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The \textit{C. elegans} Touch Response Facilitates Escape from Predacious Fungi

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Summary

Predator-prey interactions are vital determinants in the natural selection of behavioral traits. Gentle touch to the anterior half of the body of \textit{Caenorhabditis elegans} elicits an escape response in which the animal quickly reverses and suppresses exploratory head movements \cite{1, 2}. Here, we investigate the ecological significance of the touch response in predator-prey interactions between \textit{C. elegans} and predacious fungi that catch nematodes using constricting hyphal rings. We show that the constricting rings of \textit{Drechslerella doedycoides} catch early larval stages with a diameter similar to the trap opening. There is a delay between the ring entry and ring closure, which allows the animal to withdraw from the trap before being caught. Mutants that fail to suppress head movements in response to touch are caught more efficiently than the wild-type. This demonstrates that the coordination of motor programs to touch are caught more efficiently than the wild-type. Our results suggest that selective pressures imposed by predacious fungi have shaped the evolution of \textit{C. elegans} escape behavior.

Results and Discussion

Escape behaviors increase the prey’s odds of surviving an encounter with a predator and have been extensively studied from an ethological, neurophysiological, and behavioral genetic perspective \cite{3–5}. However, the dichotomy between field and laboratory studies makes it difficult to unify investigations of both proximate and ultimate causes of behavior. Furthermore, because phenotypic selection is usually measured in natural populations, our current understanding of genotypic selection is largely correlative. Unraveling of causative relationships between phenotypic and genotypic selection requires the ability to experimentally manipulate behavioral traits. The ability to genetically manipulate behavioral traits and the defined neural architecture of the nematode \textit{Caenorhabditis elegans} provides a unique opportunity for a comprehensive neuroethological analysis of behavior \cite{6}.

\textit{C. elegans} moves on its side by propagating a sinusoidal wave of body wall muscle contractions along the length of its body. Locomotion is accompanied by exploratory head movements, in which the tip of the nose moves rapidly from side to side (Figure 1A) \cite{7}. Gentle touch elicits an escape response in which \textit{C. elegans} quickly moves away from the stimulus \cite{1}. The animal backs up in response to light touch on the nose or anterior half of the body, whereas it quickly moves forward when touched on the posterior half. Exploratory head movements continue in response to nose or tail touch but are suppressed during backing when the animal is touched on the anterior region of its body, between the pharyngeal bulb and the midbody (see Figure S1 available online) \cite{2}. The \textit{C. elegans} touch response has provided one of the few examples for which the entire sensorimotor circuit has been defined \cite{1, 2, 8}. However, the ecological significance of the touch response is unclear.

Why would \textit{C. elegans} specifically suppress exploratory head movements in response to touch on the anterior region of its body, but not when it is touched on the nose or the tail end? In soil and decaying organic material, nematophagous fungi prey on nematodes as their main food source \cite{9–11}. Some of these fungi have developed distinctive trapping devices along their hyphae to catch nematodes. Trapping structures include adhesive nets, knobs or branches, and constricting and nonconstricting rings. Predatory fungi that use rings as a trapping mechanism have been found along with nematodes in 100-million-year-old amber \cite{12}, indicating a long evolutionary predator-prey relationship. The constricting rings used by fungi such as \textit{Drechslerella doedycoides} are composed of three cells that form a closed ring at the end of a short hyphal branch (Figures 1F–1H). When a nematode passes through the ring, gentle friction induces the cells of the ring to rapidly inflate inwards and catch the nematode (Movie S1). Once a nematode is captured, hyphae penetrate the cuticle and digest the worm.

To determine whether the nematode’s touch response is important to avoid predation, we analyzed predator-prey relationships between fungi that use constricting rings and \textit{C. elegans}. The average inner diameter of constricting rings of the different fungi ranged from 10 \textmu m to 25 \textmu m (Figures 1B and 1G). The diameter of \textit{C. elegans} increases throughout development, from approximately 12 \textmu m shortly after hatching to 50 \textmu m in the young adult (Figure 1C). To determine which life stages were caught by fungal traps, we inoculated agar plates that contained the fungus \textit{D. doedycoides} with different developmental stages of \textit{C. elegans} (Figure 1D). L1 larvae were small enough to pass through constricting rings, often without setting off the trap. Because \textit{C. elegans} is tapered on either end, the slightly larger L2 and L3 larvae could enter a ring until the body diameter was too large to continue forward movement. L4 larvae and adult animals were too large to enter the ring during forward movement and usually swept the rings aside with foraging head movements. However, because the tail of \textit{C. elegans} tapers more narrowly than the head region, larger animals could get caught while reversing into a ring. Whereas adult animals were rarely caught, the majority of L2 larvae were caught within 4 hr of inoculation. Most animals were caught at the anterior half of the body (Figure 1E), indicating that animals with a diameter similar to or slightly larger than the trap aperture are caught most efficiently. Because both \textit{C. elegans} \cite{13} and nematophagous fungi \cite{14} have been found worldwide in decaying organic matter, it is likely that \textit{C. elegans} faces fungal predation in its natural habitat.

Can \textit{C. elegans} escape from a constricting hyphal ring? Although the inflation of the ring cells occurs very quickly
once initiated (approximately 0.1 s) [15], there is a slight delay between an animal entering a trap and trap closure (Figure 2A). We found that on average, the latency between initial contact of the worm with the inside of the ring until inflation occurred was 5 s, long enough to allow nematodes to enter and withdraw from the ring and escape without setting off the trap (Figure 2B). We monitored individual trap encounters and found that the large majority of wild-type animals that entered a ring managed to exit the ring without getting caught (81% ± 4%). Occasionally, animals set off the fungal trap but still managed to withdraw and escape the constricting ring before it inflated (Figure 2C; Movie S2). Much like the anterior touch response elicited by a hair, the tightening noose around the worm’s neck induced a quick reversal and the suppression of exploratory head movements. To determine whether mechanosensory perception of the fungal hyphae increases the animal’s chance of escaping from constricting rings, we analyzed trap encounters of touchInsensitive mec-4 mutant animals. The MEC-4 DEG/ENaC channel is expressed in the touch sensory neurons and is required for mechanotransduction [16]. Because mec-4(u253) complete loss-of-function animals are lethargic, we analyzed mec-4(e1339) partial loss-of-function mutants, which have a strong reduction in the anterior touch withdrawal response [17]. The fraction of
mec-4(e1339) mutant animals that managed to escape from traps was drastically reduced (43% ± 8%) compared to the wild-type (Figure 2B). osm-9(n1603) mutants are nose touch defective [18, 19] but did reverse and suppress head movements in response to anterior touch. Once osm-9(n1603) mutant animals entered a trap, they escaped as frequently (81% ± 6%) as the wild-type. Thus, anterior touch, but not nose touch, sensation is crucial for C. elegans’ ability to escape from constricting rings. Does the suppression of head movements increase the animal’s ability to escape from predacious fungi? We have previously shown that the biogenic amine tyramine is required to coordinate head and body movements in the C. elegans anterior touch response [8]. The anterior touch sensory neurons activate backward locomotion command neurons, which are electrically coupled to the tyraminergic neurons. Tyramine release inhibits forward locomotion as well as exploratory head movements through the activation of the tyramine-gated chloride channel LGC-55 [20, 21]. Like the wild-type, tyramine-deficient tdc-1(n3420) and lgc-55(tm2913) mutants reverse normally in response to touch. However, tdc-1 and lgc-55 mutant animals back up slightly less far than the wild-type and fail to suppress exploratory head movements during these reversals. We found that tdc-1 and lgc-55 mutants initiated reversals when wedged in a constricting ring but escaped less frequently than the wild-type (Figure 2B; tdc-1, 49% ± 10%; lgc-55, 60% ± 6%). unc-4(e120) mutants can move forward and suppress head movements in response to touch but have severe defects in backward locomotion [22]. unc-4(e120) mutant animals escaped less frequently (59% ± 7%) than the wild-type, indicating that the coordination of a reversal with the suppression of head movements is required for an efficient escape. To test whether increased capture of tyramine signaling mutants is caused by increased attraction to fungi or by a defect in the motor program used to extricate from noose-like structures, we mimicked C. elegans trap encounters with a nylon mesh. We compared how young adult wild-type and lgc-55 mutant animals crawled through a narrow opening of a nylon fence (Figure 3). Young adult animals can pass through these openings, but this requires body contact with the threads of the mesh. lgc-55 mutant animals are indistinguishable from the wild-type with respect to developmental timing, size, locomotion rate, locomotion pattern, touch sensitivity, and exploratory behavior (Figures S2 and S3). We found that once they entered the mesh, lgc-55 mutants took more than
that were not caught were washed off the plate, and the numbers of captured GFP-positive and GFP-negative animals were counted. The capture index was calculated by subtracting the fraction of the caught tested genotype from the fraction of the caught control animals. A positive capture index indicates a selective disadvantage compared to the wild-type strain (Figure 4). In these competition experiments, a larger fraction of lgc-55 mutants was caught than the wild-type (capture index 0.36 ± 0.02). Expression of wild-type copies of lgc-55 under its endogenous promoter completely rescued the selective disadvantage of the lgc-55 mutants (capture index 0.02 ± 0.02). Our previous studies have shown that lgc-55 is expressed in neck muscles and neurons that receive presynaptic inputs from the tyraminergic RIM neurons (Figure S1) [20]. lgc-55 expression in neck muscles is required to suppress head movements, and expression in the AVB forward locomotion command neurons is required to suppress forward locomotion, which leads to longer reversals in response to touch. Whereas the suppression of head movements may allow a smooth retraction, long reversals may also facilitate escapes once the animal has moved far into the ring. To determine whether the suppression of head movements alone aids in the escape, we performed competition assays with lgc-55 mutant animals in which wild-type copies of lgc-55 were expressed in muscles using a Pmyo-3::LGC-55 transgene. lgc-55; Pmyo-3::LGC-55 animals are not rescued for reversal defects but do suppress their head movements in response to anterior touch [20]. We found that the capture index of lgc-55 mutants in which only the defect in the suppression of head movements was rescued was significantly lower than that of the original mutant strain (capture index 0.12 ± 0.02). This demonstrates that the suppression of head movements in response to anterior touch increases the animal’s chance of smoothly reversing without getting caught in the deadly noose (Figure 4B). Therefore, the coordination of motor programs that control head movements with locomotion is of vital importance for C. elegans to evade predation in the wild.

The suppression of head movements in response to anterior touch is conserved in related Caenorhabditis species (data not shown). Moreover, phylogenetic analyses of predacious fungi and morphology of fungal trapping devices suggest

Figure 3. Tyramine Signaling Facilitates Extraction from a Noose
(A) Young adult animals moving through a nylon mesh. Animals make contact with the threads of the mesh when they pass through the 37 μm openings.

(B) The time that animals spent in a 37 μm opening of the nylon mesh before exiting: wild-type (N2), 9 ± 1 s (n = 156); lgc-55(tm2913), 23 ± 4 s (n = 274), p = 0.0059; Plgc-55::LGC-55, 10 ± 1 s (n = 418), p = 0.1675. Error bars represent SEM. **p < 0.005 versus wild-type by two-tailed Student’s t test.

Twice as long to extricate themselves as did wild-type animals (WT, 9 ± 1 s; lgc-55, 23 ± 4 s). A transgenic lgc-55 rescuing construct restored the behavior of lgc-55 mutants back to that of the wild-type (P(lgc-55::LGC-55, 10 ± 1 s). Thus, our findings indicate that the tyraminergic coordination of backward locomotion with the suppression of head movements facilitates retraction from a narrow ring.

To directly test whether the suppression of head movements provides a selective advantage, we performed a competition experiment. We inoculated D. doedycoides cultures with a mix of wild-type and lgc-55 mutant L2 larvae (Figure S4). We used a transcriptional Plgc-55::GFP reporter to label one of the strains in the selection experiments. The green fluorescent protein (GFP) marker did not affect capture. A mix of equal amounts of staged L2 larvae of each genotype was transferred onto the hyphal mats of D. doedycoides. After 90 min, animals caught in the deadly noose (Figure 4B). Therefore, the coordination of motor programs that control head movements with locomotion is of vital importance for C. elegans to evade predation in the wild.

The suppression of head movements in response to anterior touch is conserved in related Caenorhabditis species (data not shown). Moreover, phylogenetic analyses of predacious fungi and morphology of fungal trapping devices suggest

Figure 4. Touch-Induced Suppression of Head Movements Facilitates Escape from Fungal Constricting Rings
(A) Animals that fail to suppress head movements are caught more often than the wild-type in competition experiments. Genotypes were mixed in a 1:1 ratio, and the caught fraction of each genotype was determined. Fluorescent reporters were used to mark the different genotypes. The capture index was calculated by subtracting the weighted fractions of the caught testing genotype from that of the control. A capture index of 0 represents an equal distribution of animals caught between the testing genotype and the wild-type. A positive capture index indicates a selective disadvantage compared to the wild-type. Wild-type (N2), 0.01 ± 0.02 (n = 14); lgc-55(tm2913), 0.36 ± 0.02 (n = 17); lgc-55; Plgc-55::LGC-55 rescue, 0.02 ± 0.02 (n = 12); lgc-55; Pmyo-3::LGC-55 rescue, 0.12 ± 0.02 (n = 17). Error bars represent SEM. ***p < 0.0001 for indicated comparisons by two-tailed Student’s t test. (See also Figure S4.)

(B) C. elegans locomotion is accompanied by exploratory head movements. When an animal wedges itself into a constricting ring, activation of the anterior touch sensory neurons induces an escape response. Wild-type animals reverse and suppress exploratory head movements, allowing a smooth exit from the constricting ring. The tyramine signaling mutants tdc-1(n3420) and lgc-55(tm2913) reverse but fail to suppress head movements during an escape, making it more likely that the animal will activate the ring cells and be caught.
that constricting rings might have evolved from nonconstricting rings [23]. This raises the intriguing possibility that the touch-induced suppression of head movements may be the consequence of an evolutionary arms race between predacious fungi and nematodes. Further characterization of the habitat of soil nematodes and predacious fungi will be key to our understanding of the selection pressures that shape behavioral adaptation. Because we know the molecular and neural underpinnings of the \textit{C. elegans} escape response in exquisite detail, comparative studies of soil nematodes provide a powerful model to study the mechanisms that underlie the evolution of escape behaviors.

**Experimental Procedures**

\textit{C. elegans} strains were grown under standard conditions. Nematophagous fungi were maintained on malt extract agar and subcultured on water agar plates to induce trap formation. Behavioral and capture assays were performed at room temperature. Different genotypes were scored in parallel.

To compare the capture rates of different developmental stages, we transferred staged animals to fungal plates with approximately 40 constricting rings per mm$^2$. After 4 hr, the numbers of caught and uncaught animals were counted. Diameter of uninflated rings, diameter of nematodes, and relative trap position were measured from still images. To score the outcome of individual trap encounters, we transferred animals to fungal plates and video recorded them. An encounter with a trap was defined as any time a worm entered a trap with the anterior portion of its body. Each encounter was scored as either a capture or an escape. In encounters that resulted in a capture, the latency from the time of trap entry until trap closure was measured. An exit from a trap by reversing qualified as an escape. To mimic trap encounters, we inserted a nylon mesh with 37 μm openings perpendicularly into the agar plate. The time spent in mesh was measured from the first entry of the animal’s nose to the time when the animal completely extricated itself.

For the competition assays, equal amounts of staged L2 larvae of the different genotypes were mixed in M9 buffer and transferred to plates with \textit{D. doedycoides} and agar plates with \textit{E. coli} (OP50). After 90 min, uncaught L2 larvae were gently washed off the plate and caught. GFP-positive and -negative larvae were counted with a fluorescence dissection microscope. The fraction of caught animals was weighted according to the fraction of each genotype in the mixed populations on agar plates without fungi. Capture index was calculated by subtracting the weighted fraction of the test genotype from the weighted fraction of the GFP-marked wild-type strain. See Supplemental Experimental Procedures for additional information.

**Supplemental Information**

Supplemental Information includes four figures, Supplemental Experimental Procedures, and two movies and can be found with this article online at doi:10.1016/j.cub.2011.06.063.

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