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The olfactory pathway for individual recognition in the American lobster *Homarus americanus*

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Summary

Individual recognition in the lobster *Homarus americanus* (Milne-Edwards), is based on detection of urine pheromones *via* chemoreceptors of the lateral antennular flagellum. The specific sensory pathway mediating this recognition is not known. Most of the chemoreceptor cells of this flagellum are found in the unimodal aesthetasc sensilla and project specifically to the glomeruli of the olfactory lobe in the brain. Additional chemoreceptor cells are located among mechanoreceptor cells in bimodal sensilla, including the guard hairs; they do not project to the olfactory lobe. This neuroanatomy suggested that aesthetascs were essential to all complex chemosensory tasks until it was shown that spiny lobsters *Panulirus argus* can still perform complex food odor discrimination and localization tasks without aesthetascs. Here, we demonstrate that the aesthetascs of *H. americanus* contain the chemoreceptors necessary for individual recognition of familiar opponents. In contrast to intact and guard hair-shaved animals, lobsters with aesthetascs removed did not recognize previous opponents as shown by second encounters statistically similar in length and aggression to first-encounter fights. Non-aesthetasc chemosensory pathways were incapable of rescuing opponent recognition. Subsequent lesion of all remaining chemoreceptor cells (by immersion in distilled water) abolished recognition and renewed fighting.

Key words: lobster, *Homarus americanus*, Crustacea, aesthetasc, chemoreceptor, pheromone, individual recognition.

Introduction

If individual recognition is considered a feature of advanced species, then some decapod crustaceans should be included in this company, i.e. at least one species of crayfish (Lowe, 1956), two species of hermit crabs (Gherardi and Atema, 2005; Gherardi and Tiedemann, 2004; Hazlett, 1969), a crab (Vannini and Gherardi, 1981), a mantis shrimp *Gonodactylus festae* (Caldwell, 1979; Caldwell, 1985) and the lobster *Homarus americanus* (Karavanich and Atema, 1998a). In many animals this individual recognition is based on chemical signals (e.g. Hurst 1990a,b,c), including crustaceans (Atema and Steinbach, in press). These species use individual recognition to recognize mates and/or maintain stable dominance hierarchies. With further study many more species may turn out to have this capability. Indeed, individual recognition may not be so specialized. Learning and remembering individual odor cues may be a common feature similar to learning other important environmental cues such as home stream recognition and imprinting on familiar and/or related individuals.

The best understood crustacean model for individual recognition in its social biological context is *H. americanus*. In typical first encounters between size-matched, naïve opponents, lobsters fight and establish dominance. In subsequent encounters, however, the previous loser avoids a fight with the previous (known) winner. Yet, this same loser will fight and can win fights against unknown winners of other fights, demonstrating that the loser recognizes the *individual* winner and not only the dominance status of *any* winning lobster, as appears to be the case in some species of crayfish (Breithaupt and Eger, 2002; Copp, 1986; Gherardi and Daniels, 2003; Zulandt Schneider et al., 2001). A separation of 1–2 weeks may be the limit of the memory of a former opponent; this memory is not affected by multiple social interactions with other lobsters (Karavanich and Atema, 1998a). Individual recognition allows lobsters to form stable dominance relationships (Jacobson, 1977; Morschauser and Atema, 2003), which have consequences for mate selection and reproductive success (Cowan and Atema, 1990) and access to shelter (Atema et al., 1979; Atema and Voigt, 1995), two of the most important aspects of lobster survival.

Lobsters are covered with chemoreceptive setae, many of which are clustered into at least five different chemoreceptor organs, each specialized for different functions (Atema and Voigt, 1995; Derby and Atema, 1982). The thoracic appendages, which include the walking legs and the maxillipeds, appear in function and neuroanatomy more like

vertebrate taste organs and serve important feeding functions. The cephalic appendages, including first and second antennae, resemble olfaction organs, as they monitor the external fluid environment (Atema, 1977) and project to specialized sensory brain areas (Sandeman et al., 1992). Physiologically, the cephalic appendages of *H. americanus* are both chemoreceptive (Voigt and Atema, 1992) and mechanoreceptive (Miller-Sims and Atema, 2004). The first antenna, known as the antennule, is composed of a lateral and a medial flagellum standing on a set of basal segments. The behavioral functions of the medial flagellum and the second antenna remain unknown, despite several lesion studies (e.g. Atema et al., 1999; Devine and Atema, 1982). The lateral flagellum is considered the organ most specialized for chemosensory detection and plays a leading role in tracking odor plumes (Devine and Atema, 1982) and individual recognition (Karavanich and Atema, 1998b). Lobster memory and individual recognition are mediated by chemical signals in urine released during a fight (Karavanich and Atema, 1998b).

The lateral antennular flagellum uses two separate chemosensory pathways: aesthetasc and non-aesthetasc. The sensory neuroanatomy of antennular pathways is best known for spiny lobsters (Schmidt and Ache 1992, 1993, 1996a,b, 1997; Schmidt et al., 1992). The aesthetasc sensilla are the most abundant setal type and are located in a distal tuft. Each of the 50 tuft annuli of the lateral flagellum of a mature lobster (*H. americanus*) carries two rows of some 12 aesthetascs, each containing ~300 olfactory receptor neurons (Atema and Voigt, 1995; Oleszko-Szuts and Atema, 1977) that project to the glomeruli of the olfactory lobes (Sandeman et al., 1992). A variety of morphologically different non-aesthetasc sensilla (Oleszko-Szuts and Atema, 1977; Guenther and Atema, 1998) contain unimodal and bimodal chemo- and mechanoreceptor neurons that project to the lateral antennular neuropils, which lack glomerular organization (Schmidt and Ache, 1992; Schmidt and Ache, 1997). The prominent guard hairs are bimodal, containing some 20 receptor neurons (Cate and Derby, 2001). In *H. americanus* each of the 50 tuft annuli carry up to four guard hairs for a total of 4000 receptor neurons, most of which appear chemoreceptive based on axon diameter (J. Atema, unpublished observation). Several other setal types are found on this flagellum, but their function is not known and homology with setae described in spiny lobsters (Cate and Derby, 2001) is still unclear.

Based on this sensory neuroanatomy it was believed that crustaceans would need functional aesthetasc sensilla to perform complex chemoreception tasks, such as discriminating odor mixtures and locating odor sources. Some behavioral results seemed to support this notion. Removal of one lateral antennular flagellum prevented *H. americanus* from making correct initial directional decisions when tracking food odor; selectively shaving off the aesthetasc sensilla of one flagellum still had a noticeable, though lesser, effect (Devine and Atema, 1982). This suggested a major role for aesthetasc sensilla and a minor role for non-aesthetasc sensilla in odor tracking. However, detailed studies on spiny lobsters, in which either aesthetasc chemoreceptors or non-aesthetasc chemoreceptors were ablated, showed that aesthetascs are not required for seemingly complex olfactory tasks. Without aesthetascs they can still discriminate between complex food odor mixtures (Steullet et al., 2002), and can locate food odor sources in low flow environments (Steullet et al., 2001) and track odor plumes in a narrow flume (Horner et al., 2004). If the aesthetascs are not essential for food mixture detection and source localization, what then is unique about this major chemosensory input system with its large glomerular olfactory lobes?

We focus here on the role of aesthetasc sensilla in individual recognition in *H. americanus.* We know that the individual recognition function is limited to the lateral flagella and cannot be supported by the medial flagella and antennae (Atema et al., 1999; Karavanich and Atema, 1998b). However, as these lesion studies were based on treatment with distilled water, which eliminates all chemoreceptor function (Derby and Atema, 1982), it remained unknown if the aesthetasc pathway is uniquely involved. Such knowledge would facilitate identification of pheromone receptors and the central processing of individual memory.

Materials and methods

Animal maintenance

Mature male lobsters *Homarus americanus* Milne-Edwards used in this study were captured by local fishermen in the waters surrounding Woods Hole, Massachusetts, USA, and ranged in size from 75 to 100 mm carapace length (CL). They were isolated in individual holding tanks with running seawater for at least 7 days before being tested. During this acclimation period in isolation, the possible memory of each other from earlier dominance fights in the field would be greatly diminished (Karavanich and Atema, 1998a). During their time in the laboratory they were fed 2–3 times a week on squid and maintained on a light cycle that approximated natural sunrise and sunset for that time of year. Water temperature during the course of the testing varied from 6° to 23°C. Several weeks after the end of experimentation all animals were released in local waters.

Experimental design

The design used to address these questions includes two separate experimental groups: (1) aesthetasc shaved and (2) guard hair shaved. Each group contains three sequential treatments: for Group 1 these are (A) first interaction, normal untreated; (B) second interaction, aesthetasc or guard hair shaved; (C) third interaction, lateral flagella lesioned using distilled water. Treatment designations for group 2 will be AA, BB and CC, respectively. The only difference between the two groups is treatment B (aesthetasc shaved) *vs* BB (guard hair shaved). If aesthetasc sensilla are important for individual recognition, we expected that their shaving would prevent recognition and result in long and intense second interactions (B) not altered by subsequent water lesion in third interactions

(C). In contrast, guard hair shaving would not affect recognition, leading to reduced second interactions (BB) and a return to long and intense interactions after water lesion (CC).

All 60 lobsters in this study were size matched so that fighting pairs were within 3 mm CL; this close matching is known to make the fight outcome unpredictable by size (Scrivener, 1971). These 30 fighting pairs were randomly assigned to either of the two experimental groups: 15 pairs to be aesthetasc shaved and 15 pairs to be guard hair shaved. Each pair, regardless of group designation, had to complete a series of three 20 min interactions in a 'boxing tank' on 3 consecutive days. In interaction A, the pair fought to establish dominance. 2–6 h later either aesthetascs or guard hairs were shaved according to group designation. One day (24±4 h) after the first interaction, the pair was brought together again in interaction B. Approximately 22 h after the conclusion of interaction B, each pair, regardless of group, was given a 10 min distilled water immersion of both lateral flagella exclusively. 2 h later the pair was reintroduced for a third and final time in interaction C. After completion of the experiments both lateral flagella were removed from all animals and preserved in formalin for future inspection of shaving efficiency. The lobsters themselves were kept in the laboratory for a few more weeks prior to release to observe their state of health and possible molting that may have affected the fight outcome.

Experimental apparatus and procedures

Testing was conducted in a 'boxing tank': a 90 cm wide×60 cm×60 cm all-glass aquarium illuminated by two 100 W bulbs suspended 1 m above the water surface. A water depth of at least 30 cm was maintained during all fights. Water was drained and replaced after each fight. The lobsters of a pair were placed in this 'boxing tank' on either side of a plastic divider for 5–10 min prior to the start of the fight in order to acclimate. Then, the divider was removed and the pair were allowed to interact for at least 20 min. All interactions were recorded with an overhead video camera for later analysis. Recording ended if the interaction had been a fight with a definitive winner or if there had been no significant interaction. However, if the lobsters were still actively engaged in fighting after 20 min, the recording was continued until a definitive outcome to the interaction was reached. A definitive outcome was defined as maintenance of a stable dominance relationship for at least 5 min, as indicated by the loser's continuous crouched position and avoidance of his opponent.

Shaving of sensilla was accomplished by first restraining the lobsters upside down on a customized barber chair. Lobsters were wrapped with a wet towel and their antennules were constantly wetted to prevent desiccation. Aesthetascs or guard hairs were shaved with a razor blade fragment under a dissection microscope. The procedure took no more than 45 min per lobster. After the shaving procedure, the lobsters were allowed to recuperate in their individual holding tanks. To determine lesion efficiency, the lateral flagella of all animals were removed after the end of experimentation and preserved in formalin. Two independent observers scored the number of intact remaining sensilla on each flagellum. Greatly shortened (<50%) or fallen sensilla were not considered intact (see Discussion).

Applying the distilled water lesion was done by first restraining the lobsters in the same fashion described above. Their lateral flagella exclusively were then briefly rinsed in deionized water and subsequently immersed in a vial of deionized water for 10 min. After this procedure the lobsters were allowed to recuperate for at least 2 h in their individual holding tanks before the beginning of interaction C.

Data analysis

The videotapes were analyzed for fight duration and intensity according to established procedure (Karavanich and Atema, 1998a) using slightly modified agonistic levels. As before, levels –1 and –2 represent avoidance (walking away) and fleeing (tail flipping, running away), respectively; level 0 means an animal not facing the other within one body length or no response; level 1 represents approach behavior and level 2 threat displays (such as meral spread and antenna and claw pointing) without physical contact. We split the former 'level 3' into a new level 3 consisting of antenna whipping (with contact) and a new level 4 consisting of claw pushing or boxing. This caused former levels 4 and 5 to become levels 5 (claw lock) and 6 (scissor, rip). We then assigned a single agonistic score for every 5 s interval of the fight. Since it was possible for a lobster to be engaged in more than one agonistic level during one 5 s interval we adopted the following ranking to assign this score. Agonistic levels 6, 5, 4, 3 and 2 outranked all other levels in decreasing order (i.e. 6 outranked all other levels, 5 outranked all other levels except 6, etc.); level 1 outranked only level 0; level –2 outranked level –1; both levels –1 and –2 outranked levels 0 and 1. Mean aggression was then calculated as the sum of all agonistic scores divided by the number of 5 s time intervals during the fight. Maximum aggression was calculated as the total number of level-6 scored during the fight. Fight duration was measured from the start of engagement at aggression level 3 until the time that aggression dropped below level 3 for 5 min.

Statistics

We measured the duration of each fight and the mean and maximum aggression of the winner and the loser. The data were not normally distributed. Therefore, each of these five parameters was evaluated first using a non-parametric χ^2 test across groups and treatments (Van der Waerden test in the program JMP 4.0.0; SAS Institute Inc., 1995). Then treatment differences within the two groups were evaluated with a Friedman ANOVA (Statistica for Windows 5; Statsoft, Inc., Tulsa, OK, USA). The experimental design allowed us to then test pairwise for differences between the three sequential treatments (Wilcoxon signed ranks test; JMP 4.0.0; SAS Institute Inc., 1995). We evaluated the effect of lesion efficiency on fight parameters of winners and losers by Spearman rank correlation (Statistica; Statsoft Inc. 1995). Mean values are shown with standard errors (S.E.M.).

Results

Fight duration

Effects of aesthetasc vs *guard hair shaving*

Fight duration was significantly different across groups and treatments (Table 1). Differences within Group 2 (guard hair shaved) were significant but not within Group 1 (aesthetasc shaved; Table 1). The major change occurred after guard hair shaving in Group 2, where the mean fight duration dropped from 901±219 s in the first ('AA') fight to 182±77 s in the second ('BB') fight (Table 2a, AA–BB). This 80% drop was due both to a large number of no-fights and much shorter remaining fights: in BB encounters, 9 of the 15 pairs (60%) did not fight at all and six pairs showed a shorter fight duration (455±130 s) than in their AA fights (1240±367 s; Table 2b, AA–BB).

In contrast, only 3 of 15 pairs (20%) did not fight after aesthetasc shaving ('B' fight; Table 2a, B–C). When we eliminated these three from analysis we did not find significant differences between treatments (Table 2b, B–C).

In sum, guard hair removal and leaving aesthetascs intact resulted in the complete absence of BB fights in nine pairs and shorter BB fights in the remaining six pairs. This greatly and significantly reduced fighting suggests normal function of opponent recognition in the second fight was retained in this group. In contrast, aesthetasc removal and leaving guard hairs intact did not lead to significantly shorter B fights in fighting animals, suggesting interference by the treatment with the process of recognizing previous opponents.

Effects of distilled water lesion

Group 1

Fight duration after aesthetasc shaving did not change significantly after subsequent treatment with distilled water (Table 2a, B–C). Mean C fight duration was also not significantly different from the original A level (Table 2a, A–C) indicative of lost recognition capability. Two of the three pairs that did not fight in the B encounter started fighting again in the C fight, suggesting that they had now lost the recognition capability they may have had in the B fight.

Group 2

Similarly, six of the nine pairs that did not fight after guard hair shaving (BB) started fighting again after distilled water lesion (CC), indicative of now lost opponent recognition. In this guard hair group, distilled water lesion also caused a significant increase in fight duration from (Table 2a, BB–CC), but the mean fight duration of CC fights remained less than in the AA fights (Table 2a, AA–CC).

In sum, most pairs that did not fight or had short fights after guard hair or aesthetasc removal started fighting again after distilled water treatment had eliminated the chemosensory capabilities of their lateral flagella.

Mean and maximum aggression of winners and losers

For analysis of mean and maximum aggression, all 17 'no fights' were excluded from calculations to reveal fight intensity in those that did fight. Overall, across all fighting pairs, regardless of treatment (N=73), both mean and maximum aggression were significantly greater for winners (W; W_{mean}=3.53±0.08; W_{max}=4.62±0.76) than for losers [L; L_{mean}=3.0±0.11; L_{max}=3.29±0.65; Wilcoxon signed rank, $(W–L)_{mean}$, rank=1057, P<0.0001; $(W–L)_{max}$, rank=416, P=0.01]. In winners and losers of both groups, a downward trend in aggression measures appeared over the three treatments, but none of the differences were statistically

Table 1. *Non-parametric statistical evaluation of fight parameters*

Fight parameter	Test	N	χ^2	d.f.	P
Fight duration					
*Overall: 2G, 3T	Van der Waerden χ^2	89**	20.35	5	0.0011
Group 1	Friedman ANOVA	15	4.5	2	<0.11
Group 2	Friedman ANOVA	15	19.6	2	<0.00006
Mean aggression winner					
*Overall: 2G, 3T	Van der Waerden χ^2	73†	2.64	5	0.76
Mean aggression loser					
*Overall: 2G, 3T	Van der Waerden χ^2	73†	4.5	5	0.48
Maximum aggression winner					
*Overall: 2G, 3T	Van der Waerden χ^2	73†	11.3	5	0.046
Group 1	Friedman ANOVA	11	7.3	2	0.026
Group 2	Friedman ANOVA	6	4.4	2	0.11
Maximum aggression loser					
*Overall: 2G, 3T	Van der Waerden χ^2	73†	6.6	5	0.25

Group 1, aesthetasc shaved; Group 2, guard hair shaved.
*Two groups (G) with three treatments (T) each.
**Excludes the pair where one animal died before the C-fight.
†Excludes the 17 pairs that did not fight after shaving and/or distilled water lesion.

Table 2. *Fight duration and paired comparison of treatment effects (Wilcoxon)*

Group	Treatment	Number of no-fights	Duration (s)	Comparison	*N*	Wilcoxon rank	*P*
(a) Including no-fights							
1 Aesthetasc shaved	A	0	632±105	A–B	15	36	0.04
	B	3	408±119	B–C	14	0	1
	C	2*	375±117	A–C	14	23.5	0.11
2 Guard hair shaved	AA	0	901±219	AA–BB	15	60	<0.0001
	BB	9	182±77	BB–CC	15	37	0.001
	CC	4	499±142	AA–CC	15	39.5	0.02
(b) Excluding no-fights							
1 Aesthetasc shaved	A	0	632±105	A–B	12	19	0.15
	B	3	510±134	B–C	10	8.5	0.42
	C	2*	403±123	A–C	13	18	0.18
2 Guard hair shaved	AA	0	1240±367	AA–BB	6	10.5	0.03
	BB	9	455±130	BB–CC	6	9.5	0.063
	CC	3	623±159	AA–CC	12	23.5	0.067

(a) Includes interactions where no fight took place. (b) Excludes 'no-fights'.
*Excludes the pair where one animal died before the C-fight (eliminated from all C-fight analyses).
Duration values are means ± S.E.M.
N, number of fighting pairs per treatment.

significant (Wilcoxon tests as above). In particular, mean aggression was remarkably stable among all treatments for both winners and losers. Winner mean aggression in the six treatments varied from a high of 3.72±0.11 in treatments A and B to a low of 3.23±0.27 in CC. Loser mean aggression varied from 3.37±0.21 in A to 2.67±0.39 in BB.

Of the four aggression parameters, only 'Maximum Aggression of Winners' showed a significant treatment effect, which occurred in Group 1 (aesthetasc shaved), but not in Group 2 (guard hair shaved; Table 1). The difference was due to a large drop from 5.8±1.2 in the A fight to 1.77±0.72 in the C fight. In the same animals, mean aggression remained nearly unchanged (3.72±0.11 in A to 3.51±0.2 in C).

In sum, treatment did not significantly affect fight intensity: if a pair fought, they did so with characteristic intensity in which winners were more aggressive than losers.

Effectiveness of aesthetasc and guard hair shaving

Guard hairs were always completely removed. However, in most cases at least a few aesthetasc sensilla were still remaining after shaving. The number of remaining intact aesthetasc sensilla per pair of antennules varied from 0–20 per winner/loser pair (0–13 per animal in winners, 0–11 in losers), representing 0–0.5% of the ~2500 aethetascs per animal.

Correlations between the number of intact aesthetasc sensilla remaining after shaving either in winner, loser or both and the duration of fights and aggression levels of winners and losers were not significant (Spearman rank correlation). Thus, the presence of a few remaining aesthetasc sensilla did not significantly affect the overall outcome of this study. Although overall not significant statistically we will discuss the possibility that, particularly in losers, a few remaining aesthetascs could have mediated recognition of a familiar opponent.

Effect of temperature on fight duration and aggression

Fight durations were shorter at the lowest (6°C) and highest (23.5°C) temperatures (quadratic regression, r^2=0.06, t=–2.11, P=0.04). A total of six interactions were conducted at 6°C. Two of these resulted in no fight, one in an aesthetasc shaved pair (B) and one in a guard hair shaved pair (BB). Both winners and losers showed greater mean aggression at higher temperatures (linear regression, winner: r^2=0.29, t=5.44, P<0.0001; loser: r^2=0.18, t=3.95, P=0.0002), but the clear differences in mean aggression between winners and losers (see above) were not affected by temperature. The maximum aggression of losers but not winners increased with temperature (r^2=0.05, t=1.99, P=0.05).

We conclude that the effects of temperature on fight parameters did not differentially impact the treatments and thus the outcome of this study.

Discussion

The results of this study indicate that aesthetasc sensilla are necessary for recognition of individual urine pheromones in the American lobster *H. americanus*; non-aesthetasc chemosensory pathways were incapable of rescuing opponent recognition. We base this conclusion on the following evidence.

Normally, in a pair of intact lobsters, the duration of their second fight 1–7 days later is greatly reduced, frequently to

zero. Because the loser of such a pair will not reduce fight duration when faced with an unfamiliar animal who has won his previous fight against another lobster, this second-fight reduction or absence has been interpreted to reflect individual recognition of the former opponent. Recognition of a former opponent is based on information transmitted by urine pheromones *via* the lateral flagella (Karavanich and Atema, 1998b), but it was not known which sensillar type is involved. In the present study the removal of aesthetasc sensilla abolished the normal fight reduction, thus showing that aesthetascs are necessary to mediate individual recognition. Both the positive control of removing all chemoreception by distilled water lesion and the negative control of no lesion had been done several times previously under similar conditions (Karavanich and Atema, 1998a,b; Atema et al., 1999).

Our results in *H. americanus* complement studies on the role of aesthetasc sensilla in spiny lobsters, *Panilurus argus* (Steullet et al, 2000, 2001), where their role in social recognition was not investigated. The importance of aesthetascs in social behavior was also found in blue crabs (Gleeson, 1982) where partial removal of aesthetascs from the lateral flagella of male blue crabs resulted in reduced courtship responses in males; the response was absent in males with total aesthetasc tuft ablation.

While this lesion study focused on aesthetasc sensilla *vs* guard hairs, there are several other setal types present on the lateral flagellum of the antennule of *H. americanus* (Guenther and Atema, 1998) and *P. argus* (Cate and Derby, 2001). Most common are the serrulate setae, distributed all over the antennule, but not among the aesthetasc/guard hair tuft. The serrulate setae were thus not affected by either of the two shaving lesions and cannot have affected the differential outcome of the present study; their chemoreceptors cells could, however, have been affected by distilled water lesion. In the tuft region, closely associated with the guard hairs, are two relatively rare and very small setal types, feathered and cork-screw shaped setae of unknown function, the latter morphologically different from but perhaps homologous to asymmetric sensilla in *P. argus* (Cate and Derby, 2001). These setae were not specifically considered in this study. Based on their location and on post-operation inspection of antennules, both types appeared to be removed during guard hair shaving, while remaining intact after aesthetasc shaving. Therefore, we interpret 'guard hair shaving' results to include removal of these additional setal types.

This study on the sensory pathway for individual recognition points to the olfactory lobe with its typical glomeruli as the initial processing center for learning and memory of odors associated with social partners. The behavioral context of individual recognition in dominance is relatively well understood in lobsters; the fact that it also occurs in other crustaceans (for a review, see Atema and Steinbach, in press) suggests that it is more common than we believed at first. Both males and females learn about each other's individual odor in a dominance context (Atema et al., 1999), but this information may be used in more than dominance relationships; for example, individual recognition in courtship has not been studied in lobsters but is known in other decapod crustaceans (Atema and Steinbach, in press). Therefore, this study brings us one step closer to elucidating the physiological mechanisms and evolution of chemical recognition of individuals in invertebrates in general.

We consider the possibility that even a few aesthetasc sensilla may suffice to process individual recognition. The contribution of only a dozen sensilla was suggested in two of the three B pairs that did not fight after incomplete aesthetasc removal: subsequent deionized water lesion caused fight resumption in their C encounter, characteristic of now abolished recognition. There was also a weak negative correlation between the number of remaining intact aesthetascs in losers and the mean and maximum aggression levels of winners and losers in B fights (N=15, R=–0.37, r^2=0.14, P=0.17). This is interesting since it is the loser's recognition of the opponent that determines first his aggression level and then, indirectly, the responding aggression level of the winner and thus the duration of the fight (Steinbach and Atema, 2004). If individual recognition might be possible using only a few aesthetasc sensilla, and if each aesthetasc can be considered a 'replicate unit' (Steullet et al., 2000; Spencer, 1986) of receptor expression across its ~300 cells, then about 10 replications of 300 receptor cells might extract sufficient information from the urine signal to identify the learned odor of a former opponent. A separate study will be necessary to determine the minimum number needed for recognition of familiar opponents.

Aesthetasc shaving and distilled water lesion treatments affected primarily the decision to fight, and only to a smaller degree the intensity of the fight. This result seemed at first surprising, since we had shown earlier (Karavanich and Atema 1998a,b) that not only fight duration but also fight intensity decreased in subsequent fights. However, in the previous work we had included 'no-fight' interactions as expressions of mean fight intensity (expressed as 'agonistic value'), so that there too the reported decrease in mean fight intensity may have been caused primarily by the effect of no-fights, i.e. zero intensity. We point out that the fights of the six pairs that still fought after guard hair shaving were significantly shorter, thus still showing recognition. We interpret this continued fighting to mean that these six pairs had not completely resolved their dominance relationship in the first interaction and required some continued fighting. Such effects have been seen commonly in groups of lobsters freely establishing dominance relationships in naturalistic tanks (Morschauser and Atema, 2003).

Interactions were conducted successfully over a wide range of temperatures (6–23.5°C), with the great majority in the range of 12–23°C. Most of the six interactions conducted at 6°C showed shorter than average fight durations, including two resulting in no-fight, one each in the aesthetasc shaved and the guard hair shaved groups. In general, below 5°C lobster behavior begins to slow down, as observed in the laboratory and in the field (Karnofsky et al., 1989) until movement virtually stops at 2°C (J.A., personal observation), leading to

hibernation. We conclude that, apparently, individual recognition occurs over a wide range of temperatures.

One additional point warrants discussion. The analysis of aggression demonstrates intrinsic winner–loser effects. Excluding 'no-fight' interactions and comparing the remaining interactions revealed that there was no difference in fight intensity resulting from treatment or group and that the winner, in all treatments across both groups, always had a higher mean and maximum aggression score. This winner effect cannot be due to differences in sex (all males), size (pairs were within 3 mm CL), or molt state (all were hard-shelled and none molted in the weeks following the fights). Apparently, eventual winners consistently fight more aggressively than eventual losers. This intrinsic dominance difference may reflect 'confidence' resulting from genetic differences and from agonistic experience. It can form the basis for dominance hierarchies without individual recognition in other species (see discussion in Gherardi and Atema, 2005). It was also noticed earlier in *H. americanus* (Breithaupt and Atema, 2000) and indicates that both confidence and individual recognition play a role in the social organization of this species.

These results of this study provide important information and considerations for studies of the identification of individual recognition pheromones and dominance pheromones where it is useful to know not only the behavioral context of signal production but also the receptor pathways involved.

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References

Atema, J. (1977). Functional separation of smell and taste in fish and crustaceans. In *Olfaction and Taste*, vol. VI (ed. J. Le Magnen and P. MacLeod), pp. 165-174. London: Information Retrieval Ltd.

Atema, J. and Steinbach, M. A. (in press). Chemical communication in the social behavior of the lobster, *Homarus americanus*, and other decapod crustacea. In *Ecology and Evolution of Social Behavior: Crustaceans as Model Systems* (ed. E. Duffy and M. Thiel). Oxford: Oxford University Press.

Atema, J. and Voigt, R. (1995). Behavior and Sensory Biology. In *Biology of the Lobster, Homarus americanus* (ed. J. Factor), pp. 313-348. New York: Academic Press.

Atema, J., Jacobson, S., Karnofsky, E., Oleszko-Szuts, S. and Stein, L. (1979). Pair formation in the lobster, *Homarus americanus*: behavioral development, pheromones, and mating. *Mar. Behav. Physiol.* **6**, 277-296.

Atema, J., Breithaupt, T., LeVay, A., Morrison, J. and Edattukaran, M. (1999). Urine pheromones in the lobster, *Homarus americanus*: both males and females recognize individuals and only use the lateral antennule for this task. *Chem. Senses* **24**, 615-616.

Breithaupt, T. and Atema, J. (2000). The timing of chemical signalling with urine in dominance fights of male lobsters (*Homarus americanus*). *Behav. Ecol. Sociobiol.* **49**, 67-78.

Breithaupt, T. and Eger, P. (2002). Urine makes the difference: chemical communication in fighting crayfish made visible. *J. Exp. Biol.* **205**, 1221-1231.

Caldwell, R. (1979). Cavity occupation and defensive behaviour in the stomatopod *Gonodactylus festae*: evidence for chemically mediated individual recognition. *Anim. Behav.* **27**, 294-301.

Caldwell, R. L. (1985). A test of individual recognition in the stomatopod *Gonodactylus festae*. *Anim. Behav.* **44**, 11-19.

Cate, H. and Derby, C. D. (2001). Morphology and distribution of setae on the antennules of the Caribbean spiny lobster *Panulirus argus* reveal new types of bimodal chemo-mechanosensilla. *Cell Res.* **304**, 439-454.

Copp, N. (1986). Dominance hierarchies in the crayfish *Procambarus clarkii* (Girard, 1852) and the question of learned individual recognition (Decapoda, Astacidea). *Crustaceana* **51**, 9-24.

Cowan, D. and Atema, J. (1990). Moult staggering and serial monogamy in American lobsters, *Homarus americanus*. *Anim. Behav.* **39**, 1199-1206.

Derby, C. D. and Atema, J. (1982). The function of chemo- and mechanoreceptors in lobster (*Homarus americanus*) feeding behavior. *J. Exp. Biol.* **98**, 317-328.

Devine, C. and Atema, J. (1982). Function of chemoreceptor organs in spatial orientation of the lobster, *Homarus americanus*: differences and overlap. *Biol. Bull.* **163**, 144-153.

Gherardi, F. and Atema, J. (2005). Memory of social partners in hermit crab dominance. *Ethology* **111**, 271-285.

Gherardi, F. and Daniels, W. (2003). Dominance hierarchies and status recognition in the crayfish *Procambarus acutus acutus*. *Can. J. Zool.* **81**, 1269-1281.

Gherardi, F. and Tiedemann, J. (2004). Binary individual recognition in hermit crabs. *Behav. Ecol. Sociobiol.* **55**, 524-530.

Gleeson, R. A. (1982). Morphological and behavioral identification of the sensory structures mediating pheromone reception in the blue crab, *Callinectes sapidus*. *Biol. Bull.* **163**, 162-171.

Guenther, C. and Atema, J. (1998). Distribution of setae on the *Homarus americanus* lateral antennular flagellum. *Biol. Bull.* **195**, 182-183.

Hazlett, B. (1969). 'Individual' recognition and agonistic behavior in *Pagurus bernhardus*. *Nature* **222**, 268-269.

Horner, A. J., Weissburg, M. J. and Derby, C. D. (2004). Dual antennular chemosensory pathways can mediate orientation by Caribbean spiny lobsters in naturalistic flow conditions. *J. Exp. Biol.* **207**, 3785-3796.

Hurst, J. (1990a). Urine marking in population of wild house mice *Mus domesticus* Rutty. I. Communication between females. *Anim. Behav.* **40**, 223-232.

Hurst, J. (1990b). Urine marking in population of wild house mice *Mus domesticus* Rutty. I. Communication between males. *Anim. Behav.* **40**, 209-222.

Hurst, J. (1990c). Urine marking in population of wild house mice *Mus domesticus* Rutty. I. Communication between the sexes. *Anim. Behav.* **40**, 233-243.

Jacobson, S. (1977). Agonistic behavior, dominance and territoriality in the American lobster, *Homarus americanus.* PhD thesis, Boston University Marine Program, Boston, Massachusetts, USA.

Karavanich, C. and Atema, J. (1998a). Individual recognition and memory in lobster dominance. *Anim. Behav.* **56**, 1553-1560.

Karavanich, C. and Atema, J. (1998b). Olfactory recognition of urine signals in dominance fights between male lobster, *Homarus americanus*. *Behaviour* **135**, 719-730.

Karnofsky, E., Atema, J. and Elgin, R. H. (1989). Natural dynamics of population structure and habitat use of the lobster, *Homarus americanus*, in a shallow cove. *Biol. Bull.* **176**, 247-256.

Lowe, M. (1956). Dominance-subordinance relationships in the crawfish *Cambarellus shufeldtii*. *Tulane Studies Zool.* **4**, 139-170.

Miller-Sims, V. and Atema, J. (2004). Tuning of mechanoreceptors in the lobster antennule. *Chem. Senses* **30**, A117.

Morschauser, K. and Atema, J. (2003). Sex, dominance and individual recognition signals in lobster hierachies. Masters thesis, Boston University Marine Program, Boston, Massachusetts, USA. 65pp.

Oleszko-Szuts, S. and Atema, J. (1977). Chemical senses in the lobster *Homarus americanus*: fine structure of aesthetasc hairs. In *Workshop on Lobster and Rock Lobster Ecology and Physiology* (ed. B. F. Phillips and J. S. Cobb), pp. 16-17. Australia: CSIRO, Division of Fisheries and Oceanography.

Sandeman, D., Sandeman, R., Derby, C. D. and Schmidt, M. (1992). Morphology of the brain of crayfish, crabs and spiny lobsters: a common nomenclature for homologous structures. *Biol. Bull.* **183**, 304-326.

SAS Institute, Inc. (1995). *JMP Statistics and Graphics Guide (Version 3.1)*. Cary, NC: SAS Institute.

Schmidt, M. and Ache, B. (1992). Antennular projections to the midbrain of the spiny lobster. II. Sensory innervation of the olfactory lobe. *J. Comp. Neurol.* **318**, 291-303.

Schmidt, M. and Ache, B. (1993). Antennular projections to the midbrain of the spiny lobster. III. Central arborizations of motoneurons. *J. Comp. Neurol.* **336**, 583-594.

Schmidt, M. and Ache, B. (1996a). Processing of antennular input in the brain of the spiny lobster, *Panulirus argus*. I. Non-olfactory chemosensory and mechanosensory pathways of the lateral and median antennular neuropils. *J. Comp. Physiol. A* **178**, 579-604.

Schmidt, M. and Ache, B. (1996b). Processing of antennular input in the brain

of the spiny lobster, *Panulirus argus*. II. The olfactory pathway. *J. Comp. Physiol. A* **178**, 605-628.

Schmidt, M. and Ache, B. (1997). Immunocytochemical analysis of glomerular regionalization and neuronal diversity in the olfactory deuterocerebrum of the spiny lobster. *Cell Res.* **287**, 541-563.

Schmidt, M., Van Ekeris, L. and Ache, B. (1992). Antennular projections to the midbrain of the spiny lobster. I. Sensory innervation of the lateral and medial antennular neuropils. *J. Comp. Neurol.* **318**, 277-290.

Scrivener, J. C. E. (1971). Agonistic behavior of the American lobster, *Homarus americanus*. *J. Fish. Res. Bd. Can. Tech. Rep*. **235**, 1-113.

Spencer, M. (1986). The innervation and chemical sensitivity of single aesthetasc hairs. *J. Comp. Physiol. A* **158**, 59-68.

Steinbach, M. and Atema, J. (2004). Individual recognition in the lobster, *Homarus americanus*: the loser remembers. *Chem. Senses* **30**, A68.

Steullet, P., Cate, H. S., Michel, W. C. and Derby, C. D. (2000). Functional units of a compound nose: aesthetasc sensilla house similar populations of olfactory receptor neurons on the crustacean antennule. *J. Comp. Neurol.* **418**, 270-280.

Steullet, P., Dudar, O., Flavus, T., Zhou, M. and Derby, C. D. (2001). Selective ablation of antennular sensilla on the Caribbean spiny lobster *Panulirus argus* suggests that dual antennular chemosensory pathways mediate odorant activation of searching and localization of food. *J. Exp. Biol.* **204**, 4259-4269.

Steullet, P., Krutzfeldt, D., Hamidani, G., Flavus, T., Ngo, V. and Derby, C. D. (2002). Dual antennular chemosensory pathways mediate odor-associative learning and odor discrimination in the Caribbean spiny lobster *Panulirus argus*. *J. Exp. Biol.* **205**, 851-867.

Vannini, M. and Gherardi, F. (1981). Dominance and individual recognition in *Potamon fluviatile* (Decapoda, Brachyura) Possible role of visual cues. *Mar. Behav. Physiol.* **8**, 13-20.

Voigt, R. and Atema, J. (1992). Tuning of chemoreceptor cells of the second antenna of the American lobster (*Homarus americanus*) with a comparison of four of its other chemoreceptor organs. *J. Comp. Physiol. A* **171**, 673-683.

Zulandt Schneider, R., Huber, R. and Moore, P. (2001). Individual and status recognition in the crayfish *Orconectes rusticus*: the effects of urine release on fight dynamics. *Behaviour* **138**, 137-153.