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Male but not Female Olfaction is Crucial for Intermolt Mating in European Lobsters (*Homarus gammarus* L.)

Malin Skog

Department of Cell and Organism Biology, Lund University, Lund, Sweden

Correspondence to be sent to: Malin Skog, Department of Cell and Organism Biology, Lund University, Lund, Sweden. e-mail: malin.skog@cob.lu.se

Abstract

Chemical signals are common in most crustacean social interactions and are often perceived via chemosensory (olfactory) organs on the first antenna. Intermolt courtship behaviors and mating were investigated in size-matched pairs of intermolt European lobsters (*Homarus gammarus*) where the olfactory receptors of either the male or the female were lesioned with distilled water (olfactory ablation) or seawater (control). Matings or advanced male courtship behaviors (mounting and turning) were common in seawater controls and olfactory-ablated females. In contrast, when male olfaction was ablated with distilled water, there was not a single mating, and the only male courtship behaviors seen were a few very brief and unsuccessful mounting attempts. Individual females mated up to 5 times with different males, showing that previously inseminated females were still attractive to males. Thus, male but not female olfaction is crucial for intermolt mating in *H. gammarus*, indicating the presence of a female sex pheromone during the entire female molt cycle, not only at the time of molting. Female sex discrimination may be based on other cues from the male in combination with typical male behaviors.

Key words: antennule ablation, chemical communication, courtship, crustacea, reproductive behavior, sex pheromone

Introduction

Chemical courtship signals are among the oldest signals in the animal kingdom. The study of such signals started in the late 1950s with the discovery of sex pheromones in the silk moth, *Bombyx mori* (Schneider 1957; Butenandt et al. 1959), and today many insect pheromones are well known and used to control pest species. In most insects the female releases a pheromone (or pheromone mixture) that the male detects with olfactory receptors on his antenna and uses to find her by searching upwind (Wright 1958; Kennedy 1983; Hansson 2002; Wyatt 2003).

Mammal pheromone research has mainly focused on social, sexual, and individual recognition in rodents. Often these are either contact signals or pheromones released into the urine and received via olfactory and/or vomeronasal receptors (Johnston 2003; Bielsky and Young 2004; Brennan and Kendrick 2006).

Despite the commercial value of many crustacean species, the molecular structure of their sex pheromones still remains mostly unknown. However, the presence and importance of chemical courtship signals, often in the urine, has been demonstrated in many taxa. For example, in several crab species, premolt female urine contains a still unknown sex pheromone, which elicits male courtship behaviors toward stones, sponges, and even other males (e.g., *Portunus sanguinoletus* [Christofferson 1978]; *Callinectes sapidus* [Gleeson 1991]; *Carcinus maenas* [Bamber and Naylor 1997; Hardege et al. 2002; Ekerholm and Hallberg 2005]; *Telmessus cheiragonus* [Kamio et al. 2000]). This chemical communication seems to have both distance (olfactory) and contact (gustatory) components.

Urine communication is believed crucial for normal courtship and mating behavior in American lobsters (*Homarus americanus*), a topic that has been recently reviewed by Atema and Steinbach (2007). American lobster female sex pheromones and mating were described for example by Atema and Engstrom (1971). Mating in *H. americanus* often takes place when the female is newly molted (soft shelled) and is associated with a 1 to 2 week long male–female pair-bond and cohabitation between a female and a dominant male, but American lobsters may also mate when the female is intermolt (hard shelled) (Atema and Steinbach 2007). Males and females that encounter each other outside shelters often fight, but intermolt females may enter male shelters with only mild male aggression, cohabit with the male for short times, and receive mating attempts. Female urine signals may help reduce male aggression toward the female and facilitate mating (Bushmann and Atema 1997).

Intermolt matings have become accepted as an alternative reproductive strategy utilized both by very large American lobster females that extrude eggs twice between molts and need to replenish their store of sperm and by smaller females that failed to mate at their molt or were inseminated with a very small spermatophore (Waddy and Aiken 1990, 1991; Waddy et al. 1995; Atema and Steinbach 2007).

The urinary communication signals in lobsters are most likely perceived via the olfactory sensory organs situated on the first antenna (antennule), possibly combined with other chemoreceptors. The use of visual cues is not very likely because lobsters are mostly nocturnal animals, and courtship and mating can occur normally in temporarily blinded lobsters (Snyder et al. 1992). The olfactory receptors on the antennules are also important for recognition of fight opponents in lobster males (Karavanich and Atema 1998).

The biology of the European lobster *Homarus gammarus*, including reproductive behaviors and communication, has received far less attention than its closely related American counterpart. Intersexual interactions in *H. gammarus* were studied by Debuse et al. (1999, 2003), determining the influence of sex ratio and shelter abundance on competition in mixed-sex groups of 6 lobsters. They regarded the courtship behavior of *H. gammarus* as "similar to that of the American lobster."

However, advanced courtship interactions often took place outside shelters in the study by Debuse et al. (2003). Courtship outside shelters is not reported in American lobsters, unless the provided shelters were too small for 2 animals (Atema 1986; Karnofsky and Price 1989; Cowan and Atema 1990). Further, shelter-owning European lobster males were not involved in courtship interactions more often than those lacking shelters, possibly demonstrating different use and/or importance of shelters for courtship and mating between the 2 species (Debuse et al. 2003).

This study investigates intermolt courtship behaviors and mating in the European lobster and how the loss of olfaction through bilateral lesion of olfactory receptors in either the male or the female affects these behaviors. Based on what is known from American lobster sexual communication, the hypothesis is that antennule ablations of both the male and the female will affect normal intermolt interactions negatively and lead to behavioral deviations.

Materials and methods

Animals

Experiments were conducted during June–August 2006 and 2007 at Kristineberg Marine Research Station in Fiskebäcks-kil, Sweden. Local commercial fishermen caught European lobsters (females 70–106 mm carapace length (CL), males 70–100 mm CL), which were given at least 1 week of acclimatization to laboratory conditions before being used in behavioral tests. All animals were released back to the approximate area where they were caught after the completion of the experiment. National guidelines for keeping invertebrates as experiment animals were followed.

Animals were housed individually in circular 80-L tanks or separated in individual mesh compartments in 300-L communal tanks, maximum 6 individuals in each tank. Thus, individuals held in communal tanks received visual and chemical information about the other lobsters held in the tank but could not interact physically. The sexes were kept in separate tanks, and each individual was provided with a polyvinyl chloride cylinder shelter. Ambient deepwater (from 35-m depth in the Gullmar fjord, temperature between 15 and 17 °C) was continuously flowing through each tank.

Lobsters were maintained at a light:dark cycle resembling the natural light (Swedish summer light regime 16–18 h light:6–8 h dark) and fed pieces of fish or mussel once a week. They were individually marked with one or several colored rubber bands placed behind the dactylus of one claw, not affecting the normal use of that claw. Only intermolt animals were used, and molt stage was evaluated weekly by examination of the pleopods (Aiken 1973). Only one of the used females molted at the end of the summer, and none carried or extruded eggs. No animals were injured during the experiment.

General procedure

One male and one female lobster was paired by size (CL difference <5 mm [Scrivener 1971]) and allowed to interact in a ~200-L glass aquarium lit from above. Foothold for the animals was provided by covering the bottom with 2–3 cm of >1-mm sieved and rinsed shell sand. The glass walls of the aquarium were covered on 3 sides by white paper sheets to prevent movements outside the tank to affect the lobsters during the interaction and video recorded through the fourth side with a digital video camcorder (Sony Handycam DCR-HC90E).

The aquarium was filled with fresh seawater prior to each interaction, and the 2 lobsters were placed on each side of a removable opaque plastic divider placed in the middle of the aquarium. The animals, separated by the divider, were allowed to acclimatize for 10 min to the new conditions before lifting the divider. Intersexual interactions were filmed for approximately 30 min, starting just before the divider was lifted.

Antennule ablations

Either the male or the female in each pair was treated with distilled water to block the olfactory input from the antennules temporarily (Karavanich and Atema 1998). The exact mechanism for olfactory blocking is unknown, but it is related to osmotic shock being induced in the olfactory (aesthetasc) hairs on the antennules by distilled water. The treatment is specific to the aesthetascs, lasts for about a day, and is reversible over the next few days (Derby and Atema 1982; Gleeson et al. 1996, 1997).

The treatment animal was wrapped in a damp cloth, and the entire antennules (outer and inner branches) were dipped in a small vial containing distilled water for 10 min. After this treatment, the animal was left for 20 min in a semidark 30-L plastic container with flow-through seawater to recover from handling, followed by the acclimatization of both animals to the interaction aquarium and subsequent interaction according to the general procedure. During the treatment and recovery time, the nontreated animal in each pair was placed in a small (30 L) glass aquarium with comparable water, light, and bottom substrate conditions to the interaction aquarium ($N = 10$ for both male and female ablations).

Sham ablations (control)

To control for handling effects, sham ablations (where the antennules were dipped in fresh seawater instead of distilled water) were performed. The treatment of animals was identical to that in the real antennule ablations in all other ways ($N = 10$ for both male and female sham ablations).

Two-day interactions

Pairs of untreated lobsters met in 2 consecutive interactions 24 ± 4 h separated according to the general procedure to evaluate possible recognition between the animals. Between the interactions, both lobsters were returned to their respective storage tanks overnight ($N = 15$).

Video analysis

The number, latency (from lifting the divider to the first start of that behavior), duration of the behavior the first time it is performed (first duration), and total duration of each behavior during the entire interaction (summed duration) of 5 intersexual behaviors (Table 1) were analyzed in all interactions by an analyzer unaware of which treatment the interactions belonged to.

Male mouthpart (maxillipede) touching could coincide with mounting and turning, but all other behaviors are treated as mutually exclusive. Mouthpart touching, mounting, and turning are together referred to as male precopulatory behaviors.

Pairs were not separated after copulation and mating success was determined by whether or not the male performed ejaculatory thrusts with his abdomen. Successful matings and mating attempts (without male ejaculation) were treated together in the analysis. The transfer of a spermatophore could often be ascertained by examination of the female after the entire interaction period, but some females may have removed the spermatophore during postcopulatory grooming.

Due to a shortage of size-matched animals, the 55 interactions were conducted using 23 females and 31 males, and most animals had to be used more than once. However, each pairing was unique, that is, the same 2 animals (male and female) never met more than once. Further, the behavior of the animals did not seem to be affected by earlier use in the experiment. For example, males performed as much mounting and turning with previously mated and inseminated females as with other females. The 19 matings and attempted matings (successful turnings) involved 14 different males and 13 different females. Females mated 1–5 (!) times during 2 months (July and August) with different males and males mated between 1 and 4 times in the same time span. Being fully aware that my data do not quite meet the assumption of independency, I still treat each unique pair as a separate replicate in the data analysis.

Table 1 Definitions of intersexual behaviors used in the video analysis

Sex[a]	Label[b]	Intersexual behaviors[c]
F	Present tail	The female turns in front of the male, positioning her tail directly in front of him, and stops moving
M	Mouthpart touching	The male uses the maxillipedes to touch the female, usually on the tail/carapace before and during mounting and turning
M	Mount	The male climbs onto the females carapace, usually from behind
M	Turn	The male uses his walking legs and maxillipedes to turn the female after mounting is completed
F/M	Copulation	The female is on her back with outstretched claws, the male is on top of her. The male inserts his gonopods into the females' spermatheca
M	Ejaculation[d]	Several rapid thrusting movements by the abdomen of the mail signify the ejaculation of his spermatophore and thus mating success

[a] The sex of the lobster that performed the behavior. F, female; M, male.
[b] The label used to refer to the behavior in the text (mouthpart touching, mounting, and turning may be referred to as male precopulatory behaviors).
[c] Adapted from Atema et al. (1979).
[d] Not included in the statistical analysis.

Statistical analyses

The data set did not conform to normal distribution after transformations, necessitating the use of nonparametric statistical tests. Two-day interactions were compared pairwise using 2-tailed Wilcoxon signed-rank tests, and the different ablation treatments were compared using 2-sample Wilcoxon rank sums tests (2 tailed). Seawater sham ablations did not differ statistically between sexes and were pooled to form seawater control ($N = 20$). Distilled water ablations for males and females were compared with the pooled seawater control data. To control for this multiple comparison, I used a Bonferroni correction of alpha ($0.05/n$, $n = 2$) to 0.025. Only P values below this corrected alpha value should be regarded as significant.

Results

Intersexual behaviors and intermolt mating

Intermolt matings have not been described previously in European lobsters, and the following is a description of some common behaviors performed by one or both sexes during the intersexual interactions (e.g., mouthpart touching, mounting, turning, copulation, and presenting tail) that are highly unusual or never seen at all in same-sex interactions.

Male-specific behaviors were more or less directly involved in the actual copulation and included male precopulatory behaviors and ejaculation. During mouthpart touching (Figure 1), the male touched or grasped the female with the insides of his third maxillipedes, usually on her abdomen or dorsal carapace. This behavior was normally closely followed by the male attempting to mount the female, that is, climbing onto her back from behind and grasping her with his walking legs. A few males unsuccessfully tried mounting females from the side or from the front.

After successful mountings, the male directly proceeded to try and turn the female around to a backdown position. For this, he used walking legs 1–3 and the third maxillipedes on both sides to grasp and turn the female.

Complete turnings were in turn followed by copulation, where the male inserted his gonopods into the females' spermatheca. The gonopods were locked in place by the fifth walking leg on either side being held horizontally in front of them. The male kept his balance by resting the tips of his claws on the substrate, his walking legs still grasping the female. Both animals usually beat their pleopods continuously during the copulation, which lasted up to several minutes. Finally, the male performed several rapid thrusting movements by the abdomen, signifying the ejaculation of his spermatophore (which could most often also be ascertained by examination by the female after the interaction).

Almost immediately after this, the female tail flipped to right herself, and both animals usually started grooming themselves using the fourth and fifth pairs of walking legs. In a few cases with ejaculation by the male, no spermatophore was found, and it was assumed that the female removed it during the postcopulatory grooming. Mouthpart touching has never been observed in same-sex interactions in our laboratory and same-sex mounting only a few times, probably used to enforce dominance relationships, and was never continued to turning.

After successful copulations (including ejaculation), the male usually became aggressive toward the female or ignored

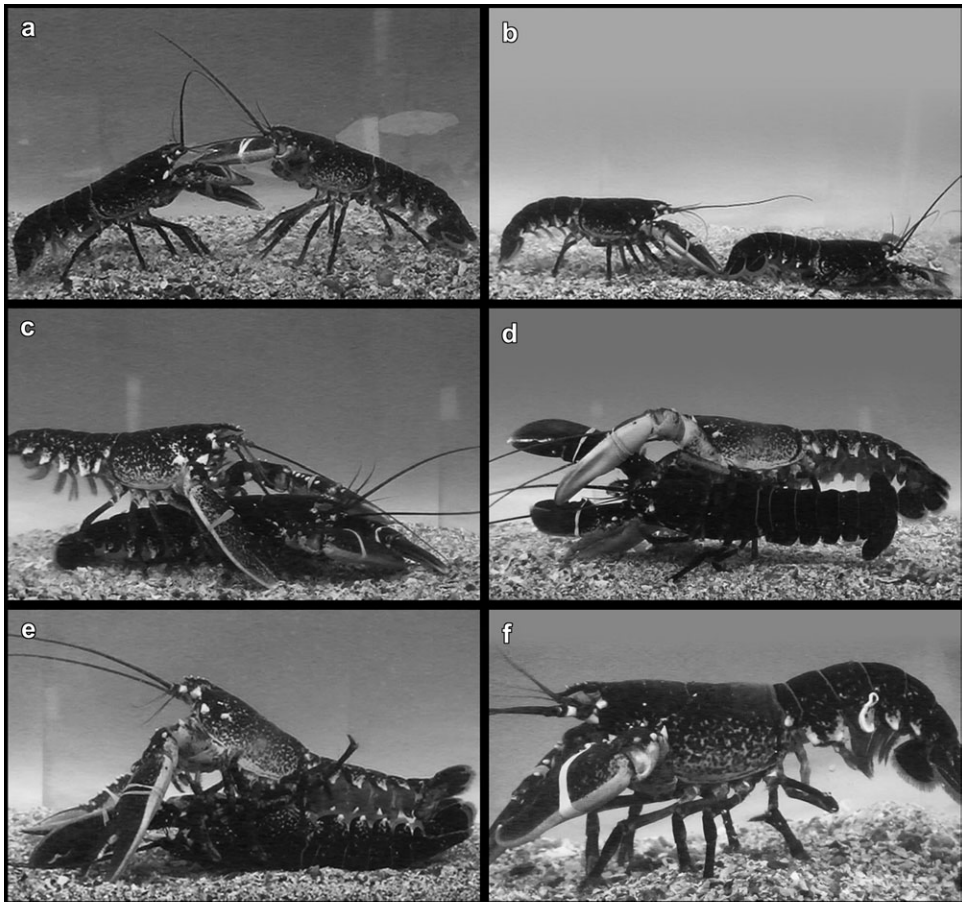

Figure 1 Examples of intersexual behaviors during European lobster (*Homarus gammarus*) intermolt mating. **(a)** Initial aggressive interaction between the male and the female. **(b)** The female (right) presents her tail to the male. **(c)** The male mounts the female while touching her with his mouthparts (third maxillipeds). **(d)** The male turns the female around. **(e)** Copulation (female below). **(f)** Postcopulatory grooming by the male.

her, but one pair copulated 3 times within one 30-min interaction, seemingly with successful spermatophore transfers (male ejaculation) all 3 times!

The female often presented her tail (Atema et al. 1979; Snyder et al. 1992; Bushmann and Atema 1997, 2000) to the male, that is, she turned around from facing the male so her tail was directly in front of him, where she halted.

Mounting and turning attempts were either accepted or actively resisted by the female. Acceptance was shown as stillness and submissive postures in the female, as well as stretching out claws and walking legs forward to allow the male to turn her onto her back. Resistance of the male's advances was demonstrated by the female tail flipping or walking away to shake off the male and spreading the walking legs and claws wide to resist turning.

In lobster pairs where the female, not the male, became dominant in the initial aggressive interaction (10 out of 55 interactions), males only very rarely (2 interactions with dominant females) showed precopulatory behaviors (mouthpart touching, mounting, and turning attempt), and there were no matings.

Antennule ablations

In the 20 seawater control (sham ablation) interactions (SW), there were 4 matings or attempted matings and 9 further mountings and/or attempted turnings. 10 interactions with female antennules treated with distilled water (dw-F) produced 3 matings/mating attempts and an additional 3 cases of mounting and/or attempted turning. In contrast, distilled water treatment of males (dw-M) resulted in 3 unsuccessful mounting attempts, but no turning attempts or matings. Males in these interactions were usually either very aggressive or very inactive.

Intersexual behaviors did not differ significantly between dw-F and SW in any way. The opposite was true when comparing dw-M and SW, which produced a number of behavioral differences (2-sample Wilcoxon rank sums tests, Table 2, Figure 2). All male precopulatory behaviors (mouthpart touching, mounting, and turning) as well as female's presenting tail were significantly fewer and had shorter first durations and summed durations in dw-M treatments than in SW treatments. The latency of those behaviors present in dw-M interactions did not differ from that in SW interactions.

Two-day interactions

In fifteen 2-day interactions (i.e., 30 interactions in all), there were 10 matings or mating attempts (successful turning of the female) and 12 other cases of mounting and/or attempted turning. Seven of the matings/mating attempts took place on the first day of the interaction, and the additional mountings/turnings were equally distributed between day 1 and day 2 (6 on each day).

Table 2 Comparisons of intersexual behaviors in distilled water treatment (olfactory ablated) males and seawater control (sham ablation) treatments

Behavior[a]	Number[b]	1st duration[c]	Summed duration[d]
Mouthpart touching	**0.0025**[e]	0.0328[e]	**0.0073**[e]
Mounting	0.0340[e]	NS	**0.0244**[e]
Turning	0.0406[e]	0.0406[e]	0.0406[e]
Copulation	NS	NS	NS
Present tail	0.0487[e]	NS	0.0483[e]

NS, not significant.

[a] For definitions of behaviors, see Table 1.

[b] The number of times each behavior was performed during the entire 30-min interaction.

[c] The duration of each behavior the first time it was performed.

[d] The total (summed) duration of each behavior during the whole 30-min interaction.

[e] *P* values from comparisons between dw-M (olfactory-ablated males, $N = 10$) and SW (seawater sham ablation, $N = 20$) using 2-sample Wilcoxon rank sums test for independent samples in European lobster size-matched male–female pairs. Alpha was Bonferroni corrected for multiple comparisons to 0.025. Only values below 0.025 (bold) should therefore be regarded as statistically significant. *P* values close to significance ($0.025 < P < 0.05$) are also presented.

The latency to the first mouthpart touching was significantly shorter in the second interaction than in the first ($P = 0.039$, 2-tailed pairwise Wilcoxon signed-rank test, Figure 3), but there were no other significant differences in the number, first duration, or summed duration of any intersexual behavior between the 2 days.

Discussion

This study presents the first description of intermolt mating in *H. gammarus* and reports several behaviors that are rare or never seen in same-sex interactions.

Reproductive behaviors in the European lobster have not received much interest but have been assumed to be similar or identical to those in the American lobster (Debuse et al. 2003). Debuse et al. (1999, 2003) studied how intersexual interactions are affected by different shelter abundances and sex ratios. Courtship and matings occurred both inside and outside shelters, but the molt stage of animals before or during these experiments was not defined. However, Debuse et al. (1999) noticed that all females produced eggs at the end of their one-summer study, which rules out the possibility of these females molting during the experiment. Therefore, all matings seen in the study by Debuse et al. (1999) must have involved intermolt females, even though this was not commented upon by the authors.

In this study, intermolt matings and mating attempts were common; mating, turning, and/or mounting by the male were seen in 60–70% of all control intermolt interactions, including those where the same pair met on 2 consecutive days.

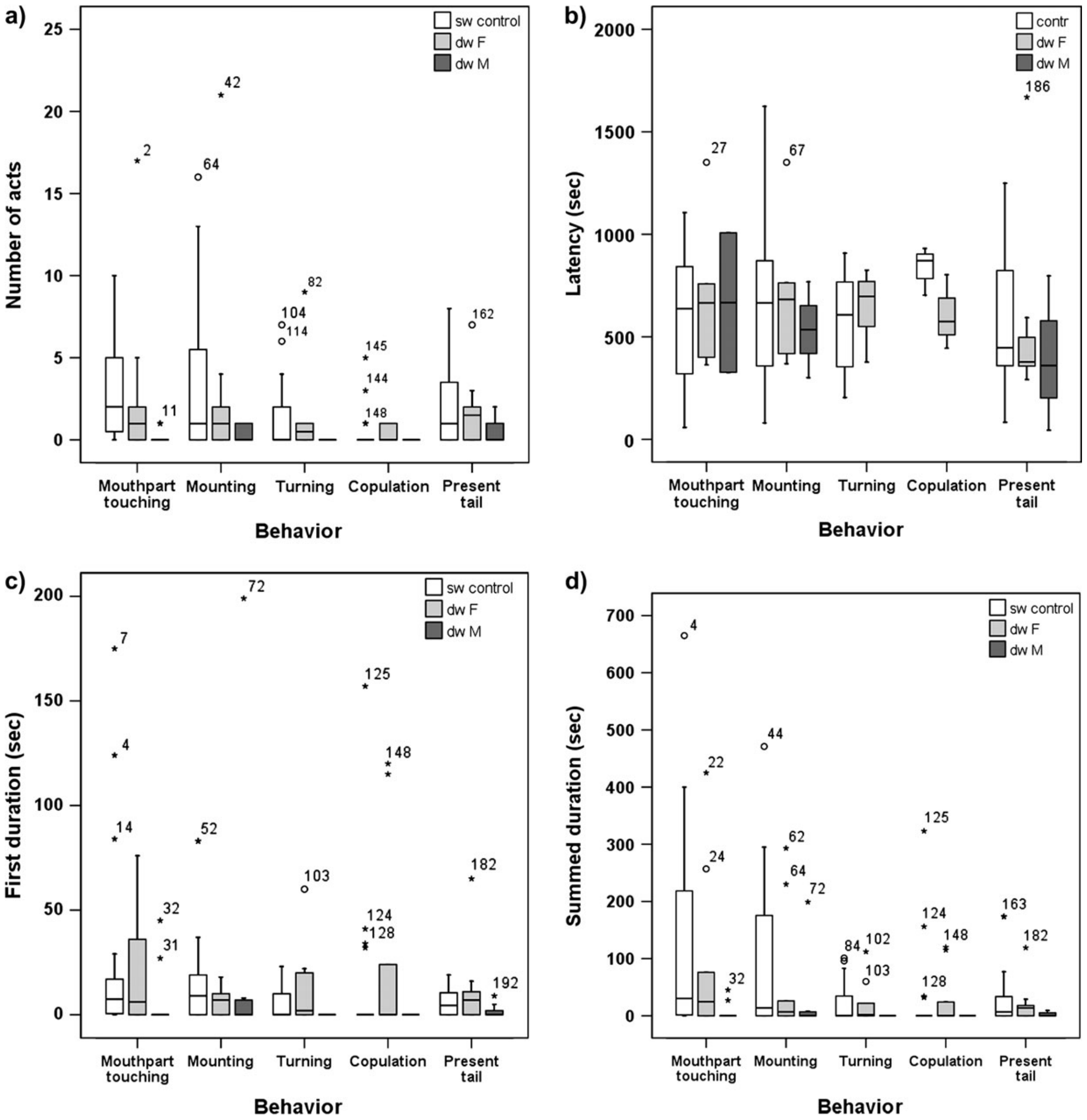

Figure 2 Boxplots over intersexual behaviors (Table 1) in size-matched pairs of one male and one female European lobster, where either the male or the female antennules are treated with seawater (control) or distilled water (olfactory ablation). Boxes represent the interquartile range of data, the mid bar is the median, and the whiskers show minimum and maximum values in the data range. Outlying data points separated from the box by more than 1.5 times the interquartile range are represented by circles and extreme outliers (separated by more than 3 times the interquartile range) by asterisks. White bars (control SW, *N* = 20): pooled data for seawater sham ablations of males or females. Light gray bars (dw-F, *N* = 10): female distilled water ablation of the antennules. Dark gray bars (dw-M, *N* = 10): male distilled water ablation of the antennules. **(a)** The number of times each behavior was performed during the whole interaction. **(b)** Latency: the time (in seconds) from the start of the interaction to the first instance of each behavior. **(c)** First duration: the duration (in seconds) of each behavior the first time it was performed. **(d)** Summed duration: the total duration (in seconds) of each behavior during the whole interaction.

Even though the forced encounters between males and females in the present study were held in a relatively small confined area, the high frequency of matings and male precopulatory behaviors may indicate that intermolt reproduction is a common phenomenon in *H. gammarus*. However, molt-related pair formation and mating, as seen in American lobsters (Atema and Steinbach 2007), could not be expressed under these circumstances. Long-term studies of European lobsters in naturalistic settings are needed to elucidate the importance of the "soft-shelled" mating strategy in *H. gammarus*. Both the smallest (CL = 70 mm) and the largest (CL = 107 mm) female in the study were mated, showing that female size and maturation stage did not affect male behaviors. Different ovary conditions in the females

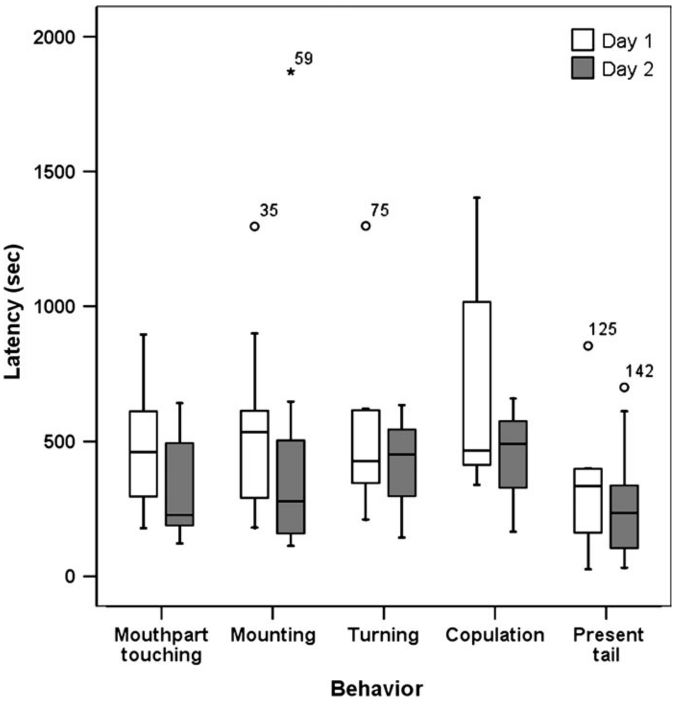

Figure 3 Boxplot of the latency (the time in seconds from the start of the interaction to the first instance of each behavior) of intersexual behaviors in size-matched pairs of one male and one female European lobster that met on 2 consecutive days (N = 15). Boxes represent the interquartile range of data, the mid bar is the median, and the whiskers show minimum and maximum values in the data range. Outlying data points separated from the box by more than 1.5 times the interquartile range are represented by circles and extreme outliers (separated by more than 3 times the interquartile range) by asterisks. White bars: first day interactions. Gray bars: second day interactions between the same pair of lobsters.

may have affected their attractiveness to males and willingness to mate.

Why intermolt mating?

In *H. americanus*, intermolt mating has become accepted as an alternative mating strategy for females that failed to mate or received an insufficient amount of sperm at the time of their molt and for very large females that spawn twice between molts and may need to replenish their amount of stored sperm (Waddy and Aiken 1990, 1991; Atema and Steinbach 2007). Female lobsters will extrude eggs whether or not they can fertilize them with stored sperm (Waddy and Aiken 1991; Waddy et al. 1995), and uninseminated females that drew close to spawning often became "desperate" in seeking a male for "last-minute" insemination, their activity intensified, and they became increasingly attractive to males, probably through some chemical signal (Waddy and Aiken 1991). Some females in this study may belong to either of these categories, but it is likely that intermolt mating may also have other explanations, for example, female reduction of male aggression and sperm competition with last-male sperm precedence.

Reduction of male aggression

Almost all intersexual interactions in this study started aggressively, and even if no animals were injured during these initial fights, this is always a risk in all well-matched lobster interactions. Thus, intermolt mating may be risky, both for lobster males and females. In American lobsters, intermolt females may enter the shelter of males, cohabit with them shortly, and receive mating attempts. This cohabitation is associated with a number of behavioral signals that induce mating and reduce aggression, thereby decreasing the risk for injury. Once inside, the *H. americanus* female displayed submissive behaviors: lying flat and presenting her tail. Female tail presentation was seen in this study as well and may be interpreted as an appeasing/aggression-reducing behavior. Possibly, the following matings also serve to reduce male aggression.

Lobster matings may be either consenting or forced from the female point of view (Waddy and Aiken 1991; personal observation), but forced matings are likely rare in nature because females can evade unwanted suitors through hiding in shelters, avoidance, or aggressive resistance. Unwilling females in this study sometimes actively resisted male mounting and turning attempts through spreading their claws and walking legs wide and tail flipping or walking away from the male, and males never used their claws to grab or try and turn females after the initial aggressive phase.

The male had to become dominant over the female in the initial fight in order to continue to precopulatory courtship behaviors and possibly mating. In the 18% of the interactions where females became dominant, there were almost no sexual behaviors, and the few subordinate males that did show precopulatory behaviors never succeeded in turning the female over and thus never mated. Dunham (1979) also noted that male American lobsters must become dominant over the female before any mating behaviors could occur. Therefore, it is likely that natural intermolt matings involve different-sized animals, with a larger male that can dominate the female.

Sperm competition and last-male sperm precedence

Sperm competition is a common phenomenon in all animals that may mate multiply. In many species, there is last-male sperm precedence, meaning that the last male to mate with a certain female fathers most (if not all) of her offspring. Such is the case in many insects like dragonflies (Córdoba-Aguilar et al. 2003), the honeybee *Apis mellifera* (Franck et al. 2002), and the cellar spider *Pholcus phalangoides* (Schaefer and Uhl 2003); in some birds (Briskie 1996); as well as in some crustaceans like the crayfish *Austropotamobius italicus* (Galeotti et al. 2007), the snow crab *Chionoecetes opilio* (Sevigny and Sainte-Marie 1996), and some fiddler crabs of the genus *Uca* (Murai et al. 2002; Dyson 2008).

In the lobster, the possibility of last sperm precedence has been proposed previously, for example by Debuse et al. (1999), who argued that the long period of receptivity in

H. gammarus may improve the chances for a lobster female to mate with a genetically superior male. Females that were inseminated with a sufficient amount of sperm at the last molt would then be expected to mate opportunistically with other, superior males encountered later to ensure the best possible fitness for their offspring. Male lobsters would likewise be expected to attempt mating with any encountered female that they encounter, willing or unwilling, regardless of her previous inseminations.

Reuse and previous insemination

The reuse of animals in this study was unfortunate but necessary due to the low number of animals caught during 2007. Both males and females were capable of mating several times, and multiple (2–5) successful spermatophore transfers by different males to the same female imply that the reuse of females, whether previously inseminated or not, did not affect male behavior significantly. In 2 cases, the same pair of lobsters copulated on both days of the 2-day interactions, even though spermatophore transfer was successful on the first day. Similarly, one pair in the seawater control treatment mated 3 times within one 30-min interaction, seemingly with successful spermatophore transfers (male ejaculation) all 3 times. Thus, previous insemination of the female in the experiment obviously did neither discourage males from displaying precopulatory behaviors or mating with a female nor affect the frequency of female resistance to male advances.

In the American lobster, Waddy and Aiken (1990) reported that inseminated females were no longer receptive and received no further mating attempts when held with the same males for several months, whereas others conclude that the previous sperm load (or even the presence of eggs) in the intermolt female did not affect entry into a male shelter and/or intermolt matings (Dunham and Skinner-Jacobs 1978; Bushmann and Atema 1997) or that postmolt *H. americanus* females were multiply receptive despite several previous matings (Snyder et al. 1992).

Pheromone communication

Urinary and pheromone communication is common in many animals including crustaceans and is used in a number of contexts, for example, mate attraction, alarm signaling, or territory marking (Lewis and Gower 1980). Urinary signals are common as territorial markings in many mammals and are involved in kin recognition and individual identification in rodents (Brennan 2004; Thom and Hurst 2004). Communication by urinary pheromones has also been convincingly demonstrated in many crab species. Male crabs can be induced to pick up sponges, stones, and even other male crabs treated with female pre- or postmolt urine in a typical precopulatory embrace, and female urine may also attract males from a distance (Kamio et al. 2000; Hardege et al. 2002; Ekerholm and Hallberg 2005). Southern temperate spiny lobster *Jasus lalandi* females and American lobster females are attracted from a distance to conspecific male shelters by chemical signals in the male urine (Bushmann and Atema 1997, 2000; Raethke et al. 2004). Chemical and/or urine signals are also important in aggressive interactions and needed for social recognition in many crustacean species (Caldwell 1992; Karavanich and Atema 1998; Breithaupt and Atema 2000; Zulandt Schneider et al. 2001; Breithaupt and Eger 2002).

Chemical communication signals are by definition perceived by olfactory (distance) or gustatory (contact) chemoreceptors. In insects, pheromones are detected by olfactory receptors on the antenna, and in terrestrial vertebrates, the vomeronasal organ and/or olfactory epithelium in the nose are responsible for pheromone reception (Hildebrand 2001; Hansson 2002; Brennan and Kendrick 2006). In fish, the olfactory epithelium is very sensitive to pheromones, but other chemosensory systems may also be involved in the perception of pheromones (Sorensen 1992; Sorensen et al. 1998). The proposed olfactory organ of crustaceans is a tuft of sensory hairs on the antennules, whereas gustatory chemoreceptor hairs are distributed mainly on the mouthparts, walking legs, and claws (Laverack 1968; Ache 1982; Atema 1985; Hallberg et al. 1992, 1997).

Male olfaction needed in *H. gammarus*

This study demonstrates that male olfaction is needed for normal intermolt interactions in *H. gammarus*. Matings and advanced precopulatory behaviors (turning/turning attempts) occurred often (60–70% of the interactions) in pairs with seawater-treated (control) males and females as well as in pairs with distilled water–treated (olfactory ablated) females. In contrast, when the male antennules were ablated with distilled water, disrupting his olfaction, there were no turning attempts and consequently no matings. A few treatment males (~ 30%) displayed mouthpart touching and unsuccessful mounting attempts, but these behaviors were significantly fewer and had shorter first durations and summed durations than in interactions with seawater-treated controls.

This dependence on male olfaction in intermolt interactions suggests the presence of a female sex pheromone that is needed for intermolt courtship and copulation. This female pheromone may be produced throughout the female molt cycle, as opposed to the female molting pheromones found in crabs (Gleeson 1991; Bamber and Naylor 1997; Kamio et al. 2000; Hardege et al. 2002). Possibly, this female sex-specific pheromone, detected by chemoreceptors on the male antennule, is enough to reduce male aggression and induce mating in lobsters at any time of the female molt cycle. Female sex discrimination may be based on other (contact) chemosensory, visual, or tactile cues from the male, in combination with typical male behaviors such as mouthpart touching and mounting.

Differing results from other studies?

Disruption of male but not female olfaction through antennule excisions also had impact on courtship and mating in the spiny lobster *J. lalandi* and produced slightly delayed matings and reduced clutch sizes (Raethke et al. 2004). In the crayfish *Procambarus clarkii*, both the outer and the inner flagellum of the forked antennule were used for chemical sex discrimination and localization of potential partners by males as well as females (Dunham and Oh 1992; Giri and Dunham 2000).

Both soft-shell and intermolt matings are considered to be associated with a number of chemical and behavioral signals in *H. americanus*. These were studied by Bushmann and Atema (1994, 1997). Male–male shelter visits were characterized by high aggression, and a visiting male only entered the shelter if the resident was evicted. In contrast, female visitors to male shelters normally elicited only mild aggression by the resident and were allowed to enter regardless of their molt stage. The urine release rate of visiting females was increased, and when female urine release was blocked by catheters, she was met with as much aggression as a visiting male (Bushmann and Atema 1997, 2000). Bushmann and Atema therefore concluded that female *H. americanus* urine contains a signal that reduces aggression and facilitates courtship and mating regardless of the female's molt stage, similar to the proposed female sex pheromone in *H. gammarus* in this study (Bushmann and Atema 1994, 1997).

Two studies of chemical signals in American lobster intersexual interactions go against the majority of evidence; Cowan (1991), who found that female but not male olfaction was important for normal cohabitation and soft-shell mating, and Snyder et al. (1993), who even doubt the role of urine communication in lobsters because catheterized pairs with an intermolt male and a soft-shell female performed normal courtship and mating behaviors in their experiments.

Newly molted females likely release a number of nonurinary chemical signals, which may be used by a male to deduce her molt state, especially in close quarters. He could then proceed with normal courtship and mating behaviors despite the female being catheterized, possibly explaining the curious results in the study by Snyder et al (1993). In the study by Cowan (1991), olfactory-ablated males were seen using their walking legs and mouthparts (third maxillipedes) to an abnormal extent, possibly compensating for their loss of olfaction through the use of contact chemoreceptors.

Thus, urinary signals perceived by the olfactory receptors on the antennules are likely a major channel in lobster sexual communication, as the majority of evidence points out, but other signals and contact chemoreception may be used as a complement in certain cases. This way, lobster males that lose their antennules in a fight may detect receptive females using other chemoreceptors, until their antennules are regrown and the olfactory sense again functioning fully.

Conclusion

From this study, we can conclude that intermolt matings may be a common phenomenon in European lobsters and that male but not female olfaction is crucial for normal intermolt courtship and mating behaviors. This may indicate the presence of a female sex pheromone that is present throughout the female molt cycle (not only at the time of molting) and is enough to reduce aggression and induce mating in *H. gammarus* and possibly in *H. americanus* as well.

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