

Protocol for performing the chemotaxis assay developed in Tasnim et al., 2025.

Worm growth and selection

Growth, pre-exposure, and assay plates were prepared using appropriate volumes of Nematode Growth Medium (NGM): 4 ml per 35 mm plate, 20 ml per 100 mm plate and 8 ml per assay plate. NGM was prepared by combining 17 g bacteriological agar, 2.5 g bacto-peptone and 3 g NaCl in 1 L water, autoclaving, cooling to 60°C, then adding 1 ml each of cholesterol (5mg/ml in ethanol), 1M CaCl₂ and 1M MgSO₄, and 25 ml of potassium phosphate buffer (pH 6.0).

Growth plates were seeded with overnight cultures of *E. coli* OP50 grown at 37°C in Luria-Bertani (LB) broth (100 µl per 35 mm NGM plate and 500 µl per 100 mm NGM plate) and left at room temperature for ~48 hrs, by when a lawn forms. These seeded plates were stored for up to 4 weeks at 4°C until use. Worm washes and transfers were done using M9 buffer prepared by adding 5 g NaCl, 11.32 g dibasic Na₂HPO₄*7H₂O and 3 g KH₂PO₄ to 1 L water, autoclaving, and cooling to ~60°C before addition of 1 ml 1M MgSO₄. A worm bleaching solution (a stock mix of 45ml 5M NaOH, 15ml Clorox® bleach (8.25% NaOCl) and 90 ml water aliquots diluted to 50% before use) was used to dissolve worms while leaving the embryos protected by the eggshells. Subsequent hatching resulted in populations of L1-staged animals that were then moved to seeded plates and grown to adulthood before their behavior was assayed.

Staging

Plates were set up on day 1 by washing an unstarved but crowded 35 mm plate of worms with 1 ml M9 and transferring 10 µl of the suspension onto 12 OP50-seeded 35 mm plates. These plates were grown for 36-48 hrs at 20°C until large numbers of gravid adults were observed, then worms were bleached by dropwise addition of 250-300 µl of worm bleaching solution, ensuring that all worms were covered using the least volume of bleaching solution. Bleached plates were kept at 20°C for 24 hrs until the surviving eggs hatched, then L1 worms were pooled from 2 plates by washing with 500 µl M9 and transferred onto a seeded 100 mm plate. These worms were then allowed to grow for an additional 72 hrs (total 96 hrs post-bleach) to get a synchronized cohort of young adult worms which were then pre-exposed and tested for their response to volatile odorant(s). Six 100mm plates of young adult worms were sufficient to pre-expose with vehicle and to measure both dispersal and chemotaxis in 8 arenas each.

Pre-exposure

M9 buffer (5-6 ml) was used to pool worms from six 100 mm plates into 1.5 ml microcentrifuge tubes and washed a total of 3 times by adding 1 ml of M9 and centrifuging at 11,000 rpm for 2 min. This use of centrifugation is different from the gravity-aided settling that was used in some assays (e.g., Kauffman et al., 2011) [We estimated gravity-aided settling until the solution above the worm 'pellet' is clear to take >7 min]. At the end of the third wash, the supernatant was discarded, retaining 200 µl of liquid with worms and one such tube of worms was used per 100 mm plate during pre-exposure. A P1000 pipette was used to transfer worms onto 100 mm plates without *E. coli* OP50 food, taking care not to transfer any bacterial pellets and pipetting from the top of the worm "pellet" so as not to transfer any worm carcasses from the bottom of the tube. 5 µl of ethanol was streaked onto the lid of the plate and the covered plate was then sealed with parafilm. Plates were kept undisturbed at room temperature for 1h, then pre-exposed worms were collected and washed three times using M9 buffer. At the end of the third wash the supernatant was discarded, retaining ~200µl of liquid with worms.

Chemotaxis and dispersal assays

Ethanol was used as a vehicle and fresh odorant dilution (10% 2-butanone) was made on the day of the behavioral assay in 1.5 ml microcentrifuge tubes. Arenas were set up as described (Figure 1A in Tasnim et al., 2025) and for each test (dispersal or chemotaxis), 2 sets of 4 rectangular arenas were used per pre-exposure treatment (N=8). A template was used to trace quadrants onto extra lids of plates and these lids were placed under each set of 4 arenas to aid transfer of worms to the origins to initiate each assay. A P20 pipette with the tip cut off to increase the bore was used to transfer 10 µl of worms from the top of the worm "pellet" onto the center

(origin) of each rectangular plate, minimizing transfer of worm carcasses or remnant bacteria from the bottom of the tube. Typically, one tube of pre-exposed worms yielded enough for testing >100 worms per plate on 4 sets of 4 rectangular arenas (N=16). After transferring worms, 2.5 μ l of the odorant in vehicle or the vehicle alone was pipetted at either end of the chemotaxis plates in opposite ends such that the worms in one set of arenas must move in the opposite direction to worms in the other set for the same response. With this orientation, if worms were responding to a gradient of an unknown cue outside the arena, then they will move in the same direction in both arenas, thereby reducing effect size in response to the odorant within the arena. The dispersal plates did not have any odorant added to them (Figure 1A in Tasnim et al., 2025). The transfer of worms and odorants/vehicle typically took a total of 5 min per 4 set of 4 rectangular arenas (N=16). The plates were left with the lids on at room temperature for 1 h. At the end of 1 h, videos were taken of the plates keeping the extra lid underneath to allow visualization of the quadrants, and the videos were used to count the numbers of young adult worms in each quadrant. The video was taken using an iPhone positioned over the objective using an adapter and typically took 5 min per 4 set of 4 rectangular arenas (N=16).

References

1. Tasnim, S., Liu, A., Jose, A. M. (2025) A simple yet reliable assay for odorant sensing in *C. elegans*. *microPublication Biology*, In submission.
2. Kauffman, A., Parsons, L., Stein, G., Wills, A., Kaletsky, R., Murphy, C. (2011) *C. elegans* Positive Butanone Learning, Short-term, and Long-term Associative Memory Assays. *J. Vis. Exp.* (49), e2490.