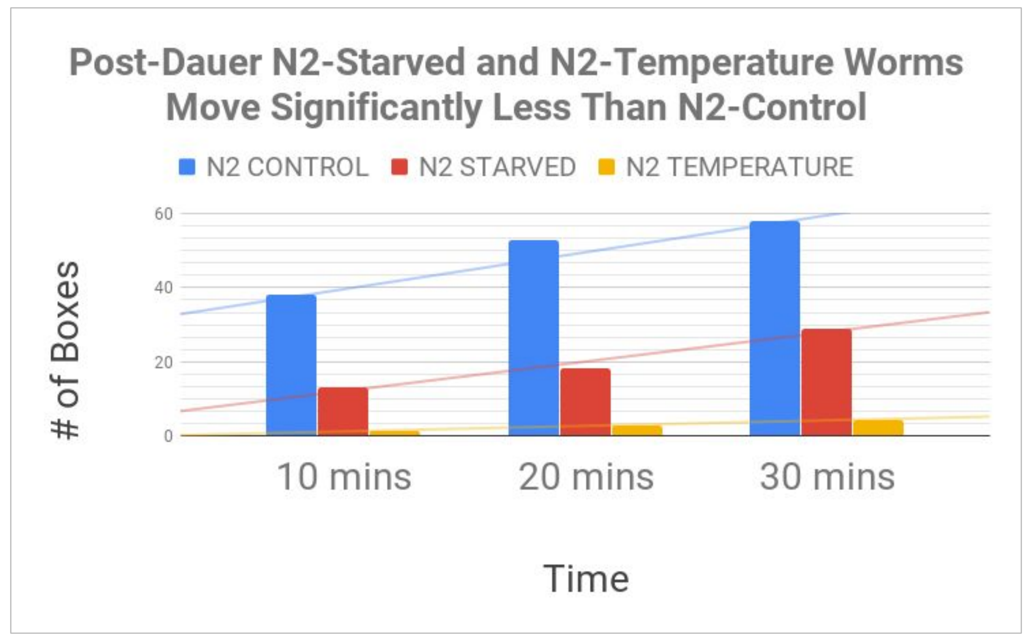

Fig. 2b

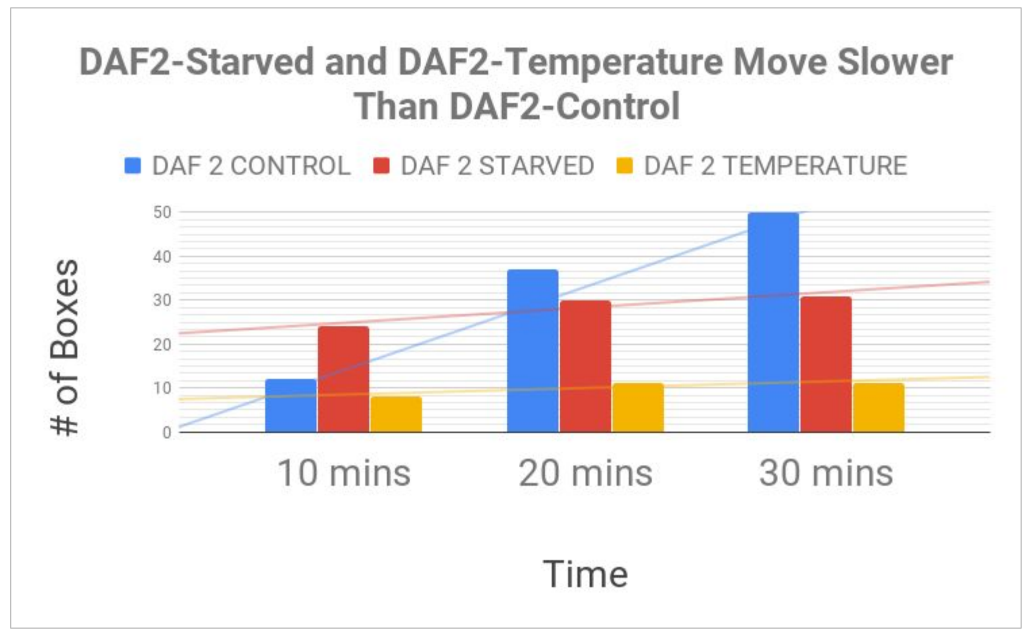

Fig. 3a

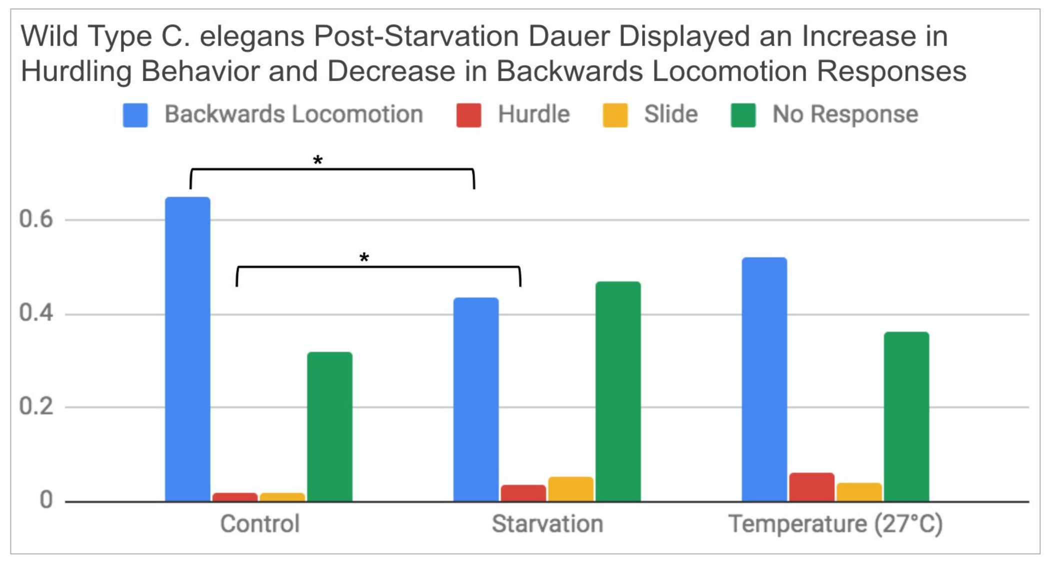

Fig. 3b

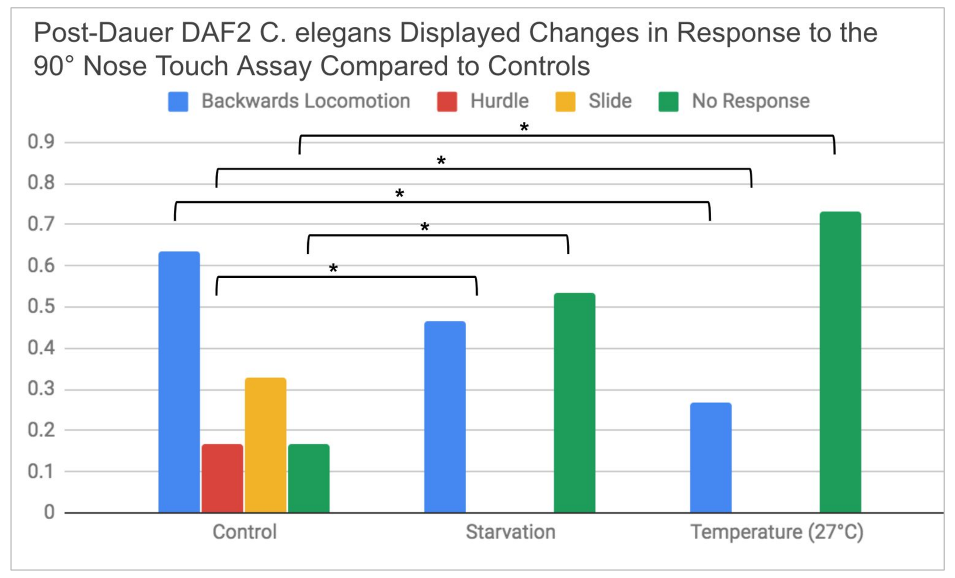

Fig. 4a

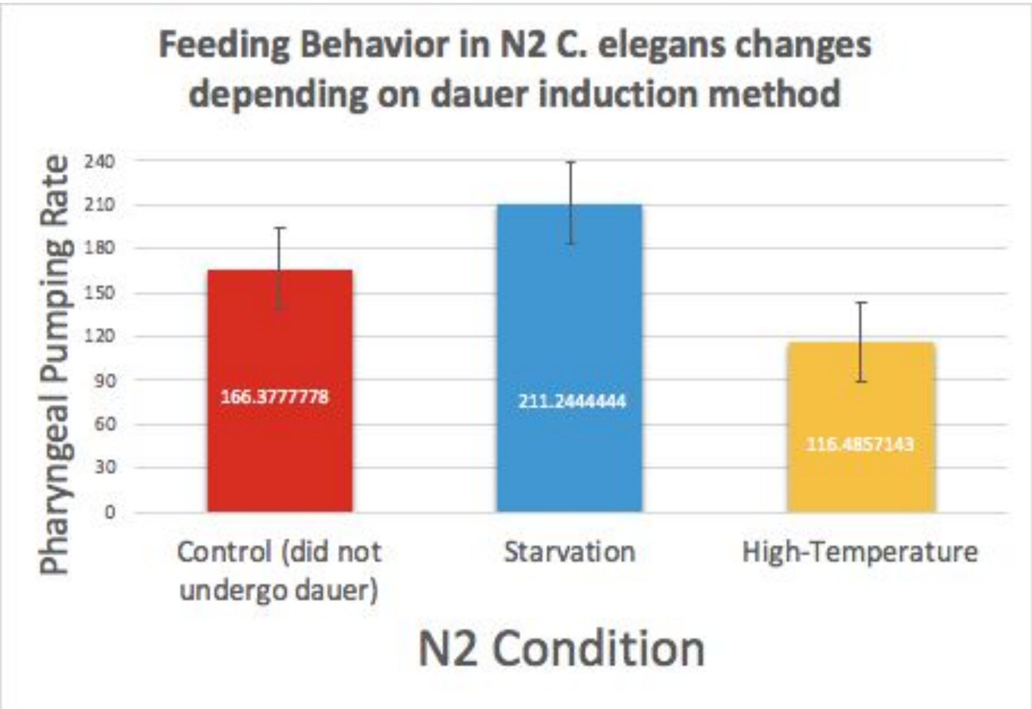

Fig. 4b

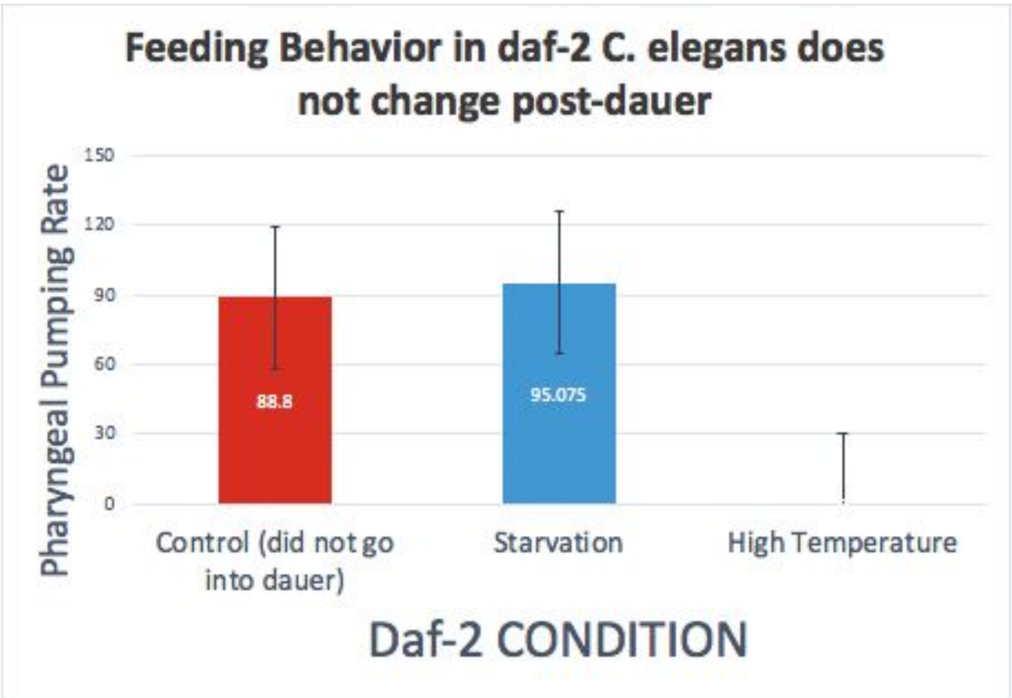

Figure 2a depicts the decrease in locomotion in post-dauer Wild-Type worms compared to controls. Figure 2b depicts the decrease in speed in daf-2 worms that underwent dauer.

Figure 3a displays an increase in hurdling behavior and decrease in backwards movement post-contact with stimuli in wild-type worms that underwent dauer, Figure 3b displays the differences in results across all response types in the daf-2 post-dauer worms.

Figure 4a depicts the feeding increase in wild-type worms post-starvation induced dauer, and decrease in worms post-temperature induced dauer. Figure 4b depicts the lack of changes between post-dauer daf-2 mutant worms, and ones that did not undergo dauer.

Figures 2a, 3a, and 4a: statistical significance was measured between N2 Control and starved, and N2 control and high temperature. In Figures 2b, 3b, and 4b, statistical significance was tested between the daf-2 Control and daf-2 starved, and daf-2 Control and daf-2 high-temp. We used $p \leq 0.05$ to determine statistical significance.

Discussion

Undergoing dauer influences behavioral traits, even after recovery, exhibiting differences in behaviors between post-dauer adults and ones that progressed through the normal life stages. Different dauer induction methods had varying effects: results display differences in how worms that underwent temperature-induced dauer exhibited certain behaviors in comparison to ones that underwent starvation-induced dauer.

- Post-dauer N2-Starved and N2-Temperature worms moved significantly less than N2-Control. DAF2 starved and DAF2 temperature move slower than DAF2 control.
- The wild type C. elegans recovered from starvation-induced dauer had a statistically significant change in behavior regarding a decrease in the proportion of backwards locomotion responses and an increase in the proportion of hurdling responses. The DAF2 animals showed statistical significance in both the starved and temperature induced plates, specifically in that after dauer no animals exhibited hurdling or sliding tendencies.
- In the N2 worms post-temperature induced dauer, there was a decrease in the pharyngeal pumping feeding behavior, whereas in the ones post-starvation induced dauer, there was an increase in the pharyngeal pumping feeding behavior. There was no significant difference in pharyngeal pumping in the daf-2 mutant strain.

Understanding effects of stressors during "adolescence" of worms and how they impact their adult lives can impart a better understanding of how our own childhood stressors can have lasting consequences.

Future Direction

To further validate our results, we could repeat our protocols with larger numbers of plates, especially with more daf-2 worms, to ensure more accurate results. Another mutant strain, Him-5, could also be incorporated into the study, as previous literature has shown that Him-5 mutants demonstrate differences in reproductive behavior between post-dauer adults and ones that haven't undergone dauer. A more in depth look into each behavioral assay could be utilized to elucidate the mechanisms behind the differences found.

Acknowledgements

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Works Cited

Porta-De-La-Riva, M., Fontrodona, L., Villanueva, A., & Cerón, J. (2012). Basic Caenorhabditis elegans Methods: Synchronization and Observation. *Journal of Visualized Experiments,* (64). doi:10.3791/4019