

The olfactory pathway mediates sheltering behavior of Caribbean spiny lobsters, *Panulirus argus* to conspecific urine signals

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Abstract The “noses” of diverse taxa are organized into different subsystems whose functions are often not well understood. The “nose” of decapod crustaceans is organized into two parallel pathways that originate in different populations of antennular sensilla and project to specific neuropils in the brain—the aesthetasc/olfactory lobe pathway and the non-aesthetasc/lateral antennular neuropil pathway. In this study, we investigated the role of these pathways in mediating shelter selection of Caribbean spiny lobsters, *Panulirus argus*, in response to conspecific urine signals. We compared the behavior of ablated animals and intact controls. Our results show that control and non-aesthetasc ablated lobsters have a significant overall preference for shelters emanating urine over control shelters. Thus the non-aesthetasc pathway does not play a critical role in shelter selection. In contrast, spiny lobsters with aesthetascs ablated did not show a preference for either shelter, suggesting that the aesthetasc/olfactory pathway is important for processing social odors. Our results show a difference in the function of these dual chemosensory pathways in responding to social cues, with the aesthetasc/olfactory lobe pathway playing a major role. We discuss our results in the context of why the noses of many animals contain multiple parallel chemosensory systems.

Keywords Aggregation · Urine · Chemical signals · Crustacea · Flume

Introduction

Chemical stimuli play vital roles in many types of animal behavior. The evolution of chemosensory systems has allowed animals to effectively detect and process these stimuli. Chemosensory systems show great organizational complexity. For instance, the rostral chemosensory organs (“noses”) of many animals are organized into multiple, anatomically distinct neuronal pathways with different peripheral sensors and different central processing centers. Partitioning noses in this way suggests that different chemosensory pathways fulfill different functional requirements for odor processing or for driving behaviors. In some organisms, this supposition is well supported. Several species of male insects have anatomically separate main and accessory olfactory systems that detect and mediate the behavioral response to general host plant odors and female sex pheromones, respectively (Hansson 1995; Hildebrand 1995; Christensen and White 2000; Hansson and Anton 2000; Christensen and Hildebrand 2002). In other arthropods and vertebrates, the functional significance of anatomically separate chemosensory pathways is less well understood. The noses of many amphibians, reptiles, and mammals also contain multiple anatomically separate chemosensory pathways, including the main olfactory system, the vomeronasal system, septal organ and/or Grueneberg ganglion (Baxi et al. 2006; Breer et al. 2006; Brennan and Zufall 2006; Breer et al. 2006; Spehr et al. 2006; Storan and Key 2006), though the functional divisions between these odor processing pathways are not always clear (Hudson and Distel 1986; Dorries et al. 1997; Eisthen 1997; Halpern

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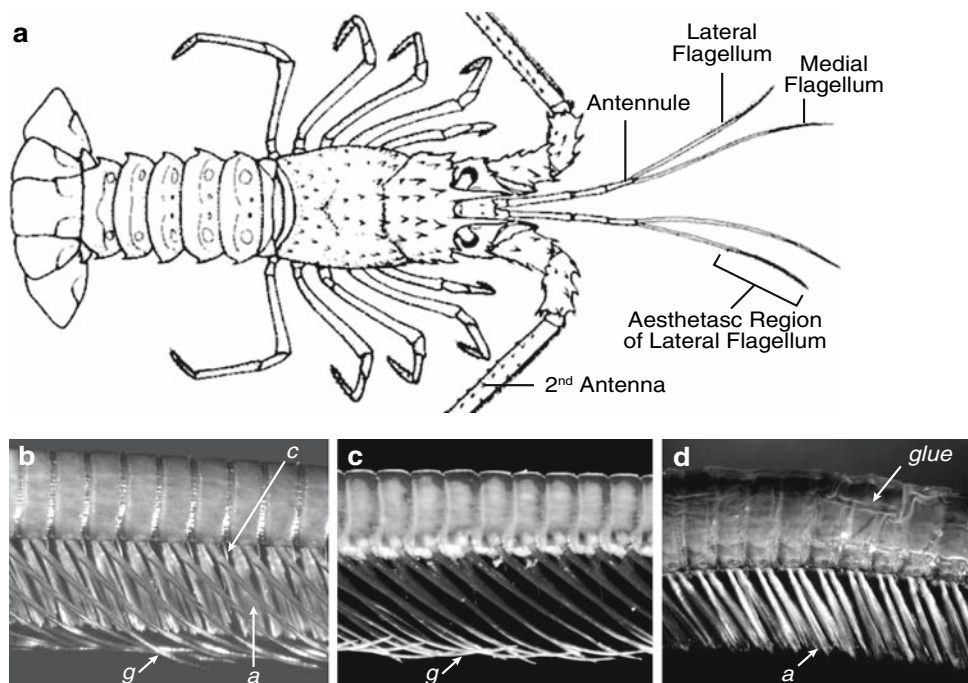
et al. 1997; Johnston 1998, 2000; Miller and Gutzke 1999; Christensen and White 2000; Ptacyk and Graves 2002; Halpern and Martínez-Marcos 2003; Restrepo et al. 2004; Lin et al. 2005; Baxi et al. 2006; Brennan and Zufall 2006; Spehr et al. 2006).

Examinations of how multiple parallel systems function in odor-mediated behaviors can be challenging, including in mammalian systems where chemosensory organs can be difficult to access. Decapod crustaceans are valuable model systems for understanding the functional organization of chemosensory systems (Ache 2002; Caprio and Derby 2007). Similar to insects and vertebrates, the noses (antennules) of decapod crustaceans, including the Caribbean spiny lobster *Panulirus argus*, which we use in this study, contain multiple anatomically distinct chemosensory pathways: the aesthetasc/olfactory lobe pathway and the non-aesthetasc/lateral antennular neuropil pathway. The aesthetasc/olfactory lobe pathway is a purely chemosensory pathway that originates in the prominent aesthetasc sensilla located in the distal region of the lateral flagella (Fig. 1a, b). Aesthetascs are innervated only by olfactory receptor neurons whose axons target the paired olfactory lobes, which show the typical glomerular organization that characterizes the first order olfactory processing centers of both vertebrates and insects (Laverack 1964; Laverack and Ardill 1965; Grünert and Ache 1988; Schmidt and Ache 1992, 1996b; Hildebrand and Shepherd 1997; Christensen and White 2000; Steullet et al. 2000; Eisthen 2002; Ache and Young 2005; Schachtner et al. 2005). The non-aesthetasc/lateral antennular neuropil pathway is a multimodal pathway that originates in a diverse

group of sensilla on the antennular flagella (Fig. 1a, b) that are collectively called “non-aesthetascs” (Schmidt et al. 1992; Schmidt and Ache 1996a). Non-aesthetasc sensilla are innervated by both mechanosensory neurons and chemosensory neurons whose axons target the paired lateral antennular neuropils, which lack glomeruli, have a stratified organization, contain arborizations of antennular motoneurons, and are sensory-motor integration centers (Schmidt et al. 1992; Schmidt and Ache 1993, 1996a; Cate and Derby 2001, 2002; Schmidt and Derby 2005). The functional significance of this parallel organization in decapod crustaceans is poorly understood. Only one study has demonstrated a unique role for the non-aesthetasc pathway—activation of a sensory-motor antennular behavior (Schmidt and Derby 2005). Only three studies have conclusively demonstrated unique functions for the aesthetasc pathway in decapod crustaceans—detecting pheromones (Gleeson 1980; Johnson and Atema 2005; Horner et al. 2008).

Caribbean spiny lobsters aggregate to chemical cues released from conspecifics (Herrnkind et al. 1975; Kanciruk 1980; Childress and Herrnkind 1997; Nevitt et al. 2000; Ratchford and Eggleston 2000). Caribbean spiny lobsters associate preferentially with shelters scented with conspecific urine over shelters scented with seawater, food odors or predator odors, demonstrating that urine is an aggregation pheromone (Horner et al. 2006). The goal of the current study is to use this urine-based signal to explore potential functional differences between the aesthetasc and non-aesthetasc chemosensory pathways in mediating the behavioral response to aggregation pheromones.

Fig. 1 Spiny lobster and antennular sensilla. **a** Diagram of *P. argus* showing the major components of the chemosensory system. **b** Light micrograph of a portion of the aesthetasc tuft region of the lateral flagellum from a control lobster. Aesthetasc (*a*), guard (*g*), and companion (*c*) sensilla are visible. **c** Light micrograph of a portion of the aesthetasc tuft region from an aesthetasc-ablated lobster. Guard and companion sensilla are intact, but aesthetascs have been removed. **d** Light micrograph of a portion of the aesthetasc tuft region of a non-aesthetasc ablated lobster. Aesthetascs (*a*) are intact, but non-aesthetascs have been removed. The cyanoacrylate glue coating is also visible



Materials and methods

Animals

Caribbean spiny lobsters, *P. argus* (Latreille, 1804), with carapace length of 59.0 ± 1.2 mm (mean \pm SEM, $N = 53$) were collected in the Florida Keys, shipped to Georgia State University, and held in 800-L aquaria containing aerated, recirculated, filtered artificial seawater (Instant Ocean[®], Aquarium Systems, Mentor, OH, USA). Animals used in our study were both males and females, and females were neither gravid nor containing spermatophores. Animals were maintained on a 12 h:12 h light:dark cycle and fed shrimp or squid three times per week. Intermolt lobsters were randomly selected for use in the experimental trials, and at least 2 days before being tested in the behavioral assay, experimental animals were transported to holding aquaria (0.90 m long \times 0.58 m wide \times 0.67 m tall) at Georgia Institute of Technology, where they were maintained throughout the course of the experiments.

Ablations

We conducted two sets of experiments to examine separately the importance of the aesthetasc or non-aesthetasc chemosensory pathway for mediating shelter selection in spiny lobsters. All ablations were performed on non-anesthetized spiny lobsters immobilized on a plastic restraining device within a shallow container of artificial seawater. Surgical ablations were performed several days in advance of the start of experimental trials, and chemical ablations (using deionized water) were performed within 12 h of the start of each trial. Within each ablation experiment, we randomized the order of testing of ablated and control animals.

Aesthetasc ablation. All aesthetasc sensilla on both lateral flagella were surgically removed using a hand-tooled narrow blade of 0.2-mm width. Removal of aesthetascs in this manner eliminates the outer dendrites of the olfactory sensory neurons, which results first in unresponsiveness to odors, and ultimately in the death and degeneration of the sensory neurons (Harrison et al. 2004). Ablated antennules were excised at the conclusion of each series of experimental trials, and the efficacy of ablation was evaluated using light microscopy to count the number of sensilla that remained intact (Fig. 1c). This analysis confirmed that shaving was a highly reliable method for removing sensilla. Shaving removed greater than 99.9% of aesthetascs. Some non-aesthetasc sensilla, particularly asymmetric, guard, and companion sensilla, were unintentionally removed during the aesthetasc shaving procedure. However, at least 30% of the asymmetric sensilla, 99% of guard sensilla and 97% of companion sensilla were still present on the antennules after the aesthetasc ablation procedure. Control animals for

the aesthetascs ablated group were immobilized in the plastic restraining device in the same manner and for the same duration as the aesthetasc ablated animals, but no sensilla were removed.

Non-aesthetasc chemoreceptor ablation. The non-aesthetasc ablation was a two-step process that was designed specifically to eliminate the function of non-aesthetasc chemosensory neurons while preserving as much as possible the function of non-aesthetasc mechanosensory neurons. In the first stage of the ablation, the lobsters were restrained and all visible non-aesthetasc sensilla located on annuli within the aesthetasc tuft region of both lateral flagella were surgically ablated. This shaved region was then coated with a thin layer of cyanoacrylate glue (Super Glue Corp., Rancho Cucamonga, CA, USA) to prevent stimulus access to any remaining non-aesthetasc sensilla. Covering the antennules with cyanoacrylate glue effectively prevents stimulation of non-aesthetasc chemosensory neurons and mechanosensory neurons that are responsive to hydrodynamic and tactile stimuli (Derby and Atema 1982). In the second stage of the ablation, the lobsters were restrained a second time and the unglued portions of the antennules (medial flagella and proximal regions of the lateral flagella) were ablated chemically by immersion in deionized water for 15 min. The glued portion of the antennule was maintained in artificial seawater during the deionized water ablation to prevent damage to the aesthetasc sensilla. Deionized water functionally inactivates the chemosensory neurons of marine crustaceans by disrupting the osmotic balance of the outer dendrites (Derby and Atema 1982; Gleeson et al. 1997). Some mechanosensory neurons with dendrites projecting up the length of the sensillum may have been exposed to, and inactivated by, the deionized water environment (Derby and Atema 1982; Garm et al. 2003), but mechanosensory neurons lacking this morphology were probably not affected. Thus, many mechanosensors along the medial flagella and proximal portions of the lateral flagella likely remained intact and functional. The deionized water ablation is temporary and reversible, lasting only about a day before the neurons once again respond to chemical stimuli (Derby and Atema 1982; Steullet et al. 2001), and thus was performed within 12 h of the start of each trial. The efficacy of the non-aesthetasc ablation in the aesthetasc tuft region and the condition of the aesthetascs was assessed with light microscopy at the conclusion of the experiment (Fig. 1d). Overall, the aesthetascs remained in excellent condition, and very few non-aesthetasc sensilla remained in this region. On average, shaving successfully removed all guard sensilla, 97% of asymmetric sensilla and 96% of companion sensilla from the aesthetasc tuft region. Control animals for the non-aesthetasc ablated group were also immobilized twice, but no sensilla were removed or inactivated.

Odor stimuli

Control stimulus was artificial seawater (Instant Ocean[®]) taken directly from the flume before the start of the trials. Experimental odor stimulus consisted of a pooled sample of conspecific urine collected from eight catheterized lobsters (four males and four females). Details of the catheterization and urine collection are described elsewhere (Horner et al. 2006). Urine was diluted 1:100 in artificial seawater taken directly from the flume at the start of the trials.

Bioassay

The shelter choice assay was conducted in a 5,000 l seawater flume located at Georgia Institute of Technology. The flume measures 12 m long \times 0.75 m wide \times 0.35 m high with a downstream working section measuring 2 m long \times 0.75 m wide \times 0.35 m high. All trials were conducted with a background flow rate of approximately 5 cm/s. Details on the flow dynamics of this flume and its use in other behavioral experiments are described elsewhere (Webster and Weissburg 2001; Keller et al. 2003; Weissburg et al. 2003; Horner et al. 2004). The flume water was filtered through biological, particulate, activated carbon and UV filters between trial days.

Two concrete blocks served as shelters for the lobsters. The block dimensions were 39.5 cm tall \times 19.5 cm wide \times 19 cm deep, and the opening size was 14 cm tall \times 13 cm wide \times 19 cm deep. The blocks were placed at the upstream end of the working section 5 cm from the wall of the flume and 26 cm apart. The area between each block and the sidewall of the flume was filled with plastic grating (1 cm \times 1 cm) to prevent the lobsters from sheltering in this area. Plastic grating spanning the width of the flume was placed behind the blocks to prevent the lobsters from escaping the working section. Odor stimuli were introduced into the flow by a dual channel peristaltic pump (Masterflex, Cole Parmer Co., Vernon Hills, IL, USA) that pumped the diluted conspecific urine through one shelter while simultaneously pumping seawater through the other shelter as a control. We randomly chose which shelter released an odorant before the start of each trial. Control and experimental stimuli were pumped into the flume at 15 ml/h. Thus with a 1:100 dilution, only 150 μ l of urine was released into the flow over the course of 1 h. Previous experiments showed this rate and dilution of conspecific urine to be sufficient to elicit sheltering behavior (Horner et al. 2006).

All trials were conducted with the room lights on and with a 60-W light mounted above the downstream end of the flume to provide constant illumination in the working section. Lobsters typically search for shelter in the early morning in their natural environment; however, spiny

lobsters will shelter in the presence of aggregation signal regardless of where they are in their circadian cycle (Ratchford and Eggleston 2000; Horner et al. 2006). All trials were conducted between 0900 and 2100 h, without special effort to run the trials at specific points in the lobster's natural light:dark cycle.

Trials began when a lobster was placed in a cage of Plexiglas and plastic grate (30.5 cm long \times 21 cm wide \times 20.5 cm high) 1.5 m downstream from the face of the concrete blocks for a 5-min acclimation period. Odor stimuli were pumped into the flume during this acclimation period. After 5 min, the cage was lifted and completely removed from the flume, thus allowing the lobster to move freely around the working section. Each trial lasted for 1 h, and the movements of the lobsters were recorded by a video camera mounted above the flume.

Video analysis

All videos were analyzed by an observer who was unaware of both the ablation condition of the lobster and which shelter released the urine stimulus. We used two criteria for including data in the analysis. This first criterion was that for data from an individual lobster to be included in the analysis, the lobster had to explore both sides of the flume and walk more than halfway upstream. This criterion ensured that a lobster had the opportunity to sample odors from both shelters, and that it was healthy and motivated to explore the flume and shelters. The second criterion was that for data for an entire day of experiments for both control and ablated animals to be included in the analysis, a majority of control animals on that day had to explore both sides of the flume and walk more than halfway upstream. This criterion ensured that any deficits in the behavior of ablated animals resulted from the ablations and not from variations in flume conditions. In all experiments, a majority of animals met the criteria for inclusion in the data set. In the aesthetasc ablation experiment, 75% of control lobsters and 80% of ablated lobsters met the criteria. In the non-aesthetasc ablation experiment, 64% of control lobsters and 67% of ablated lobsters met the criteria.

The sheltering behavior of the lobsters was quantified by recording the number of times each shelter was entered and by measuring the total amount of time spent inside (all body parts except antennae completely within the shelter) or within 30 cm of each shelter. Sheltering time included the time spent within 30 cm of each shelter because sometimes lobsters positioned themselves outside, but in contact with, the shelter such that their antennules could still sample water passing through the shelter opening. Although these animals were not inside the shelter, they still derived protection from it. Each trial produced two values for total sheltering time: one for time spent in or around the

seawater-emanating (control) shelter, and one for time spent in or around the urine-emanating (experimental) shelter. Each trial also produced two values describing the number of entries into each shelter.

Statistical analysis

The shelter preference of each of the four groups of lobsters (aesthetascs ablated, non-aesthetascs ablated, and the two control groups) was analyzed separately using a Wilcoxon matched pairs test. For each group, we compared the amount of time the lobsters spent in and around the shelter emanating urine to the amount of time they spent in and around the shelter emanating seawater. The same comparison was made for the number of entries into each shelter. Data from each of the control groups were analyzed using a one-tailed Wilcoxon matched paired test because previous experiments demonstrated consistently that lobsters with intact antennules significantly prefer shelters emanating conspecific urine (Horner et al. 2006). Data from each of the ablated groups were analyzed using a two-tailed Wilcoxon matched-pairs test since we did not have any precedent for the behavior of these groups.

A second analysis determined whether the ablations had a general effect on the tendency of the lobsters to shelter. We calculated the total amount of time each treatment group spent sheltering by summing the time spent in and around the control shelter and the time spent in and around the urine-emanating shelter. The total number of shelter entries was calculated in the same way. A Mann–Whitney *U* test was used to determine if there were statistically significant differences in either the total time spent sheltering or in the total number of shelter entries between each ablated group and its respective control group.

Results

Role of the aesthetasc pathway

Control lobsters often explored the shelters and the working section of the flume extensively after being released from the cage. As the trial progressed, most animals began to show a clear interest in the shelter emanating conspecific urine over the control shelter. Overall control lobsters spent significantly more time in or around the shelter emanating urine than the shelter emanating seawater (Fig. 2a). They also entered the shelter emanating urine significantly more often than the shelter emanating seawater (Fig. 2b).

Aesthetasc ablated lobsters also actively explored the flume and shelters after being released from cage. However, unlike the control lobsters, aesthetasc ablated lobsters as a group did not show a statistically significant preference for

either shelter. Approximately half of the animals showed more interest in the shelter emanating seawater than the shelter emanating urine whereas the other half showed the opposite pattern of behavior (data not shown). Overall, aesthetasc ablated animals spent approximately equal amounts of time in or within two body lengths of both shelters (Fig. 2c) and entered both shelters with similar frequency (Fig. 2d).

There were no statistically significant differences in the overall tendency to shelter between control and aesthetasc ablated lobsters. The two groups spent similar total amounts of time sheltering (Fig. 3a) and entered the shelters with similar frequency (Fig. 3b). This suggests that the urine signal plays a more important role during the final stages of shelter selection—after the lobsters have started actively searching for a suitable shelter—and a less important role in the initiation of searching behavior.

Role of the non-aesthetasc pathway

Control lobsters in this experiment behaved similarly to control lobsters in the aesthetasc ablation experiment. They spent significantly more time in and around the shelter emanating conspecific urine than the shelter emanating seawater (Fig. 4a) and they also entered the urine-emanating shelter significantly more often than the seawater-emanating shelter (Fig. 4b).

Non-aesthetasc ablated lobsters showed a similar pattern of behavior to control lobsters, in some respects. They spent significantly more time in and around the shelter emanating conspecific urine than the shelter emanating seawater (Fig. 4c). However, although non-aesthetasc ablated lobsters often approached the shelters, they entered them infrequently. There were no statistically significant differences in the number of entries into the control and urine-emanating shelters (Fig. 4d). Clearly, the non-aesthetasc ablation affected sheltering behavior; however, the ablated animals still retained some ability to distinguish between the two shelters. Thus, the non-aesthetasc pathway does not play a critical role in sheltering behavior, but it may play a supporting role.

Analysis of overall sheltering behavior revealed no significant differences in either the total time spent sheltering (Fig. 5a) or in the total number of entries into the shelters (Fig. 5b) between control and non-aesthetasc ablated lobsters.

Discussion

Aesthetascs are the sensors of the urine-based conspecific sheltering signal

Caribbean spiny lobsters use chemicals from conspecifics in locating available shelters (Nevitt et al. 2000; Ratchford

Fig. 2 Sheltering behavior of control (**a, b**, $N = 8$) and aesthetasc ablated lobsters (**c, d**, $N = 10$) in response to dilute conspecific urine. *Box plots* show median (*solid black line*), interquartile range (*box length*), and minimum and maximum values (*error bars*) for time spent inside or within two body lengths of each shelter (**a, c**) or number of entries into each shelter (**b, d**). Statistically significant results are indicated by “*asterisk*” (Wilcoxon matched-pairs test, $P < 0.05$)

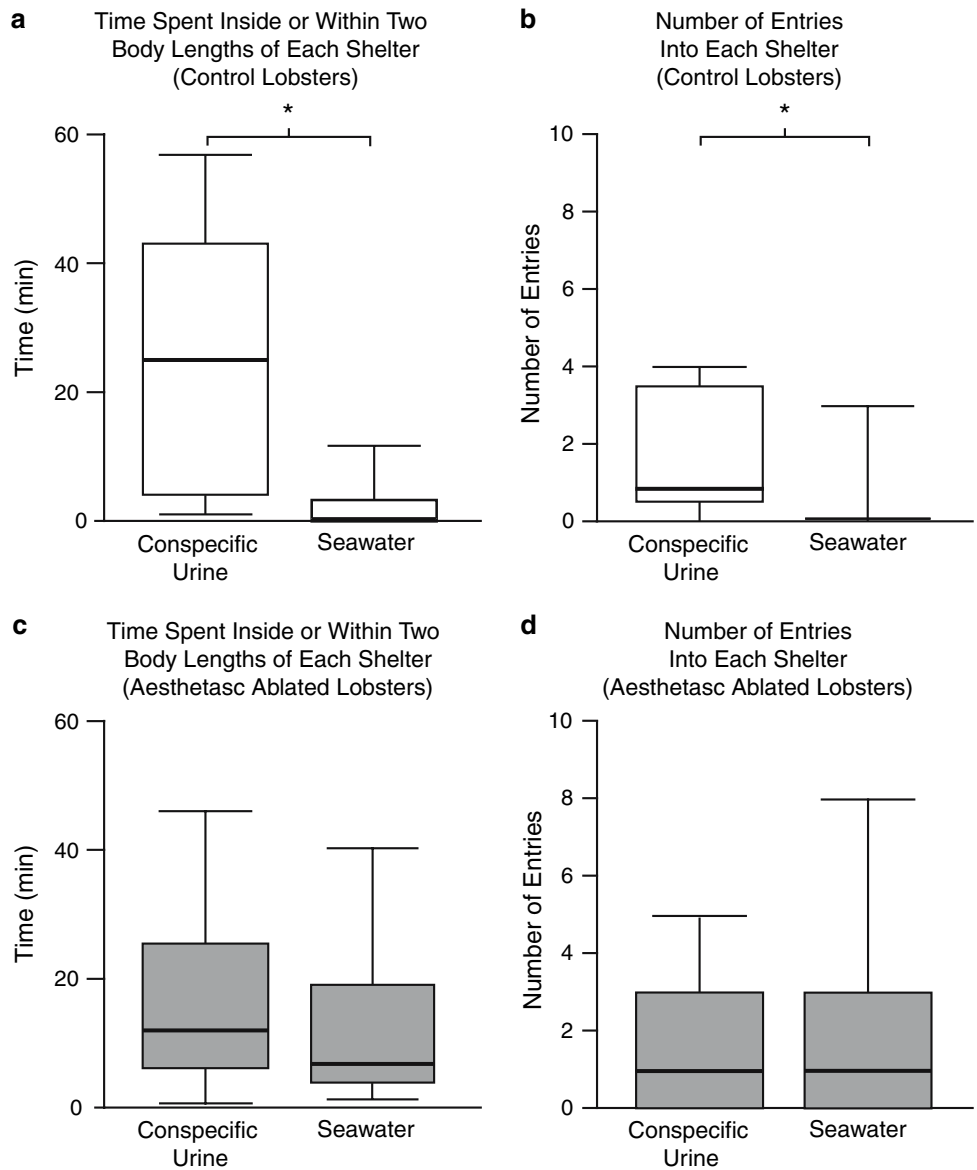


Fig. 3 Comparison of overall sheltering behavior between control ($N = 8$) and aesthetasc ablated ($N = 10$) lobsters. *Box plots* show median (*solid black line*), interquartile range (*box length*), and maximum and minimum values (*error bars*) for total time spent inside or within two body lengths of both shelters (**a**) and total number of entries into both shelters (**b**). No statistically significant differences were observed (Mann–Whitney U Test, $P > 0.05$)

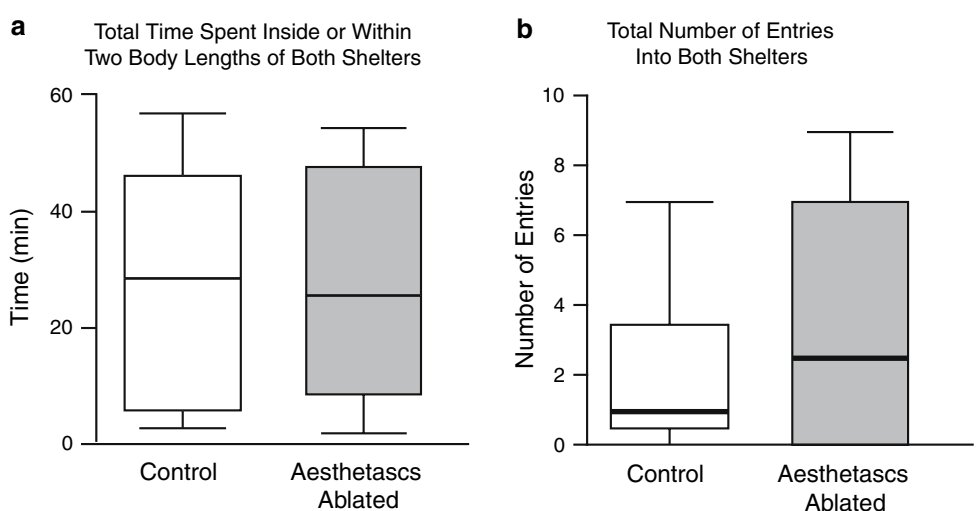
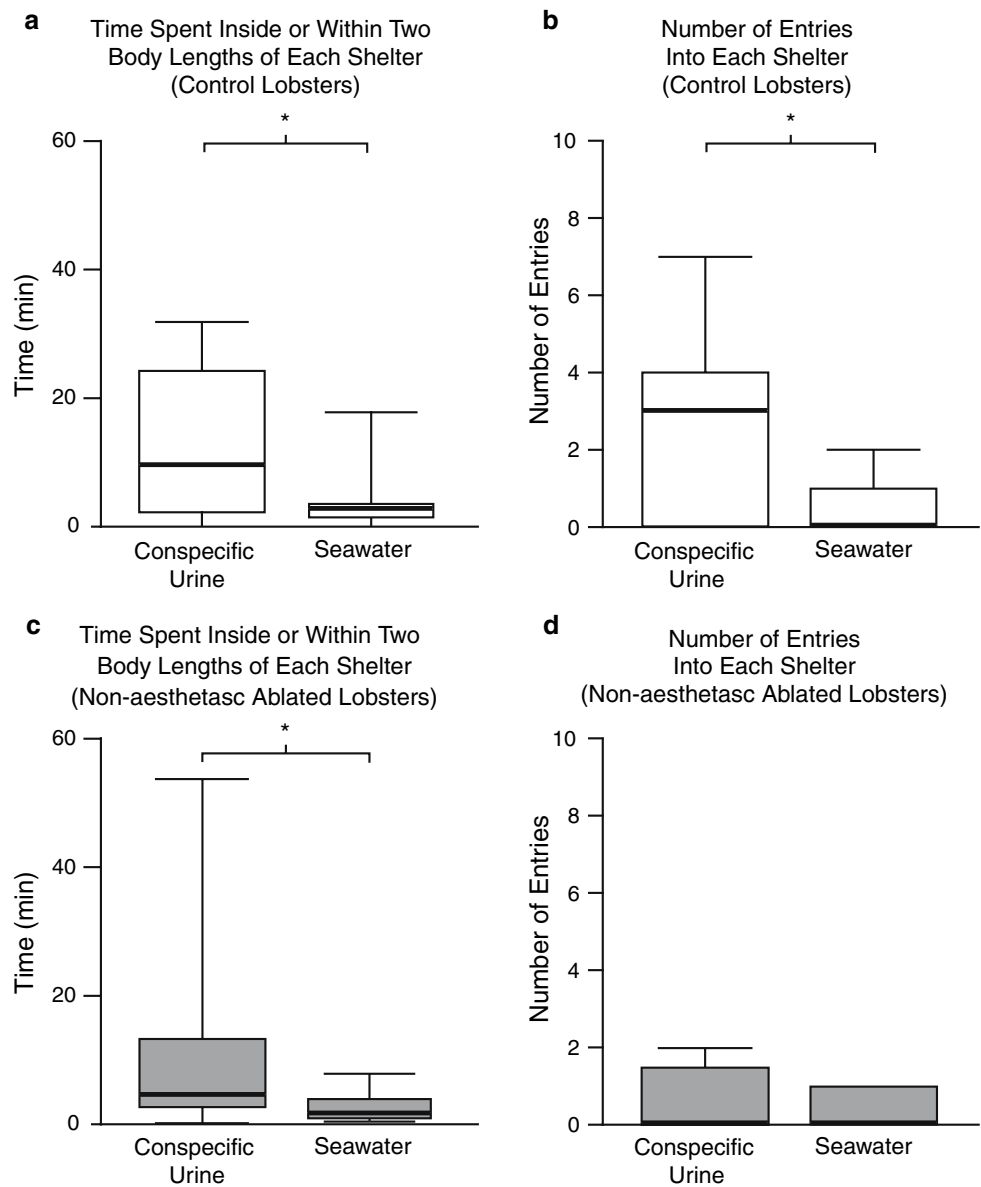


Fig. 4 Sheltering behavior of control (**a, b**, $N = 9$) and non-aesthetasc ablated lobsters (**c, d**, $N = 8$) in response to dilute conspecific urine. *Box plots* show median (*solid black line*), interquartile range (*box length*), and minimum and maximum values (*error bars*) for time spent inside or within two body lengths of each shelter (**a, c**) or number of entries into each shelter (**b, d**). Statistically significant results are indicated by “*asterisk*” (Wilcoxon matched-pairs test, $P < 0.05$)



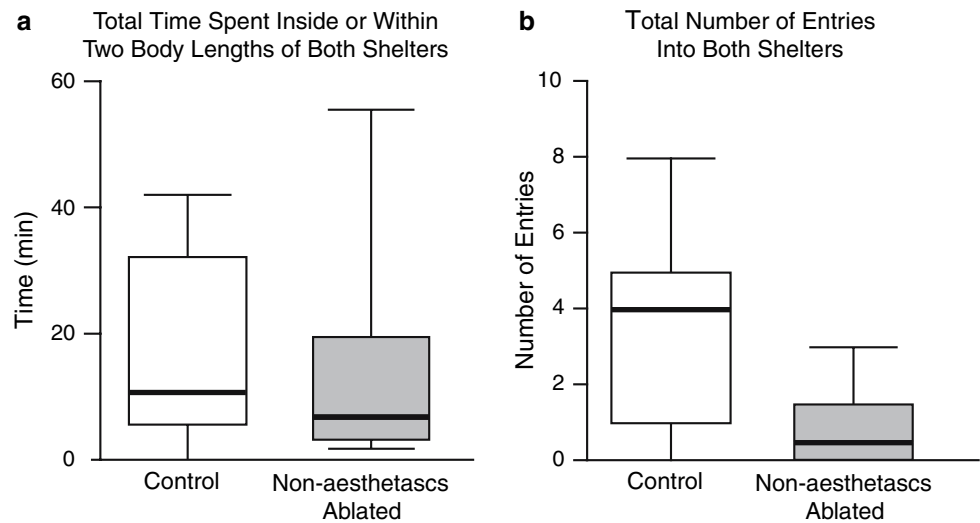
and Eggleston 2000). Urine is one source of this pheromone (Horner et al. 2006). Our study extends these findings by identifying the aesthetascs as the primary chemosensors mediating the behavioral response to this urine pheromone. Control lobsters significantly preferred shelters emanating urine over the control shelter, whereas aesthetasc ablated animals did not (Figs. 2, 3). Non-aesthetasc ablated animals were like control animals in spending significantly more time in and around the shelter containing urine than the control shelter (Figs. 4, 5). However, non-aesthetasc ablated lobsters infrequently entered the urine emanating shelter, suggesting that the non-aesthetasc ablation may have some effects on the sheltering behavior of these animals. We conclude that although the non-aesthetasc pathway does not play a critical role in sheltering behavior, it may play a supporting role in shelter selection in the natural

environment by enhancing or otherwise complementing the response to urine signals by the aesthetasc chemosensory pathway.

Aesthetasc/olfactory lobe pathway functions in crustacean social behaviors

The most significant finding of this work is that the aesthetasc/olfactory pathway is the major chemosensory pathway involved in urine-evoked shelter selection. This is the first description of a unique role for the aesthetasc pathway in this species, and it is the first description in any species that this pathway mediates this form of social behavior, gregarious sheltering. Only three other published studies have conclusively demonstrated specific behavioral roles for the aesthetasc pathway in any decapod crustacean. In the first

Fig. 5 Comparison of overall sheltering behavior between control ($N = 10$) and non-aesthetasc ablated ($N = 8$) lobsters. Box plots show median (solid black line), interquartile range (box length), and maximum and minimum values (error bars) for total time spent inside or within two body lengths of both shelters (a) and total number of entries into both shelters (b). No statistically significant differences were observed (Mann–Whitney U Test, $P > 0.05$)



case, aesthetascs play a critical role in the courtship display of male blue crabs, *Callinectes sapidus*, which occurs in response to pubertal female sex pheromones (Gleeson 1980, 1982, 1991). Removal of the aesthetascs resulted in a loss in this behavior even in the presence of female pheromones. In the second case, the aesthetasc chemosensory pathway plays a critical role in individual recognition in clawed lobsters, *Homarus americanus* (Johnson and Atema 2005). Removal of the aesthetascs resulted in longer and more intense second encounters between pairs of ablated lobsters than intact control pairs. In the third case, recognition of social status of crayfish, *Procambarus clarkii*, requires aesthetascs (Horner et al. 2008).

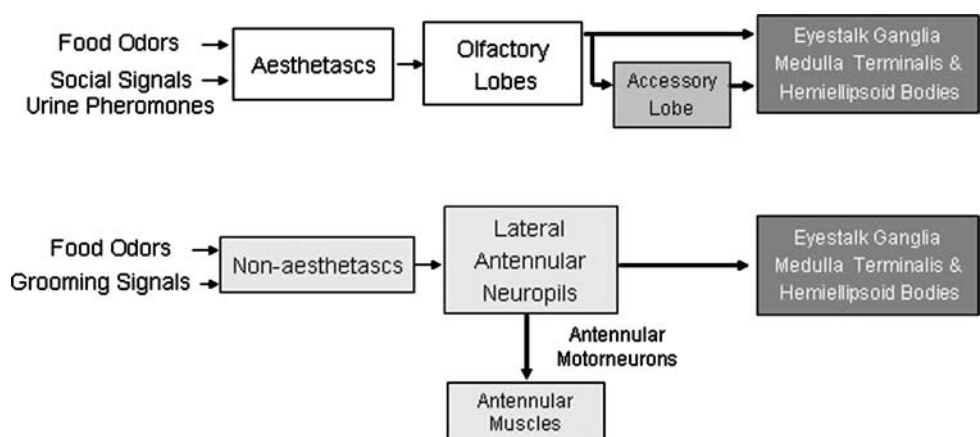
The aesthetasc chemosensory pathway is not simply a pheromone processing system. It also functions in a variety of food odor mediated behaviors that can also be activated by the non-aesthetasc chemosensory pathway in the absence of aesthetasc input. Thus, the two pathways have overlapping roles in behaviors such as activation of food search, food odor discrimination, and learning and orientation to a distant food odor source (Derby et al. 2001; Steul-

let et al. 2001, 2002; Horner et al. 2004). Functional overlap is an important feature of many sensory systems and can benefit an organism in several important ways, including allowing an animal to continue to function normally in the event of loss or damage to a subset of sensors, increasing the probability of stimulus detection, and enhancing the overall sensitivity of the system (Derby and Steullet 2001). In addition to this functional overlap, the aesthetasc pathway appears to be the exclusive domain of pheromone processing, and the non-aesthetasc pathway plays an essential role in antennular sensory-motor activities such as chemically evoked antennular grooming behavior (Schmidt and Derby 2005). A summary of the crustacean chemosensory pathways and their functions is presented in Fig. 6.

Why do many “noses” have multiple chemosensory pathways?

Many taxa have “noses” with anatomically separate chemosensory pathways. Different pathways often contain different

Fig. 6 Diagram of the crustacean chemosensory pathways and their functions



complements of receptor neurons with both overlapping and non-overlapping odor sensitivities and specificities. Packaging neurons with different sensitivities and specificities into differently organized pathways allows for different processing requirements to be met and also potentially allows for a greater range of behavioral responses.

The olfactory system of some insects provides one of the best examples of a clear functional separation between pathways based on the types of odorants detected and the types of behaviors mediated. The main olfactory pathway, which projects to generalist antennal lobe glomeruli, mediates the response to general odorants whereas the male specific pathway, which projects to a specific subset of glomeruli (the macroglomerular complex), responds exclusively to female sex pheromones (Hansson 1995; Hildebrand 1995; Christensen and White 2000; Hansson and Anton 2000; Christensen and Hildebrand 2002). Organizational features of the male specific pathway, including the high affinity of the pheromone receptors and the massive convergence of the axons of pheromone sensitive neurons onto a few specific glomeruli, make it a highly sensitive and selective pheromone processing system. Packaging pheromone sensitive neurons into a separate pathway allows these signals to be detected and discriminated with much greater sensitivity than general odorants.

In addition to the purely chemosensory pathways, some insects also have an antennal contact chemo-mechanosensory pathway. The sensory neurons in this pathway project to the dorsal lobe of deutocerebrum and the subesophageal ganglion, with a somatotopic organization (Nishino et al. 2005). This arrangement of chemosensory and chemo-mechanosensory pathways thus allows for the insect antennae to discriminate in parallel sexual odors and general odors, and to locate chemo-tactile stimuli in space. Similarities between this organization and that of the decapod crustacean antennular pathways are noteworthy.

The noses of many vertebrates also have multiple pathways, the most well studied of which are the main olfactory system, consisting of the olfactory epithelium and main olfactory bulb, and the vomeronasal or accessory olfactory system, consisting of the vomeronasal organ and the accessory olfactory bulb (Eisthen 1997; Christensen and White 2000; Baxi et al. 2006; Breer et al. 2006; Spehr et al. 2006). The role of these organs has become clearer in recent years, and this includes considerable functional overlap in the types of odorants detected. Pheromones, once thought to be the exclusive domain of the vomeronasal system, can be discriminated by the main olfactory system in some species (Hudson and Distel 1986; Dorries et al. 1997; Johnston 1998, 2000; Restrepo et al. 2004; Lin et al. 2005; Baxi et al. 2006; Brennan and Zufall 2006; Spehr et al. 2006). General odorants can also be discriminated by either system depending on the species examined (Halpern et al. 1997;

Johnston 1998, 2000; Miller and Gutzke 1999; Ptacyk and Graves 2002; Halpern and Martínez-Marcos 2003; Baxi et al. 2006; Spehr et al. 2006). In some instances, both pathways can function together to mediate the response to a particular odorant (Johnston 1998, 2000; Restrepo 2004; Lin et al. 2005). Thus, a single and clear functional difference between the two pathways is not apparent. Additional chemosensory pathways such as the trigeminal system, which provides sensitivity to nociceptive chemicals, and the septal organ and Grueneberg ganglion, whose behavioral functions are not well understood (Storan and Key 2006), further increase the organizational complexity of the vertebrate nose.

The clustering of chemosensory organs in the nose is undoubtedly related to cephalization, but the separation of these organs within the nose may have to do with providing a differential access to particular stimuli, and hence functional differences. For example, the septal organ's function may be related to its location near the nasopalatine duct that delivers stimuli to it, and the vomeronasal organ is located at end of duct through which stimuli are delivered using a pumping action.

Our finding that the parallel antennular chemosensory pathways of decapod crustaceans show functional differences in chemically mediated behaviors opens up the exciting possibility of examining their reasons for the difference. One possibility is that the aesthetasc pathway is better able to detect pheromone signals because it has a greater number and diversity of chemosensory neurons and can thus detect lower concentrations and/or a broader range of chemicals. Additionally, the glomerular organization of central targets of the aesthetasc pathway, the olfactory lobes, may play a critical role in allowing this pathway to process pheromone signals. Glomeruli are considered to be functional units of olfactory coding and play an important role in odor discrimination and signal amplification (Christensen and White 2000; Eisthen 2002; Ache and Young 2005; Chen and Shepherd 2005). Although the reasons underlying the differences in pheromone processing between the two pathways are currently just speculation, future studies examining the physiological responses of the sensory neurons in each pathway to the active components of the urine signal are feasible. Any insight into why certain odorants are processed preferentially in one or more pathways can help us understand why the crustacean chemosensory systems are organized as they are. Although the chemosensory systems of crustaceans seem different from those of mammals or other animals, many principles of olfactory coding and organization are similar across taxa. Thus, insights gleaned from the crustacean model systems can help generate testable hypotheses for understanding the functions of multiple chemosensory pathways in other animal systems.

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