Dauer Development Modulates Olfactory Behavior
Johnny Vertiz¹, Heather Carstensen¹ and Ray Hong¹§
¹Department of Biology, California State University, Northridge, Northridge, USA
§To whom correspondence should be addressed: ray.hong@csun.edu

Abstract
Reproductive adults and developmentally arrested larvae often occupy different ecological niches and thus are expected to respond differently to environmental stimuli. To understand the genes that coordinate dauer development and olfactory behavior, we examined adult and dauer C. elegans in wild-type and dauer constitutive mutants (Daf-c). We found all dauers showed decreased attraction to all three odorants tested compared to adults, with daf-7 dauer larva (DL) exhibiting a concentration-dependent preference shift towards isoamyl alcohol, suggesting that the TGF-ß pathway is involved in both dauer regulation and dauer-specific odorotaxis.
Figure 1. Olfactory responses of *C. elegans* dauer larvae: Chemotaxis between adults and dauer larvae differ significantly in response to (A) 1% 2,3-butanedione, (B) 1% 2,3-pentanedione, and (C-D) 1% and 0.1% isoamyl alcohol in wild-type N2, as well as in the Daf-c mutants daf-2 and daf-7. For (A) and (C): One-way ANOVA between adults and dauer with Kruskal-Wallis test. Blue circles denote young adults and orange circles denote dauer larvae. For (B) and (D): Two-way ANOVA between adults and dauer with Sidak’s multiple comparisons test. *P*<0.05, **P*<0.01, ****P*<0.0001, ns: not significant. Error bars indicate the standard error of the mean. The number of assays performed is indicated at the base of each bar.

Description

In *C. elegans*, the dauer larva (DL) is a non-feeding and stress-resistant stage that can respond to environmental cues differently from adults. For example, *C. elegans* adults avoid CO$_2$ while DL are attracted to CO$_2$ (Hallem and Sternberg 2008; Hallem et al. 2011). Similarly, adults of the nematode *Pristionchus pacificus* are mildly attracted to a beetle host pheromone while its dispersive DL are highly attracted to the pheromone (Carstensen et al. 2021).

To determine if genes that affect dauer development also modulate olfactory behavior in *C. elegans*, we compared the odor response profiles of young adults versus DL in *C. elegans* of two well-studied Daf-c mutants, *daf-2* and *daf-7* (Riddle et al. 1981; Swanson and Riddle 1981; Kimura et al. 1997) towards three odors. In *C. elegans* adults, the AWA neurons detect 2,3-butanedione (diacetyl), while the AWC neurons sense isoamyl alcohol (IAA) and 2,3-pentanedione (Sengupta et al. 1996; Chalasani et al. 2007). We found that wild-type DL showed less odor attraction than corresponding adults for 2,3-butanedione, 2,3-pentanedione, and IAA, consistent with the results of a previous study that included 2,3-butanedione (Hallem et al. 2011) (Fig. 1A-C). However, this adult-DL difference was not observed in *daf-2* towards IAA or *daf-7* towards 2,3-butanedione and 2,3-pentanedione. Furthermore, the response to IAA changed from attractive to repulsive in the DL of two *daf-7* alleles (e1372, ok3125). To evaluate if the avoidance response to IAA is due to hypersensitivity to IAA in the DL, we also tested their response to a 10-fold lower IAA concentration (0.1%). We found that the strong avoidance response was no longer observed, and instead the dauer IAA attraction became significantly reduced in both *daf-7* alleles compared to adults, resembling the wildtype DL (Fig. 1D). In *C. elegans*, odor concentration-dependent preference shift has been observed for IAA for adult worms and is dependent on *odr-3* function (Yoshida et al. 2012). Thus, *C. elegans* wild-type and Daf-c DL showed decreased attraction compared to adults to all three odors tested, with the *daf-7* mutations producing an accentuated concentration-dependent response to IAA in the DL, suggesting that the TGF-ß pathway is involved in both dauer regulation and dauer-specific response to IAA.

While remodeling of the AWC dendritic ends that increase their surface area have been speculated to heighten odor sensitivity in DL (Albert and Riddle 1988), our results show that only *daf-7* alleles exhibited a potentially higher sensitivity to isoamyl alcohol, while wild-type and *daf-2* DL were actually less attracted than adults to several odors. Mutation of the *daf-2* gene also mostly eliminated acute CO$_2$ avoidance in adult *C. elegans*, while the *P. pacificus* Daf-c mutant *Ppa-hsd-2* exhibited greatly enhanced adult attraction to a host odor (Hallem and Sternberg 2008; Carstensen et al. 2021). These results suggest that dauer development has multiple effects on olfactory behavior, such that both wild-type and Daf-c DL should be assessed when surveying species-specific responses.

Methods

Request a detailed protocol

Nematodes were cultured on OP50-seeded NGM plates and assayed at ~22°C. The chemotaxis assays for *C. elegans* were set up in a quadrant with worms loaded onto the center of 6 cm plates containing MOPS/Tween agar, along with two opposing pairs of odors and controls (Nuttley et al. 2002; Margie et al. 2013; Carstensen et al. 2021). 0.5 µl of 1 M sodium azide was spotted onto each odor or counter-attractant. Since *C. elegans* DL are hydrophilic, we collected DL primarily from the underside of the lids of recently starved plates by washing the lids with sterile spring water (Arrowhead, CA). For assays with Daf-c mutants, each plate was scored for either DL or YA using visual confirmation. Chemotaxis assays lasted 1-1.5 hours.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB1370</td>
<td>daf-2(e1370)III</td>
</tr>
<tr>
<td>CB1372</td>
<td>daf-7(e1372)III</td>
</tr>
<tr>
<td>RB2302</td>
<td>daf-7(ok3125)III</td>
</tr>
<tr>
<td>N2</td>
<td>wildtype</td>
</tr>
</tbody>
</table>
Reagents

- MOPS buffer (pH 7)
- Tween 20 (Polyoxyethylene 20-Sorbitan Monolaurate) CAS 9005-64-5
- 2,3-butanedione, isoamyl alcohol, and 2,3-pentanedione (Sigma-Aldrich)

References


Funding: NIH TL4GM118977 to JV; NIH GM105579 to RLH

Author Contributions: Johnny Vertiz: Investigation, Methodology. Heather Carstensen: Writing - review and editing, Investigation, Data curation. Ray Hong: Data curation, Methodology, Conceptualization, Funding acquisition, Supervision, Writing - original draft.

Reviewed By: Ian Chin-Sang

History: Received September 8, 2021 Revision received September 22, 2021 Accepted September 22, 2021 Published October 14, 2021

Copyright: © 2021 by the authors. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.