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Investigation

Natural variation in *C. elegans* 1-octanol olfactory avoidance behavior

Emily A. Polk,[1] Robyn E. Tanny ,[2] Amanda O. Shaver ,[2] Erik C. Andersen ,[2] Omer Gokcumen ,[1] Denise M. Ferkey [1,*]

[1]Department of Biological Sciences, University at Buffalo, The State University of New York, Buffalo, NY 14260, United States
[2]Department of Biology, Johns Hopkins University, Baltimore, MD 21218, United States

*Corresponding author: Department of Biological Sciences, University at Buffalo, The State University of New York, 109 Cooke Hall, Buffalo, NY 14260, United States. Email: dmferkey@buffalo.edu

The detection of chemical stimuli is a fundamental driver of animal behavior. Odorants and tastants provide essential information about not just food availability and quality, but also about environmental conditions and safety. However, much of what we know about how animals respond to their surroundings comes from the study of a limited number of laboratory-adapted strains of model organisms. In the case of *Caenorhabditis elegans*, chemosensory research has focused almost exclusively on the N2 strain first isolated in Bristol, England, in 1951, and which has been deemed the wild-type reference for studies of the species since that time. A collection of hundreds of wild *C. elegans* strains gathered from around the globe and whole-genome sequence data are now available through the *Caenorhabditis* Natural Diversity Resource. Using these resources, we assessed natural variation in behavioral sensitivity to the aversive odorant 1-octanol. We found that the wild strains either responded similarly to N2 or were less sensitive. No hypersensitive strains were seen among those tested. Using genome-wide association studies we identified a single quantitative trait locus (QTL) on the right arm of chromosome II correlated with diminished behavioral avoidance of 1-octanol and confirmed its role using near-isogenic lines. Building upon our recent report of wild strain taste responses, this study is only the second of this scale to investigate how natural genetic variation shapes *C. elegans* chemosensory behavior, focusing for the first time on olfaction.

Keywords: *C. elegans*; wild strain; olfaction; avoidance; behavior; 1-octanol; GWAS

Introduction

The senses of smell and taste (combined, referred to as chemosensation) provide animals with important information about the availability of food and potential mates, or the presence of harmful/toxic compounds and possible predators, in their environment. Over many decades, genetic screens and targeted experiments in model organisms have revealed key signaling molecules and neurons required for distinct chemosensory modalities in animals ranging from worms and insects to mammals (Prasad and Reed 1999; Bargmann 2006; Gerber and Stocker 2007; Ferkey et al. 2021; Keesey 2022). However, a limitation of these studies, as with most animal models, is that they rely primarily on a limited number of laboratory strains for analysis. As a result, relatively little is known about how naturally occurring genetic variation influences these behavioral traits across species.

In the case of *Caenorhabditis elegans*, the isogenic laboratory-adapted strain named N2 was first isolated in 1951 from mushroom compost in Bristol, England (Brenner 1974; Sterken et al. 2015) and has been a particularly fruitful model for the study of chemosensory behaviors for over 6 decades (Bargmann 2006; Ferkey et al. 2021). Although early experiments focused on identifying attractive and aversive chemicals to which the nematode responded to in the laboratory (Dusenbery 1973; Ward 1973; Dusenbery 1974; Dusenbery 1975; Bargmann et al. 1993), we now know that *C. elegans* is found worldwide in rotting plant environments that are rich in microbes associated with decaying vegetable matter (Frezal and Felix 2015). Among the bacterial strains associated with *C. elegans* in the wild are nutritious species that likely emit volatile chemicals that serve as long-range attractive olfactory cues to drive chemotactic behavior (Choi et al. 2016; Samuel et al. 2016; Schulenburg and Felix 2017; Worthy et al. 2018; Siddiqui et al. 2025). However, some soil microbes are also harmful to *C. elegans*, including pathogenic and nematocidal bacteria that release soluble chemicals that could alert the nematode to their presence and trigger avoidance (Pradel et al. 2007; Meisel et al. 2014; Tran et al. 2017). Aversive odorants, including 1-octanol and 2-nonanone, might also indicate the presence of fungi or pathogenic bacteria (Kaminski et al. 1974; Sharpell 1985). However, with limited exceptions (Volkers et al. 2013; Glater et al. 2014; Crombie et al. 2022; Lansdon et al. 2022; Amoroso et al. 2024; Polk et al. 2025), little is known about how genetically diverse wild *C. elegans* strains respond to potential environmentally relevant chemical cues.

The detection of noxious/aversive stimuli (nociception) is evolutionarily important for animal survival (Sneddon et al. 2014; Sneddon 2015; Tracey 2017). The N2 laboratory strain of *C. elegans* avoids many soluble and volatile compounds (Bargmann 2006; Ferkey et al. 2021; Fryer et al. 2024), including the bitter plant alkaloid quinine (Hilliard et al. 2004), heavy metals such as copper (Bargmann et al. 1990; Sambongi et al. 1999), the detergent SDS

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(Bargmann et al. 1990; Hilliard et al. 2002), alcohols such as 1-octanol (Bargmann et al. 1993; Troemel et al. 1995, 1997; Chao et al. 2004), ketones including 2-nonanone (Bargmann et al. 1993; Troemel et al. 1997; Tanimoto et al. 2017), and even high concentrations of several odorants that are normally attractive at low concentrations (Bargmann 2006; Ferkey et al. 2021). Self-protective reversal responses are initiated by activation of nociceptive sensory neurons (primarily the ASH pair), which relay signals to downstream interneurons and motor neurons that drive backward locomotion (White et al. 1986; Bargmann et al. 1990; Troemel et al. 1995; Sambongi et al. 1999; Hilliard et al. 2002, 2004; Bargmann 2006; Liu et al. 2018; Ferkey et al. 2021).

In 1998, *C. elegans* became the first multicellular eukaryotic organism to have its whole genome sequenced, with focus on the commonly used laboratory-adapted strain N2 (Berks 1995; Consortium 1998). Release of these sequence data revolutionized genetic studies in the animal, but with the limitation that they were not reflective of the genomic variation that exists among diverse individuals of the species. More recently a collection of >650 wild strains, along with whole-genome sequence data, has become available through the *Caenorhabditis* Natural Diversity Resource (CaeNDR, https://caendr.org), positioning *C. elegans* as a powerful model for genome-wide association (GWA) studies (Cook et al. 2017; Crombie et al. 2024). We recently reported natural variation in response to 3 aversive tastants (quinine, copper, and SDS) and identified associated quantitative trait loci (QTL) for each (Polk et al. 2025). Using the wild strains and resources available from CaeNDR, we have now investigated natural variation in behavioral sensitivity to the aversive olfactory stimulus 1-octanol across >150 wild *C. elegans* strains. Surprisingly, we found that among the strains assayed, response sensitivity to 15% 1-octanol only ranged from hyposensitive to an N2-like response, without any observed hypersensitivity.

Among the suite of stimuli that have now been tested, only 1-octanol and quinine are thought to signal through G protein-coupled receptor (GPCR) pathways. We uncovered a correlation between responses to this odorant and tastant that was not seen in any other pairwise comparison. Using genome-wide analysis, we identified a chemosensory QTL correlated with dilute 1-octanol response and validated its contribution to this behavioral trait. This 1-octanol QTL overlaps with a QTL that we previously identified for differential quinine responses (Polk et al. 2025), suggesting that some of the genomic variants across this region could affect genes encoding signaling and/or regulatory molecules shared by the 2 G protein-coupled pathways, although changes that uniquely affect 1-octanol and quinine responses are also likely. Taken together, our work has begun to uncover how naturally occurring genetic diversity influences how animals interact with their environment, while linking distinct behavioral modalities (smell/taste) that arise from shared signaling networks.

Materials and methods

C. elegans culture

Wild strains and N2 used for behavioral analysis were maintained under standard conditions on NGMA (nematode growth medium containing 1% agar and 0.7% agarose) agar plates (Andersen et al. 2012) seeded with the OP50 strain of *Escherichia coli* bacteria (Brenner 1974). NGMA plates were used to minimize burrowing by the wild strains.

Strains

For lists of strains used in this study (all wild strains used, the subset of strains used for GWA, and near-isogenic lines [NILs]), see Supplementary File 1. Behavioral response to 15% 1-octanol is also given for all wild strains (time to respond in seconds) in the first tab.

Octanol assays

Wild strains were freshly thawed, bleached to get rid of any contamination, and subsequently maintained for 3 generations at 20 °C on NGMA (Andersen et al. 2012). Octanol avoidance assays were performed as previously described (Troemel et al. 1995; Hart et al. 1999; Chao et al. 2004; Ezak and Ferkey 2010; Ezak et al. 2010; Krzyzanowski et al. 2013; Likhite et al. 2015). Briefly, young adult *C. elegans* were moved from NGMA plates with OP50 to transfer plates without OP50 to remove residual bacteria, and then to NGMA plates lacking the bacteria and allowed to acclimate "off food" for 20 min prior to assaying. A hair dipped in octanol was placed in front of a forward-moving animal, and response was scored as the amount of time it took for the animal to initiate backward locomotion. Time was kept with a metronome set to 60 beats per minute. Assays were stopped at 20 s because animals typically spontaneously reverse ~3 times per minute as they explore their environment. Thus, for these assays, a longer time to respond is indicative of diminished behavioral response. Wild strain response assays (15% 1-octanol, Fig. 1 and Supplementary File 1) were performed in triplicate (10 to 15 animals per plate) over the course of several months, all by the same researcher. Response assays for 100% 1-octanol (Supplementary Fig. 3) were performed in duplicate on 3 separate days (6 replicates total for each). Each animal was tested only once and the average time to respond to the 3 plates is reported (without relative calculations). The N2 strain was included as the control for behavioral reproducibility each day, with the final N2 data representing the average time to respond of all N2 plates assayed in parallel to the wild strains. Assays performed using the NIL strains (Fig. 4) were performed in duplicate over 3 d (6 replicates total for each), in parallel with N2. Following outcrossing to generate the NILs, strains were maintained and assayed on NGM plates (Brenner 1974). Control experiments indicated no significant differences between behavioral responses of animals grown on NGM vs NGMA plates. 1-octanol was purchased from Argos Organics (catalog number 150632500) and diluted in 100% ethanol for 15% 1-octanol avoidance assays.

Correlations

Correlation graphs were plotted in R using the package ggplot2 with the line of best fit setting (Wickham 2016). Correlation statistics, the R^2, and *P*-values for each stimulus comparison were calculated using the summary statistics in base R.

GWA studies

To test for correlations, GWA analyses were performed using the CaeNDR "Genetic Mapping" tool (https://caendr.org), as previously described (Cook et al. 2017; Zdraljevic et al. 2019; Lee et al. 2021; Widmayer et al. 2022). To minimize the effect of the high variability in Hawaiian genomes, a subset of the wild strains tested for 1-octanol response was used (1 Hawaiian strain, 1 from an unknown location). We used 2 GWA methods that both correct for individuals' relatedness between populations (Crombie et al. 2024). The INBRED association mapping uses a genomic relatedness matrix made specifically for organisms that are inbred (such as *C. elegans*) and corrected for genetic stratification. LOCO (leave-one-chromosome-out) excludes markers on the chromosome being left out, and associations are determined from the remaining chromosome markers. Both associations were visualized using

Manhattan plots. For full GWA results with test statistics, see Supplementary File 2.

Near-isogenic lines

NILs were made as previously described (Zdraljevic et al. 2017; Evans et al. 2018; Zdraljevic et al. 2019; Nyaanga and Andersen 2022; see also Polk et al. 2025). NILs were created using a wild strain with a common haplotype in the QTL (WN2066) crossed with N2 animals. After strains were backcrossed 4 times, the NILs were whole-genome sequenced (Zdraljevic et al. 2017; Evans et al. 2018; Zdraljevic et al. 2019). Other regions of donor strain sequence were present in other spots of the genome, but for N2 > WN2066, these small regions were not shared between the 2 NILs. For the 2 WN2066 > N2 NIL lines, 2 shared regions of WN2066 genomic DNA were outside the QTL (position 1 to 591,019 of chromosome III, which corresponds to 4.3% of the chromosome, and position 5,379,628 to 10,590,675 of chromosome X, which corresponds to 29.4% of the chromosome). However, neither the INBRED nor LOCO GWA analyses revealed significant peaks in these regions.

Results

Response to dilute 1-octanol varies among *C. elegans* wild strains

We previously reported a large range in sensitivity among hundreds of wild *C. elegans* strains to the ASH-detected aversive tastants quinine, copper, and SDS (Polk et al. 2025), and a small pilot study comparing 10 wild strains to N2 suggested that natural variation might also exist in response to the aversive odorant 1-octanol (Crombie et al. 2022). That study examined 100% 1-octanol, the concentration to which the N2 laboratory strain typically responds within 2 to 3 s. Therefore, in an effort to assess both decreased and increased response sensitivities to 1-octanol relative to N2, we diluted 1-octanol to 15%, the concentration to which N2 reliably responded within ~10 s, and assayed 156 wild strains obtained from the CaeNDR (Cook et al. 2017; Crombie et al. 2024). Within this wild strain set, all responded with either similar or diminished sensitivities compared to N2, with wild strain responses ranging from 8 to 19 s (Fig. 1, Supplementary File 1) and showing a normal distribution (Supplementary Fig. 1). Interestingly, we did not identify any hypersensitive wild strains here, in contrast to wild strain responses to quinine, copper and SDS, which ranged from hyposensitive to hypersensitive, with N2 at the approximate median of response sensitivity for those stimuli (Polk et al. 2025). These data reveal variation in responses to the aversive odorant 1-octanol, but with a lack of hypersensitivity among the strains tested.

Wild strain responses to dilute 1-octanol are moderately correlated with responses to quinine

We previously found only weak correlations between wild strain responses to quinine, copper, and SDS (Polk et al. 2025). However, these stimuli are thought to signal through distinct signal transduction cascades, with only quinine likely activating a GPCR pathway (Bargmann 2006; Ferkey et al. 2021). Although neither the quinine nor the 1-octanol receptors have been identified in *C. elegans*, response to 1-octanol is mediated by G protein-coupled signaling (Bargmann 2006; Ferkey et al. 2021). To determine if a wild strain's response to 1-octanol is correlated with its response to aversive tastants, each pairwise combination was plotted (Fig. 2). We found that 1-octanol and quinine responses were moderately correlated ($R^2 = 0.111$, $R = 0.33$), while 1-octanol response was only weakly correlated with responses to copper and SDS ($R < 0.16$ for each). This result suggests that the genetic factors contributing to variation in avoidance behaviors among wild strains are largely unique to each stimulus, although some commonalities might exist—especially for stimuli that signal through signal transduction pathways with shared components and/or regulatory molecules.

GWA analysis revealed 1 QTL for dilute 1-octanol response

To determine if genetic variation underlies the varied response sensitivity to 15% 1-octanol, we used the wild strain whole-genome variant data available from CaeNDR (20250625 release), in conjunction with the behavioral data, to perform GWA analyses. We performed both INBRED and LOCO association mappings using a subset of 71 strains to correct for population structure (Widmayer et al. 2022). The INBRED approach revealed

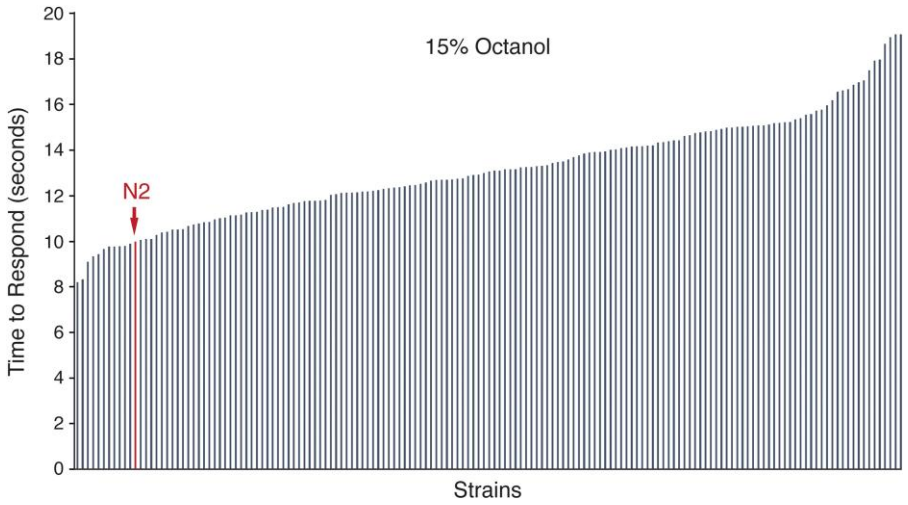

Fig. 1. Variation in *C. elegans* wild strain responses to 15% 1-octanol. A range of behavioral sensitivity was observed across the 156 wild strains when assayed for avoidance of the aversive odorant 1-octanol. The time to respond (seconds) is shown. Data represent the average of behavioral experiments performed in triplicate for each wild strain.

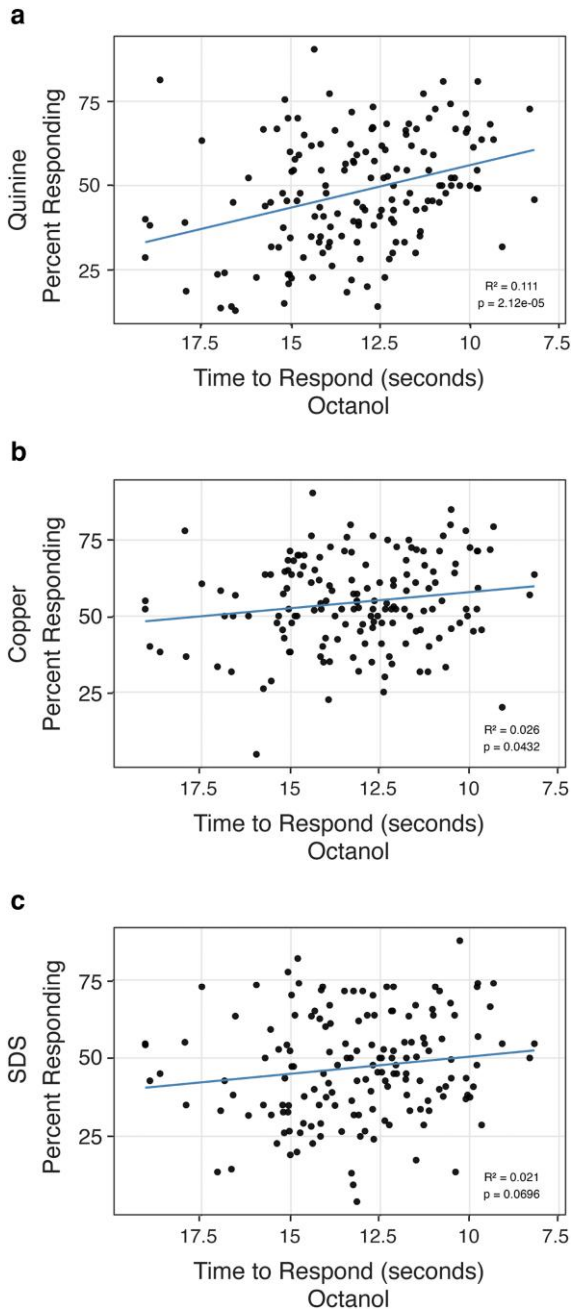

Fig. 2. Behavioral correlations between 15% 1-octanol and aversive tastants. Correlations of the 156 wild strain responses between 15% 1-octanol and 2.5 mM quinine (a), 15% 1-octanol and 0.5 mM copper (b), and 15% 1-octanol and 0.001% SDS (c) are shown. Each point represents an individual strain's response to 1 stimulus plotted along the *x* axis and its response to the second stimulus plotted along the *y* axis. 1-octanol responses are given as time to respond (seconds). Responses to the aversive tastants are plotted as percent responding. The line of best fit, along with the R^2 and *P*-values are shown for each comparison. We considered $R < 0.3$ as only weakly correlated (Cohen 1988).

a single highly significant QTL on the right arm of chromosome II (referred to as II-R, Fig. 3). This region also approached significance when the LOCO approach was used (Fig. 3).

To determine if the most significant single nucleotide variant (SNV; position 12,620,507) within the II-R QTL was associated with increased vs decreased behavioral sensitivity to 1-octanol, the responses of strains with the reference (REF) allele (ie same as N2) were compared to the responses of strains with the alternate (ALT) allele at this position (Supplementary Fig. 2). Because *C. elegans* is a self-fertilizing species, all SNVs are assumed to be homozygous in the wild strains maintained and distributed by CaeNDR (Frezal and Felix 2015; Cook et al. 2017; Crombie et al. 2024). Wild strains with the alternate SNV at this position displayed decreased behavioral sensitivity to 1-octanol (Supplementary Fig. 2). Taken together, we identified a single QTL on the right arm of chromosome II that is associated with decreased response sensitivity to 15% 1-octanol.

Behavioral responses of NILs to dilute 1-octanol

To validate the effect of the QTL II-R (Fig. 3) on differential 15% 1-octanol responses, NILs were created by crossing the laboratory strain (N2) to the wild strain WN2066, followed by subsequent rounds of self-fertilization by hermaphrodites (see Materials and methods for details). This approach generally leads to megabase-sized haplotype blocks containing hundreds of SNVs, making it challenging to identify individual causal nucleotide variants within a QTL (Andersen and Rockman 2022; Widmayer et al. 2022). We therefore tested the entire II-R QTL for its contribution to 1-octanol avoidance, rather than individual variants within this region (Fig. 4). When the wild strain QTL was crossed into the N2 genomic background (WN2066 > N2), diminished 1-octanol sensitivity was observed, with the NILs responding similarly to the WN2066 wild strain (Fig. 4). In this direction, the QTL explained 89% of the difference between N2 and WN2066. However, when the reciprocal NILs were analyzed, with the N2 region crossed into the wild strain background (N2 > WN2066), response sensitivity remained similar to WN2066 (Fig. 4). This difference suggests that, although variants within the WN2066 QTL region are sufficient to dampen the response sensitivity of N2 animals, additional variants outside of the QTL can also contribute to the diminished response sensitivity of this strain, and the N2 genetic sequence of this QTL is not sufficient to overcome them.

Discussion

In the wild, animals must detect potentially harmful cues and respond appropriately to survive. At its most basic level, this usually translates into stimulus avoidance. For *C. elegans*, the odorant 1-octanol is thought to indicate the presence of fungi (Kaminski et al. 1974). Several nematode-trapping fungi have been shown to prey on *C. elegans* in a laboratory setting, and predatory fungi and nematodes (including *C. elegans* and other *Caenorhabditis* species) have been found to co-exist in the wild (Vidal-Diez de Ulzurrun et al. 2024). Although the N2 strain of *C. elegans* was first shown to avoid 1-octanol in 1993 (Bargmann et al. 1993), only a few wild strains since that time have been tested for response to this aversive odorant (Crombie et al. 2022). Taking advantage of the wild strain collection and genomic resources available through CaeNDR (Cook et al. 2017; Evans et al. 2021; Andersen and Rockman 2022; Crombie et al. 2024), we performed a large-scale comparison of 1-octanol responses using genetically diverse strains isolated from varied habitats around the world.

We found a range of behavioral sensitivities to 15% 1-octanol, with time to respond ranging from 8 s (similar to N2) to 19 s (Fig. 1, Supplementary File 1). Assays were stopped at 20 s because animals spontaneously reverse ~3 times per minute as they explore their environment. Thus, this upper limit is typically considered completely defective. We did not identify any strains that responded significantly better than the N2 strain. This result is in contrast to wild strain responses to the aversive tastants quinine, copper, and SDS, which ranged from hyposensitive to

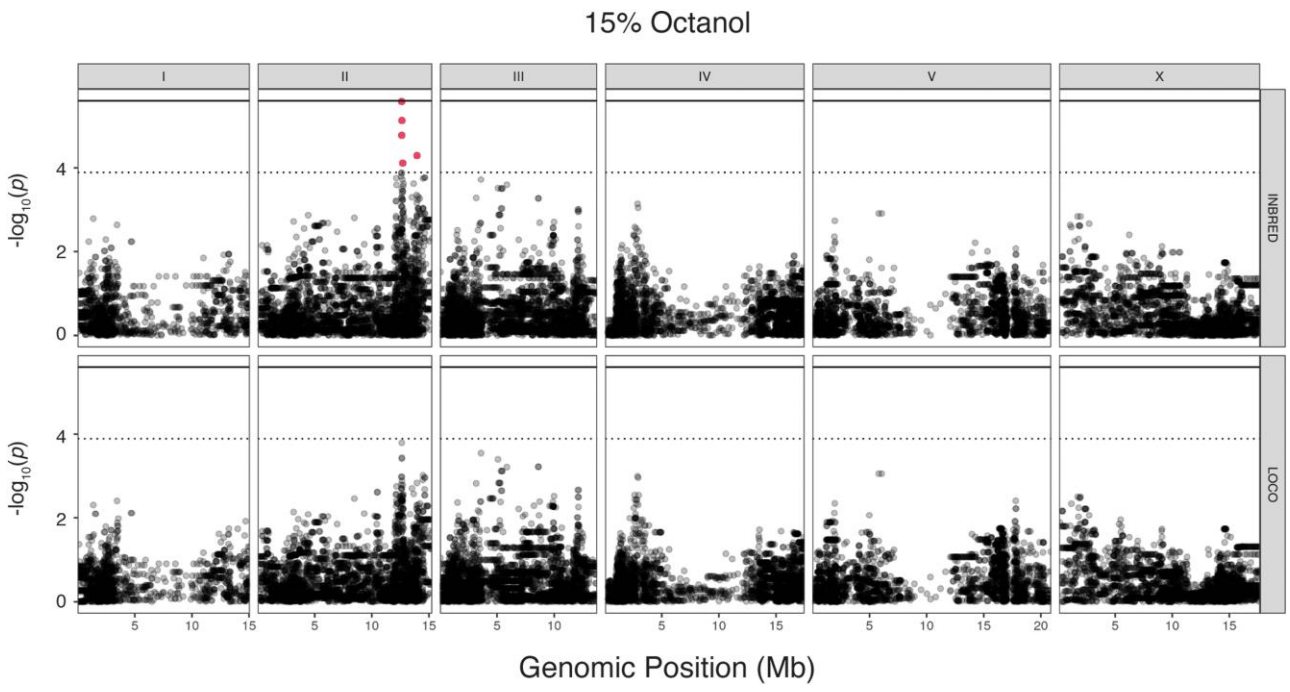

Fig. 3. Genome-wide associations (GWAs) for 15% 1-octanol response. A significant QTL was identified from the INBRED GWA mapping (71 strains, 1 Hawaiian) for 1-octanol avoidance that ranged from position 12,322,998 to 14,448,319 (peak position 12,620,507). A peak in the same region, but just below the significance threshold, was also identified from the LOCO association mapping. The x axis displays the chromosome position of the variants, and the y axis shows the significance of each variant. In each Manhattan plot, chromosomes I through V and X are shown. The horizontal dashed lines indicate the EIGEN threshold of significance and the horizontal solid lines correspond to the Bonferroni threshold in each plot (Zdraljevic et al. 2019). Each point represents 1 SNV, identified from the variant data from CaeNDR (20250625 release) (Cook et al. 2017; Crombie et al. 2024). Red points indicate significant SNVs.

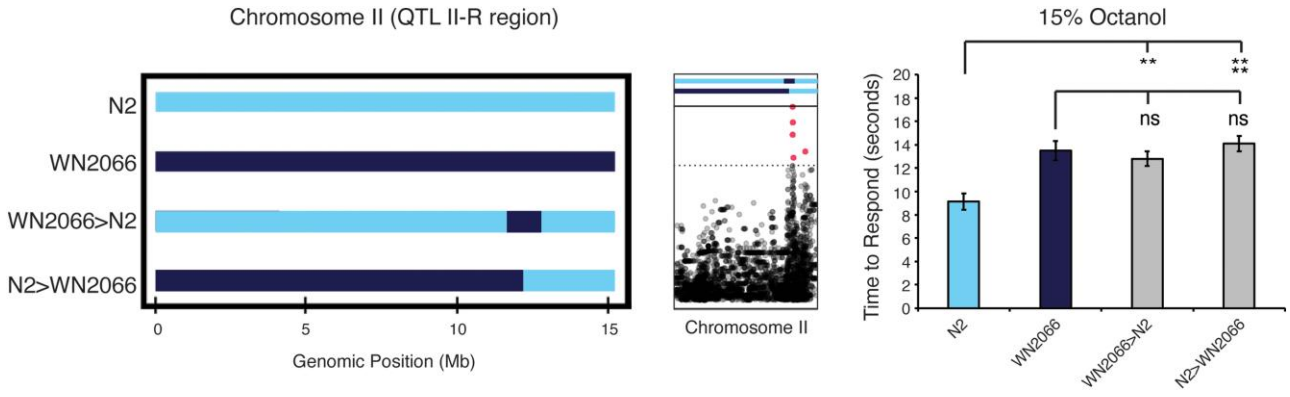

Fig. 4. Response of NILs to 15% 1-octanol. NILs were made for the significant 1-octanol response peak on the right arm of chromosome II (II-R). NILs were made such that some had the N2 genome background with the wild strain sequence in the QTL only, and the reciprocal strains containing the wild strain genome background with the N2 sequence in the same QTL. WN2066 > N2 indicates the wild strain QTL introgressed into the N2 genomic background. N2 > WN2066 indicates the N2 QTL introgressed into the wild strain genomic background. The size of QTL shown is based on only the shared genomic regions of 2 NIL strains' whole-genome sequence data, with N2 genomic sequence shown in light blue and wild strain sequence in dark blue. Only the genomic regions shared across NIL lines are indicated. See Materials and methods for additional information. The center panel shows the position of the NIL regions relative to the GWA peak. The corresponding behavioral responses of N2 (laboratory strain, light blue), the WN2066 wild strain (dark blue), and complementary NILs (light gray bars) are shown in the panel on the right. Each behavioral response bar shows the pooled data of 2 NILs and ≥ 120 animals tested over 3 d. The error bars indicate the standard error of the mean (SEM). Each NIL type was pooled (ie both lines with the wild strain QTL in the N2 background), and 1-way ANOVA with Tukey's Honestly Significant Difference (HSD) was performed for significance. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$. ns denotes $P \geq 0.05$ (not significant).

hypersensitive (Polk et al. 2025). Given that nociception is a protective behavioral response to potentially harmful stimuli, we had expected that some wild strains would be more sensitive to 1-octanol than the N2 strain. However, our data suggest that the laboratory strain might represent maximal sensitivity for this stimulus.

To determine whether 1-octanol responses correlated with the environments from which wild strains were isolated, we ran regression analyses using all available ecological variables annotated in CaeNDR and previous work (eg landscape, substrate, and geographic information) (Cook et al. 2017; Evans et al. 2017; Crombie et al. 2024). Similar to our previous work examining aversive tastants (Polk et al. 2025), we did not find any that strongly predicted behavioral response sensitivity to this odorant. However, detailed information about the composition of the

substrates from which the strains were isolated is not available, such as the presence of chemicals, bacteria or fungal species, or potential predators. Each of these has the potential to act as a selective pressure to shape the response profiles and fitness of wild strains over time. Consistent with this possibility, 12 genetically divergent *C. elegans* strains displayed differences in avoidance of the bacterial parasite *Serratia marcescens*, and strains with higher avoidance also had higher fitness in the laboratory setting, using population growth rate as the measure (Amoroso et al. 2024). Given that *C. elegans* is a self-fertilizing species, it is plausible that common haplotypes vary among populations, including variants that affect behavior, and could be adaptive within the local context of the niche.

To determine if *C. elegans* have a similar variability in their response to high concentrations of 1-octanol as they do to dilute (15%), and whether strains with diminished response to 15% 1-octanol might have increased sensitivity to a higher concentration, a small group of wild strains was also assayed for response to 100% 1-octanol. Wild strains were binned into 2-s categories based on their responses to 15% (Fig. 1, Supplementary File 1), and 2 to 3 strains were randomly selected from each bin to be representative of that bin. Of the 12 wild strains assayed, only one (ED3049) showed marked improvement in its response to 100% 1-octanol, and a few strains showed very modest improvement in their responses to 100% (Supplementary Fig. 3). However, most strains were similarly decreased in their responses to both dilute and concentrated 1-octanol. This result suggests that a strain's ability to respond to 1-octanol is generally not set by this aversive odorant's concentration simply crossing a threshold for detection.

To assess the impact of genetic variation on differential behavioral responses to 15% 1-octanol, we performed GWA mapping. Using a subset of the wild strains to minimize bias in population structure (Widmayer et al. 2022) and the resources available on CaeNDR, we identified 1 significant QTL on the right arm of chromosome II (II-R) (Fig. 3, Supplementary File 2). We validated the contribution of this genomic region to 1-octanol response differences by generating and testing NILs made between N2 and WN2066, a wild strain with a common alternative haplotype in the QTL represented by the most significant SNV. We found that when the wild strain genomic region was crossed into the N2 background, it was sufficient to decrease the behavioral sensitivity of the laboratory strain to a level comparable to that of the parent wild strain (Fig. 4). However, the reciprocal NILs, which had the N2 genomic region crossed into the wild strain background, did not display increased behavioral sensitivity (shorter time to respond). This difference could be caused by a background epistatic effect that only affects behavioral responses in 1 direction, such that the WN2066 allele(s) in the N2 background can cause the octanol response difference, but other WN2066 alleles outside of the genomic region impact the effect of the N2 allele(s). Alternatively, because of linkage disequilibrium and residual structure, the causal variant(s) might be to the left of where the significant peak marker was detected. Consistent with this possibility, we note that our WN2066 > N2 and N2 > WN2066 NILs are not exact reciprocals, with WN2066 > N2 extending further to the left.

Among the aversive stimuli that we examined previously (quinine, copper, SDS) (Polk et al. 2025) and here (1-octanol), only quinine and 1-octanol are thought to both signal via GPCR pathways (Ferkey et al. 2021). When comparing the responses of individual strains to these 2 stimuli, we found a moderate degree of correlation (Fig. 2). Interestingly, the QTL on II-R that we identified for 1-octanol (nucleotides 12,322,998 to 14,448,319, peak SNV at 12,620,507) overlaps with a QTL for quinine response (Polk et al. 2025) spanning nucleotides 13,455,815 to 14,582,528 (234 strains, CaeNDR 20250625 release). However, this region was not analyzed further for its contribution to quinine response because the QTL was represented by only a single significant SNV (nucleotide 13,906,945). We note that the previously identified QTL was associated with decreased response to quinine, and WN2066 (the wild strain used to create the NILs for 1-octanol response) did not have a diminished quinine response (Polk et al. 2025). In fact, WN2066 had a somewhat elevated response to quinine compared to the N2 strain (Polk et al. 2025). Consistent with this, WN2066 was not among the group of strains with the alternate (ALT) genotype within the QTL (Polk et al. 2025). Taken together, these data suggest that multiple unique changes on the right arm of chromosome II, likely targeting distinct genes, contribute to varied response sensitivity to 1-octanol and quinine, even though their signal transduction pathways share at least some common components and regulatory molecules (Ferkey et al. 2021).

In the verified 1-octanol response QTL on II-R, we identified 269 genes annotated in the N2 reference genome that contain variants in the WN2066 genome that could putatively affect function (Supplementary Table 1). This number does not include additional/alternate genes that might be absent in the reference genome. Genes encoding GPCRs that could potentially function as chemosensory receptors are highly represented (13%) in the QTL. However, 50% of the genes are from diverse categories, and 37% remain unnamed—obscuring predictions of their function. Additionally, variation in noncoding regulatory regions of genes could underlie evolutionarily important phenotypic variation (Ayyadevara et al. 2001, 2003; Vertino et al. 2011; Evans et al. 2021; Zhang et al. 2022). Further, association studies in humans (van Rheenen et al. 2019; Abdellaoui and Verweij 2021) suggest that changes in multiple genes likely account for differences in sensory sensitivity and response. Thus, changes in either the coding or noncoding regions of multiple genes within the QTL could contribute to differential 1-octanol sensitivity.

Underscoring the broad importance of olfaction to overall well-being, olfactory disfunction is present in at least 139 neurological, somatic, and hereditary disorders (Leon et al. 2024), with several studies suggesting that olfactory loss increases the risk of developing diverse medical conditions ranging from Parkinson's and Alzheimer's disease (Serby et al. 1991; Peters et al. 2003; Walker et al. 2021), schizophrenia (Kamath et al. 2012), and depression (Kamath et al. 2024), to major cardiac events (Chamberlin et al. 2024) and multiple sclerosis (Constantinescu et al. 1994). Olfactory ability also can predict the probability that older adults will later develop mild cognitive impairment (Wheeler and Murphy 2021) and a number of studies indicate that olfactory ability is a strong predictor for all-cause mortality up to 17 yr later (Leon et al. 2024). However, despite these findings, the sense of smell lags behind the senses of sight and hearing in perceived importance to public health (Boesveldt and Parma 2021; Philpott et al. 2025). Our work is important not only for understanding how naturally occurring genetic diversity influences how animals interact with their surroundings, but by identifying an olfactory sensitivity QTL, also will inform future studies aimed at identifying individual causal genes/variants that affect olfactory sensitivity in species ranging from invertebrates to humans.

Data availability

Plasmids and NIL strains are available upon request. All data necessary for confirming the conclusions of the article are present

within the article, figures, tables, and supplemental files. Supplementary File 1 is a list of strains and their origin, with Tab 2 denoting the strain subset used for GWA mapping. Supplementary File 2 is the INBRED GWA results with test statistics.

Supplemental material available at G3 online.

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Conflicts of interest

The authors declare no conflict of interest.

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