Chemical Communication in Crayfish

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Chapter 13
Chemical Communication in Crayfish

Thomas Breithaupt

Abstract Crayfish are a species rich group of large decapod crustaceans that inhabit freshwater environments. Having served as important models for the study of the neural and hormonal control of behavior crayfish were among the first crustacean taxa that were reported to use sex pheromones. Decades of research on crayfish chemical communication have, after initial controversies, now generated a comprehensive picture of the role of pheromones in resolving combats and in initiating sexual interactions. Moreover, the structures involved in chemical signal emission and reception have been identified in most cases. Urine, released in the head region, conveys the chemical messages and is directed via water movements such as gill currents or maxilliped generated currents to the receiver. Chemo-receptors on the first antennae were shown – in most cases – to be responsible for pheromone detection. Urinary signals reduce the duration of aggressive interactions and are crucial for the development of a linear dominance hierarchy. The social hierarchy is based on chemical recognition of the dominance status between combatants. Males are more active than females in initiating reproductive interactions. They recognize a female sex pheromone contained in the urine pulses that females release during the initial aggressive bout preceding mating. Female assessment of male quality is multimodal, involving tactile, visual, and in some cases also chemical cues. The recent development of context-specific and less ambiguous bioassays will facilitate the future purification of the molecules that mediate sexual receptivity and social status in crayfish. These pheromones could be valuable for application in the control of alien invasive crayfish species that cause environmental damage.

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13.1   Introduction

Ever since the first reports of crayfish sex pheromones in the early 1970s there has been a strong interest in the question how crayfish use chemical signals. My own interest in the chemical communication of crayfish was initiated after I had spent 2 years in the lab of Jelle Atema in Woods Hole studying the role of urinary signals in lobsters. In Atema's lab, I learned to be guided by the natural behavior of animals when investigating the role of the particular sense organs or behavioral mechanisms. In order to study lobster chemical signaling in natural aggressive interactions I used catheters to monitor urine release (Breithaupt et al. 1999; Breithaupt and Atema 2000). This technique allowed – for the first time – to quantify urine output in different behavioral contexts. The disadvantage of this technique is that the signal is collected in the catheters and does not arrive at the receiver thereby preventing any natural response. The solution to this problem was to visualize the chemical signal by injecting fluorescent dye into the circulatory system so that urine release could be quantified and still elicit a response. But it took a very gifted student with a lot of finesse to make the technique work. Petra Eger was trained as a nurse before she studied biology in Konstanz. She could handle crayfish, syringe, plasticine, and tape – this is what is needed for dye injection and subsequent sealing of the injection hole – with only two hands to test the administration of different types of dye for the visualization of urine release in crayfish. She made the method work and for the first time allowed us to actually see underwater chemical signaling in action and study when and how they are sent and received in social interactions (Breithaupt and Eger 2002). This study also gave the unquestionable evidence for chemical communication in crayfish by showing signal release as well as receiver's response. For me it further fueled my interest into the field of crustacean chemical communication.

13.2   Crayfish Diversity, Biology and Evidence for Pheromones

Crayfish are the only large decapod crustaceans to occur in freshwater environments and there are more than 640 species in three different families, Astacidae, Cambaridae and Parastacidae. The Astacidae with 39 species is the smallest family with species occurring in Europe/Asia (31 species) and North America (8 species). Cambarid crayfish (>420 species) live in North America while the distribution of Parastacidae (>170 species) is restricted to the Southern hemisphere including Madagascar, South America and Australasia (Crandall and Buhay 2008). Crayfish species have been translocated to other parts of the world mainly for aquaculture and stocking purposes and have become invasive in the new areas (Gherardi and Holdich 1999). Some of the invasive crayfish species cause considerable ecological damage in the new areas not least by transmitting the crayfish plague, a fungal disease lethal to the native crayfish species. One motivation of pheromone research is its potential

application in the control of invasive crayfish species emulating the success of pheromone use in insect pest management (see Baker, Chap. 27).

Earlier studies of crayfish suggested that chemical signals play important roles in various aspects of their life including courtship, agonistic interactions, alarm and brood care (e.g. Ameyaw-Akumfi and Hazlett 1975; Little 1976; Hazlett 1985). Little is known about the chemical communication underlying alarm and brood care and the interested reader is referred to the original literature (Little 1976; Hazlett 1985). The bulk of knowledge about crayfish chemical communication comes from research into aggressive and reproductive interactions. Therefore, in this chapter I will review the evidence that pheromones (chemical signals used in interactions between members of the same species; see Wyatt, Chap. 2) are used for the communication of dominance and sex.

Aggression is a regular part of crayfish social behavior. Crayfish generally live in high population densities and encounters between individuals are frequent (Nystrom 2002). Interactions are often of agonistic nature and escalate into fights (Moore 2007). As a result of repeated fighting, they develop a linear dominance hierarchy (Goessmann et al. 2000). Dominant animals were suggested to have an advantage over other males in gaining better access to shelter, food and mating partners (Bergman and Moore 2003; Herberholz et al. 2007; Moore 2007). Cambarid crayfish males undergo a molt from non-reproductive (Form II) to reproductive morphology (Form I). Form I has larger chelae relative to body size and fully functional reproductive appendages (Reynolds 2002). Courtship generally starts with aggressive behavior of the female toward the male. Mating commences once the male manages to overcome the resistance of the female. The male turns and mounts the female and deposits spermatophores under her abdomen (Mason 1970). Upon release, eggs are fertilized by the stored sperm and attached to the female pleopods under the abdomen. As in most other crustacean species, females carry and ventilate the eggs for extended time periods, thus investing substantially more time and energy into single offspring than males. During this time females are more susceptible to predation because the tail flip mediated escape behavior is severely restricted (for lobsters see Cromarty et al. 1998).

Ameyaw-Akumfi and Hazlett (1975) were the first to report a sex pheromone in crayfish. When tested in an aquarium *Procambarus clarkii* males showed submissive behavior (chelae down, telson curled) in response to female water and displayed aggressive behavior (raised chelae) in response to male odor (Ameyaw-Akumfi and Hazlett 1975). The authors suggested that these differential responses were caused by sex specific pheromones. This study was criticized by Dunham (1978) due to the lack of controls and "blind" observations. Subsequent studies produced contradictory findings and generated a debate about the significance of pheromones in crayfish (for review see Bechler 1995).

Studies over the past 20 years have brought considerable insight into the use of chemical signals in crayfish social behavior. The current review will particularly concentrate on the role of chemical communication in dominance and courtship interactions.

13.3 Chemical Signals: Release, Dispersal, and Detection

More recent studies of crayfish chemical communication suggest that similar to lobsters and crabs (Atema and Steinbach 2007) many chemical signals are contained within the urine. Urine is released anteriorly through a pair of nephropores (Fig. 13.1). The anterior position is advantageous as it facilitates the frontal dispersal of pheromones towards conspecifics (see Fig. 13.2). As in other crustaceans the nephropores are opened and closed by a sphincter muscle (Bushmann and
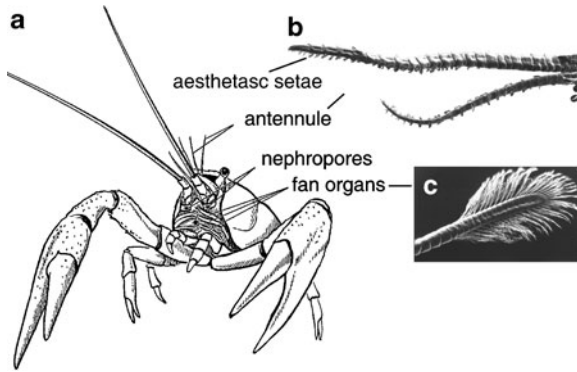

Fig. 13.1 Crayfish appendages involved in chemical communication. (**a**) Position of appendages; (**b**) lateral (*upper*) and medial (*lower*) filament of antennule (Scanning Electron Microscope (SEM) picture). Note the aesthetasc hairs on the lateral filament (**c**) fan organ (SEM picture). Figure modified after Breithaupt (2001)

Fig. 13.2 Photograph showing chemical communication during aggressive interaction of two male *Astacus leptodactylus*. Both males were blindfolded using opaque film wrapped around the eyestalks. Urine signals were visualized using Fluorescein dye injected into the heart (for methodology see Breithaupt and Eger 2002)

Atema 1996) providing the crayfish with the possibility to control the timing of pheromone release. Urine, once released, is drawn into the water currents generated by the animal itself. These are gill currents, expelled from the frontal opening of the gill chambers just underneath the nephropores, and currents generated by fan organs (Fig. 13.1; Breithaupt 2001). Whilst gill currents are always directed forward, fan currents can be directed forward, laterally, upward and backward (Breithaupt 2001). Hence, crayfish have excellent possibilities to control both the timing and the direction of chemical signals comparable to those of lobsters and hermit crabs (Atema and Steinbach 2007).

The fan organs also play a role in the detection of chemical signals. Fan generated currents facilitate acquisition of chemical signals by drawing odor molecules to the antennules, the major chemoreceptors (Denissenko et al. 2007). Fanning may be essential for odor detection in lakes and ponds but less important for detecting odors in a flow environment of a river. This difference in chemical communication between riverine and lacustrine freshwater environments have also been reported from other crustacean species (Thiel, Chap. 10). Multiple studies suggest the aesthetasc hairs on the first antennae (antennules) as the site of pheromone reception in crustaceans (Fig. 13.1; Hallberg and Skog, Chap. 6). Ablation of antennular flagellae in *P. clarkii* eliminated or reduced the sex-specific responses to female and male water (Ameyaw-Akumfi and Hazlett 1975; Dunham and Oh 1992) or impeded localization of odor from the opposite sex (in *Orconectes propinquus*: Tierney and Dunham 1984; in *P. clarkii*: Giri and Dunham 2000). In contrast, other studies of *P. clarkii* concluded (based on ablation experiments in males and in females) that presence of antennules is not necessary for mating behavior to take place (Corotto et al. 1999). In one study, the major chelae were suggested as alternative sites of sex pheromone receptors (Belanger and Moore 2006). While the significance of aesthetasc receptors for the detection of sex pheromones in crayfish is still unclear, their role in the perception of dominance odors is undisputed. Blocking of aesthetasc hairs inhibits the maintenance of dominance relationships in *P. clarkii* (see below; Horner et al. 2008).

13.4 Fighting Behavior, Social Hierarchies, and the Role of Chemical Signals

The behavioral elements of fighting in crayfish are similar to those of American lobsters (Atema and Voigt 1995) and are described in detail in Moore (2007). Fighting is initiated with one animal approaching the other and includes ritualized non-damaging behaviors (meral spread: a threat displays with animals raising the body and extending the chelipeds; tapping/pushing: tactile displays towards the opponent's body using second antennae or chelipeds; claw locking: claws used to grasp appendages of the opponent). If none of the individuals retreats, fights might escalate to potentially damaging behaviors (claw ripping: a combination of claw
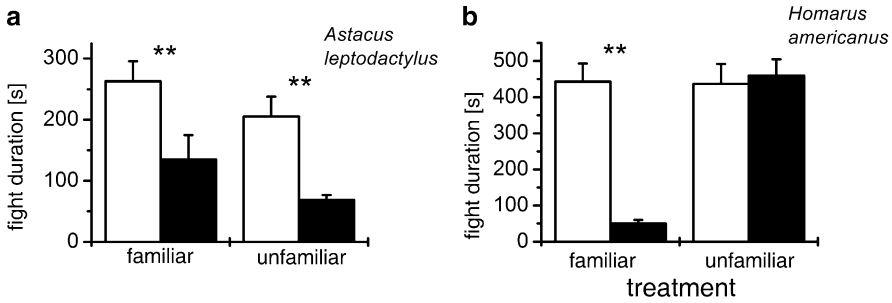
Fig. 13.3 Influence of familiarity on duration of repeated fights between crayfish (**a**) and American lobsters (**b**). "Familiar" denotes that the same pair of size-matched animals fights on two consecutive days: first and second fight. "Unfamiliar" denotes that the loser of the first day fights an unknown winner drawn from a separate fight on the second day. *White bars* denote first fights, *black bars* denote second fights. In crayfish, but not in lobsters, unfamiliar treatments leads to a decrease in fight duration on the second day. This indicates that crayfish recognize the winner-status of their opponent, but not the identity of a familiar opponent. (**a**) Drawn from data in Breithaupt and Eger 2002; (**b**) modified after Karavanich and Atema (1998)

locking and tailflip behavior that can result in removal of the opponent's appendage). Important determinants of winning are body size, claw size, win/lose history and physiological condition (Moore 2007). The closer these conditions are matched between combatants the longer and more intense the fights will be (Schroeder and Huber 2001). First fights of size-matched crayfish last several minutes (Fig. 13.3a; Breithaupt and Eger 2002; see also Horner et al. 2008). Second fights (either on the same or on the subsequent day) are about 50% shorter than first fights, indicating that dominance is maintained over extended time in crayfish (Fig. 13.3a; Breithaupt and Eger 2002; Horner et al. 2008). The previous winner will generally be dominant again (Bergman et al. 2003). In groups of four or five juvenile individuals, fighting activity is high initially, declines after the first hours and drops to low levels after 1 day (Issa et al. 1999). In adults, the gradual decrease in the frequency and mean duration of fights reflect the formation of a stable linear dominance hierarchy with the win/lose ratio being highest for the dominant animal and decreasing towards the most subordinate animals (Goessmann et al. 2000).

13.4.1 The Role of Urine Signals in Dyadic Fights

The significance of urine-borne chemical signals in fighting behavior of crayfish became obvious through blocking and visualization of urine release: Blocking of nephropores leads to a significant increase in fight duration between size-matched males of *Orconectes rusticus* (Zulandt Schneider et al. 2001). This suggests that urine signals are important for crayfish in establishing a dominance relationship with a competitor. Visualization of urine in blindfolded crayfish *Astacus leptodactylus* (Breithaupt and Eger 2002) provided new insight into the use of urine signals in
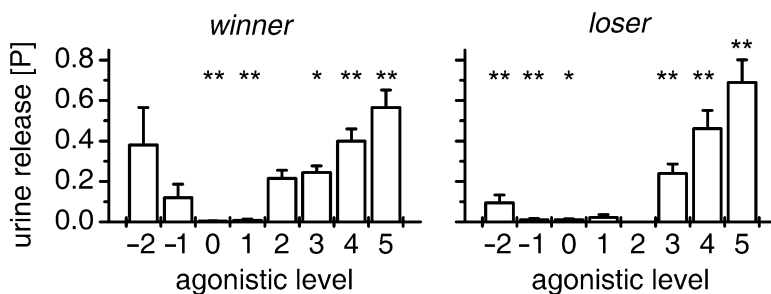
Fig. 13.4 Probabilities (P) of urine release at different agonistic levels in prospective winners (*left*) and prospective losers (*right*). Agonistic levels -2, -1 indicate defensive behaviors and agonistic levels 1–5 indicate aggressive behaviors of increasing intensity (1 = approach, 2 = meral spread, 3 = tapping/pushing, 4 = claw lock, 5 = claw ripping). *Asterisks* denote significant differences with respect to the mean probability over all agonistic levels (*asterisk* $p < 0.05$, *double asterisk* $p < 0.01$; modified after Breithaupt and Eger 2002). Probability of urine release is significantly increased only during physical aggression (levels 3, 4 and 5) while it is decreased during defensive (-2, -1) and neutral behavior (level 0) of losers and during neutral and approach behavior (level 1) of winners
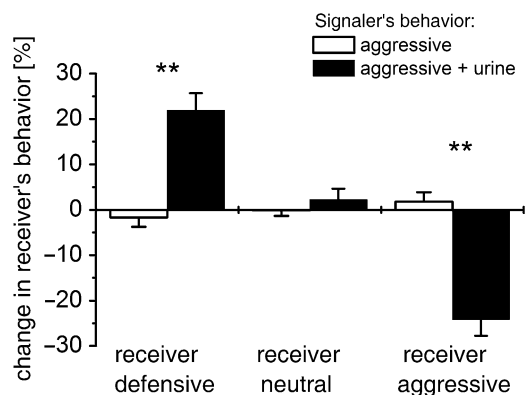

Fig. 13.5 Changes in the receiver response to offensive behavior without urine release (*open bars*) and to offensive behavior accompanied by urine release (*solid bars*). Responses are measured as changes in the relative frequency of occurrence of defensive (levels -2, -1), neutral (level 0) and aggressive (levels 1–5) behaviors. Asterisks denote significant differences between the two conditions (*double asterisk* $p < 0.01$; modified after Breithaupt and Eger 2002)

aggressive interactions: (i) urine is more likely to be released in social interactions than during non-social activities or inactivity, (ii) the eventual winner releases more urine than the eventual loser, (iii) most urine release is coupled to offensive behavior (see Fig. 13.4), and (iv) urine output increases with increasing level of aggression (Fig. 13.4). Since urine is linked to aggressive behaviors we investigated its effect on the receiver by comparing aggressive behaviors that were accompanied by urine release with aggressive behavior not accompanied by urine. Lag sequential analysis revealed that the receiver responds to the combination of offensive behaviors and

urinary signals (but not to offensive behavior alone) by decreasing its offensive and increasing defensive behaviors (Fig. 13.5). Together, urine blocking and urine visualization experiments revealed that chemical signals are essential components of fighting behavior in crayfish. Urinary components appear to transfer crucial information in crayfish aggressive interactions (perhaps about resource holding potential; Briffa and Sneddon 2006) promoting faster resolution of the combat.

13.4.2 The Role of Chemical Signals in the Regulation of Social Hierarchies

Chemical signals not only are important in modulating initial encounters between combatants. They also appear to be largely responsible for the maintenance of lasting dominance hierarchies in crayfish. When the olfactory asthetasc sensilla on the antennules were removed in previously fought pairs of red swamp crayfish (*P. clarkii*) the encounter frequency and fight time was significantly altered (Horner et al. 2008). Crayfish lacking olfactory receptors showed similar amounts of fighting within 1 h pairings on three successive days whereas control crayfish decreased the encounter frequency and fight duration in repeated pairings. The authors suggest that dominance is maintained by chemical recognition of the social status of the opponent and that the aesthetascs are a crucial component in this process (Horner et al. 2008). A similar role of the aesthetasc hairs in chemical dominance recognition had previously been shown in American lobsters (Johnson and Atema 2005). Also, in *O. rusticus* previous winners have a higher chance of winning again when paired with a naïve opponent within 20–40 min after the initial fight but have no advantage if antennular receptors are blocked (Bergman et al. 2003). The authors concluded that – in this species – the winner effect lasts less than 60 min and is mediated by antennular receptors. The perception of social odors also appears to have a priming effect (see Wyatt, Chap. 2) on crayfish promoting a prolonged alteration of aggressive behavior. Crayfish (*O. rusticus*), after exposure to odors from a loser, tend to win a subsequent fight while animals exposed to winner chemicals tend to lose (Bergman and Moore 2005). The combined findings from these three studies suggest that olfactory recognition of social status is a key mechanism responsible for the maintenance of social hierarchies in crayfish. The perception of the opponent's odor interacts with the animal's own fight history in influencing the decision to escalate or retreat an ongoing fight.

There is contradictory evidence from different crustacean species as to whether the chemical signals mediate individual identity or social rank of combatants. Experiments on American lobsters and hermit crabs indicate that dominance is maintained by the loser recognizing the individual scent of the familiar opponent it has lost to (Fig. 13.3b; Karavanich and Atema 1998; Gherardi and Tricarico, Chap. 15). Previous losers surrender early on in a fight when paired with a familiar winner. Unfamiliar winners, however, are challenged and these subsequent fights last as

long as the first fight (Karavanich and Atema 1998). This is different in the crayfish *A. leptodactylus* (Breithaupt and Eger 2002). When a dominant crayfish who won a fight on a previous day is paired with a subordinate crayfish that lost on the previous day this second fight of both combatants is generally shorter than the first fights (Fig. 13.3a). The decrease in fight duration occurs even in pairings of unfamiliar crayfish, a loser and a winner of previous fights against other opponents. This suggests that *A. leptodactylus* does not depend on individual recognition for the maintenance of dominance relationships. Similar results were obtained in *O. rusticus* (Zulandt Schneider et al. 2001) and *P. acutus* (Gherardi and Daniels 2003). All three studies found no difference in the dynamics of repeated fights between familiar and unfamiliar crayfish pairs rendering individual recognition unlikely as the mechanism for the maintenance of dominance in crayfish. Instead of the individual identity, crayfish appear to recognize the social status (dominance rank) of their opponents as suggested in an earlier study by Copp (1986). In accordance with this result, when tested in a flow-through Y maze, male *P. clarkii* responded more aggressively to dominant male odor than to subordinate odor (Zulandt Schneider et al. 1999).

Contrary to these findings, Seebacher and Wilson (2007) concluded from their study that the Australian crayfish species *Cherax dispar* maintain dominance through individual recognition of familiar opponents. In the highly aggressive *C. dispar*, the closing force of the chela is an important determinant of winning in size matched pairings (Seebacher and Wilson 2006). However, even with disabled chelae previous winners keep winning in subsequent encounters when matched against an unrestrained familiar opponent suggesting that dominance is recognized. Previous winners did not have a higher chance of winning against unfamiliar naïve individuals that had not fought before. Similarly, previous losers were not more likely to lose against unfamiliar naïve individuals (Seebacher and Wilson 2007). Unfortunately, in this study the controls – pairing winners or losers with naïve individuals with no prior fight experience – are not sufficient to exclude the possibility that losers would recognize the status of the claw-restrained dominant rather than its individual identity. This can only be demonstrated by pairing losers with unfamiliar winners. Hence, the current evidence favors status recognition over individual recognition as the mechanisms to maintain dominance relationships in crayfish.

13.4.3   What is Communicated by Urine Signals in Agonistic Interactions?

Urine includes metabolites of current and recent biochemical processes and therefore may open a window for the receiver to the physiological state and resource holding potential of the signaler. Biogenic amines such as serotonin and octopamine have been related to aggressive motivation in crustaceans (Kravitz 2000). In green crabs, high serotonin levels were found in winners of fights (Sneddon et al. 2000).

Injections of serotonin cause *P. clarkii* to adopt a dominance display (meral spread) while octopamine injection triggers a typical subordinate stance (Livingstone et al. 1980) but this study was criticized as it used unnaturally high doses of the biogenic amines. Continuous infusion of low doses of serotonin into subordinate crayfish *A. astacus* caused more subtle changes in the behavior (Huber and Delago 1998). Infusion into the circulatory system was applied throughout the fight using a cannula attached to the carapace. Infused animals showed a reduction in the tendency to retreat during a combat. These neurohormones could also affect the behavior of the opponent if they leak into the environment. Metabolites of serotonin are released with the urine (Huber et al. 1997) and therefore may be a candidate to mediate aggressive motivation of the dominant crayfish to the subordinate receiver. When tested on crayfish with prior social experience, injection of serotonin or octopamine resulted in changes in aggression but did not lead to a permanent inversion of dominance hierarchies as expected (Tricarico and Gherardi 2007). Similarly, when fighting over a resource serotonin-injected American lobsters did not win over sham-injected individuals (Peeke et al. 2000). It still needs to be established whether metabolites of biogenic amines released in the urine act as dominance pheromones in fights or whether other chemical components are involved. Hence, the chemical identity of the pheromones that regulate fighting and social hierarchies in crayfish is still elusive. To date, no bioassay has been established that could serve to guide fractionation of urinary compounds mediating dominance in crayfish (see Hardege and Terschak, Chap. 19).

13.5   The role of Chemical Signals in Reproductive Interactions

13.5.1   *Evidence for Female sex pheromones*

In some decapod crustaceans including green crabs (see Hardege and Terschak, Chap. 19) and blue crabs (Kamio and Derby, Chap. 20) the existence of female sex pheromones is assured by unambiguous male courtship responses to female odorants (e.g. "cradle carrying of a pheromone labeled object" in green crabs or "courtship stationary paddling" in blue crabs). Male crayfish do not display specific courtship behaviors and their responses to female stimuli are ambiguous, a fact which has hampered research progress into the significance and chemical identity of female chemical signals. In contrast to species such as green crabs, blue crabs and lobsters, where females are mated immediately after having molted, female crayfish mate during the intermolt period, i.e., months after their last molt. Therefore, crayfish females do not profit as much as soft-shelled females from male protection exercised by cradle carrying (in crabs: Hardege and Terschak, Chap. 19) or shelter sharing (in lobsters: Atema and Steinbach 2007) and hence may not actively seek presence of a courting male.

In most crayfish species studied, mating develops from aggressive interactions between the sexes (Gherardi 2002; but see Barki and Karplus 1999). During the agonistic interactions, the male upon recognizing sex and receptivity of the female initiate mating by seizing her rostrum or antennules with the major chelae. He turns the female by rolling her body upside down using chelae and walking legs. The male then mounts the female and deposits spermatophores to the ventral side of her cephalothorax (in Astacidae) or into sperm-receptacles (in Cambaridae) (Reynolds 2002; Stebbing et al. 2003).

Following the controversy about the existence of sex pheromones in crayfish (summarized in Bechler 1995) more recent studies provide new support for female release of and male response to chemical signals during reproductive interactions. Using visualization techniques adopted from Breithaupt and Eger (2002), Simon and Moore (2007) and Berry and Breithaupt (2010) showed that in pairings of receptive females and reproductively active males of the species *O. rusticus* and *P. leniusculus*, respectively, urine is released by both sexes (Fig. 13.6). Males of both species exhibit responses to artificially introduced female stimuli by increasing general activity or by handling the odor source (an aquarium air stone) more readily when conditioned water from receptive females was released but not in response to water from juvenile females (Stebbing et al. 2003) or to male conditioned water (Belanger and Moore 2006). In two cases, males attached spermatophores to the airstone in response to water from receptive females (Stebbing et al. 2003) suggesting a sexual nature of the response. Similarly, male *P. clarkii* appeared to recognize
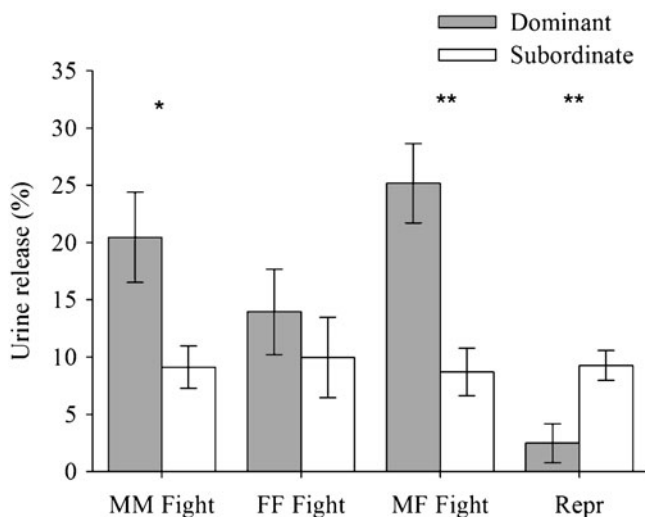

Fig. 13.6 Mean (±SEM) urine release by dominant (*gray bars*) and subordinate (*white bars*) crayfish in male fights (MM Fight), female fights (FF Fight), mixed-sex fights (MF Fight) and reproductive interactions (Repr). In reproductive interactions males are labeled as dominant (*gray bars*) and females as subordinate (*white bars*). *Asterisks* indicate differences between interactants (*asterisk p* < 0.05, *double asterisk p* < 0.01, paired *t*-test). From Berry and Breithaupt (2010)

a conspecific's gender by chemical stimuli alone. Males showed increased locomotion to both male and female stimuli but responded with aggressive postures to male stimuli only (Aquiloni et al. 2009). Our own initial approaches to bioassay development tested male responses (heart rate and behavioral changes) to a more natural device for odor delivery, a female dummy made of a molt shell (Berry and Breithaupt 2008). Males exposed to female urine showed changes in heart rate as well as significantly increased durations of mounting behavior in comparison to male urine or control water (Berry and Breithaupt 2008). However, other sexual behaviors such as turning and spermatophore deposition were not observed in this study. Perhaps the male needed tactile or visual feedback from a female before continuing mating behavior. Although these studies indicate that males discriminate odor of receptive females from that of unreceptive females or males, the elicited responses (handling of odor source, increased locomotion, mounting of a dummy, changes in heart rate) may still be considered ambiguous as they could occur in reproductive as well as in non-reproductive contexts.

In a recent study we addressed the shortcomings of previous bioassay approaches by studying in *P. leniusculus* the responses of blindfolded males to live females rather than to inanimate odor sources (Berry and Breithaupt 2010). We blocked the natural urine release of the female and compared the effect of artificial urine introduction with introduction of control water. Female urine or water was introduced through a syringe during the initial aggressive bout. In crayfish (*P. leniusculus*), during sexual interactions the male has to overcome resistance of the female and uses a specific sequence of behaviors to seize, turn and mount the female (see above). We scored the presence of any of these behaviors as a sexual response. Males were more likely to show sexual responses toward urine-blocked female when urine from a receptive female was introduced than when control water was introduced (Berry and Breithaupt 2010).

Together, these results provide clear evidence for the existence of female sex pheromones in crayfish. In some species multimodal (chemical and visual) stimulation is required to enable sex recognition by males (see Hebets and Rundus, Chap. 17). In *Austropotamobius pallipes*, a species that mainly inhabits turbulent flow environments, chemical stimuli alone do not elicit male response. Only when accompanied by the sight of a crayfish (male or female), odor was effective in causing increased activity and in reducing the time the male spent under a shelter (Acquistapace et al. 2002). The authors discussed ecological constraints (turbulence causing stronger dispersal of chemical signals) as a cause for the multimodal strategy.

13.5.2 Are Chemical Signals Involved in Female Assessment of Male Quality?

Due to their strong reproductive investment, female crayfish are expected to be highly selective in the choice of the mate. Female quality assessment could include size and dominance status of males, which both affect the resource holding potential

of the males. Mate choice based on these characters – if heritable – should be beneficial to the females by increasing her fitness. While the initial aggression of the female to male mating attempts indicates her resistance to mating, there is evidence that females recognize male gender and are able to conduct mate choice.

Sex identification in *P. clarkii* involves both chemical and visual cues (Aquiloni et al. 2009). Females do not show differences in response to male and female conspecifics, if they receive odor alone or if they can see but not smell the other individual. However, when they receive bimodal (chemical and visual) stimuli they display aggressive postures only to females not to the males. How important are chemical stimuli for females to evaluate male quality? To date there is little evidence that chemical stimuli play an important role in mate selection by females. Aquiloni et al. (2008) concluded that female red swamp crayfish (*P. clarkii*) do not show a preference when allowed to select between a dominant and subordinate male of similar size. Only when allowed to watch the fighting interaction between males prior to being exposed to them, female *P. clarkii* display a preference for the dominant male. Hence the previous visual experience was necessary to recognize a dominant male. For the assessment of male size, female *P. clarkii* appeared to require both visual and chemical information. Females approached the larger of two males hidden in plastic containers only when they could see and smell them (Aquiloni and Gherardi 2008b). It is likely that in this experiment, the chemical information was necessary for sex identification while the sight of the males allowed discrimination of size. These results imply that males release some chemicals prior to or during reproductive interactions with females. Urine visualization during reproductive interactions of *P. leniusculus* revealed that males release significantly less urine during sexual interaction than during aggressive interactions (Fig. 13.6; Berry and Breithaupt 2010); 30% of the males did not release any urine when attempting to mate a female. Females, in contrast, readily release urine during their aggressive behavior towards males prior to mating (Fig. 13.6). Figure 13.7 shows an artist illustration of inter- and intrasexual chemical signaling in a crayfish population. Since mating is always preceded by aggressive interactions between the sexes, females get additional tactile information about size and strength of the male by physically interacting with him. Crayfish have very keen tactile senses to explore their environment (McMahon et al. 2005). Due to the nocturnal activity period of most crayfish species tactile information may be even more important than visual information to assess male quality.

Females are also able to perform cryptic mate choice. After mating, females adjust their reproductive effort in relation to male traits. Galeotti and coworkers showed that female crayfish *Austropotamobius italicus* produce larger but fewer eggs for small males with large claws and more numerous but smaller eggs for large-sized, small-clawed males (Galeotti et al. 2006). Similarly, female *P. clarkii* invest in larger eggs after having copulated with large males (Aquiloni and Gherardi 2008a). The authors speculate that crayfish have the ability to reabsorb part of the nutritive substances in the cytoplasm of the oocytes before they spawn. In conclusion, mate choice in crayfish is often cryptic. Chemical and visual stimuli may provide partial information for sex identification but are not always available to the female. Tactile stimuli perceived during physical interaction with the male as well as

Fig. 13.7 Artist impressions of the social interactions between crayfish in a population of high density. Aggressive interactions between males (on the *right*) involve physical contact of the claws (claw lock) and urine release. Reproductive interaction (*bottom*) involve male mating attempt (starting with the male seizing female antennae with left claw) and female aggressive behavior (female claw lock) with urine release. Drawing courtesy of Jorge Andrés Varela Ramos

visual stimuli indicating size of the male appear to be more important than chemical stimuli for females to discriminate quality of males and to display mate choice.

13.6 Crayfish as Model Organisms

In his influential book on "The crayfish," Thomas H. Huxley (Huxley 1879) used the crayfish as a model for the introduction to the study of zoology. This book, due to its integrative approach had a great impact on various zoological disciplines. Particularly the neurosciences have successfully used crayfish preparations to understand the functioning of the nervous system and the link between neural function and behavior ("neuro-ethology") (Edwards et al. 1999). Today, there is an extensive literature on the neurobiology, behavior and ecology of crayfish providing a rich basis for further exploration of their chemical ecology. As freshwater organisms, crayfish are easy to maintain in captivity, have an ideal body size for the study of their social behavior, can be experimentally manipulated (e.g. by blindfolding, dye visualization, urine collection) for laboratory studies and their behavior can be tracked in the field. This makes them stand out as ideal model organism for behavioral studies. In the past 15 years much research effort has been devoted to studies into the neural and behavioral mechanisms of fighting behavior

(e.g. Yeh et al. 1997; Herberholz et al. 2001). Fighting behavior in crayfish is easily elicited. Two size-matched crayfish introduced to separated halves of an aquarium will almost always start fighting as soon as the separating divider is lifted. Hence, crayfish may be better suited than many other aquatic organisms including fish and polychaetes to unravel the complex role chemical signals may play in the resolution of animal combats as well as the underlying neural mechanisms. Because neurological methods, e.g. electrophysiological recordings and activity labeling in the brain, are well established (see Schmidt and Mellon, Chap. 7), the identification of crayfish sex or dominance pheromones will enable new insight into the brain function of this highly chemoreceptive group of animals.

Furthermore, crayfish show a high species diversity (>640 species) making them ideal candidates for studies into the evolution of pheromones, a field that so far depended mostly on research of insect pheromones, particularly moths and fruit flies (Wyatt 2003). Crayfish inhabit ecosystems as diverse as wetlands, stagnant ponds and lakes, turbulent creeks and rivers, providing opportunities to disentangle the ecological and phylogenetic constraints of chemical signal evolution.

13.7 Future Directions

While some basic questions about the behavioral significance of sex- and dominance pheromones in crayfish appear to be solved, questions into the exact function of the pheromones are still unanswered and new challenges are waiting to be explored. We now have good evidence that chemical signals are essential for the maintenance of dominance hierarchies in crayfish. Several studies indicate that dominance recognition is mediated by status indicating pheromones and not by individual odors. Status indicating pheromones are expected to consist of a "defined combination of molecules eliciting a particular behavior or response" (Wyatt, Chap. 2) and therefore lend themselves much better for chemical characterization than the highly variable individual odors (signature mixtures; see Wyatt, Chap. 2). Variations in odor composition between individuals such as in lobsters (Atema and Steinbach 2007) render it almost impossible to identify the molecules conveying the signal. Therefore, at the moment, crayfish provide the best opportunity among crustaceans and other aquatic animals to discover the chemical identity of molecules mediating dominance recognition. Once the chemical composition is known, new questions concerning the production and the metabolic costs can be addressed as well as the information content and the possibility of deception by chemical signals (see Christy and Rittschof, Chap. 16).

A better link between endocrinological and pheromone research may lead to new insight about the mechanisms of chemical communication. Barki and coworkers showed that implantation of the androgenic gland into a juvenile female induced male characteristics in the female once it was fully matured (Barki et al. 2003). Normal females accepted these implanted females as males, since they allowed them to mate and deposit spermatophores. This discovery suggests that implanted

females may release sex-identifying pheromones typical for males. Further research may allow investigating the potential hormonal origin of sex pheromones. Many fish species have been shown to use hormonal metabolites as pheromones (see Chung-Davidson, Huertas and Li, Chap. 24) but to date there is little evidence for hormonal pheromones in crustaceans (Chang, Chap. 21).

The recent development of context-specific and therefore less ambiguous bioassays (see "evidence for female sex pheromones") will pave the way for research into the chemical purification of female crayfish sex pheromones. Already at an early stage of chemical purification the question can be addressed whether sex pheromones are species specific. As the identity of the molecules unfolds, further questions to their function can be addressed, e.g. whether the molecules specifying sex, receptivity and species are identical such as in fruit flies (Billeter et al. 2009) and how they relate to female reproductive metabolism.

The transport of crayfish species between countries and continents has led to invasions of alien crayfish species with dramatic ecological consequences. Invasive crayfish can cause physical damage (by destroying river banks) as well as ecological damage to the ecosystem including the displacement of the native crayfish species (Hill and Lodge 1999). Protocols including the use of pheromones for species attraction and eradication need to be established to control the invasive crayfish. Here, the knowledge about insect pest management (Baker, Chap. 27) will be important as it may at least in part be transferable to design efficient strategies for the management of invasive crayfish.

13.8 Summary and Conclusions

Decades of research on crayfish chemical communication has, after initial controversies, generated a comprehensive picture of the role of pheromones in resolving combats and in initiating sexual interactions. Moreover, the structures involved in signal emission and reception have been identified in most cases. Urine, released in the head region, conveys the chemical messages and is directed with water currents to the receiver. Aesthetasc receptors on the first antennae were shown – in most cases – to be responsible for pheromone detection. Urinary signals reduce the duration of aggressive interactions and allow the development of a stable linear hierarchy. The social hierarchy is based on the chemical recognition of the dominance status between combatants. Males are more active than females in initiating reproductive interactions. They recognize the female sex pheromones. Female assessment of male quality is multimodal, involving tactile, visual and chemical components. The recent development of context-specific and less ambiguous bioassays will facilitate the future purification of the molecules that constitute the sex and dominance pheromones in crayfish. These pheromones could be valuable for application in the control of alien invasive crayfish species that cause environmental damage.

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