

# Chapter 2

## Pheromones and Behavior

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**Abstract** Chemical communication is widely used by crustaceans, for example, in sexual interactions, larval release, and planktonic settlement. However, we know the identity of very few of the molecules involved. In this chapter, I introduce pheromones and contrast them with signature mixtures. Pheromones are molecules that are evolved signals, in defined ratios in the case of multiple component pheromones, which are emitted by an individual and received by a second individual of the same species, in which they cause a specific reaction, for example, a stereotyped behavior or a developmental process. Signature mixtures are variable chemical mixtures (a subset of the molecules in an animal's chemical profile) learned by other conspecifics and used to recognize an organism as an individual (e.g., lobsters, mammals) or as a member of a particular social group such as a family, clan, or colony (e.g., mammals, desert woodlouse *Hemilepistus reaumuri*, ants, bees). A key difference between pheromones and signature mixtures is that in all taxa so far investigated it seems that signature mixtures need to be learnt (unlike most pheromones). These signature mixtures may be best thought of as cues. Pheromones evolve from molecules which give a selective advantage to the receiver and signaler. The evolution of pheromones is facilitated by the combinatorial basis of the olfactory system found in crustaceans and other animals. In crustaceans, some pheromones are also detected by the distributed chemosensory system. Crustaceans have great potential as model organisms for chemical communication research, in particular now that the *Daphnia pulex* genome has been sequenced.

### 2.1 Introducing Chemical Ecology

Like other animals, crustaceans live in a chemosensory world full of chemical information and signals from conspecifics, prey, and predators (Fig. 2.1). To a human

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**Fig. 2.1** Just as peacock males display their fan tails, the male lobster (*left*) displays with pheromones in the directed jet he sends toward the female. Drawing by Jorge A. Varela Ramos (Picture inspired by the mouse pheromone “peacock tail” in Penn and Potts 1998)

it may be strange to think of a world of smells underwater (as we experience smells in air) but chemical communication is widely used by aquatic animals including crustaceans. However, the types of molecules used by crustaceans for communication underwater are likely to be different from those used in terrestrial environments by most insects and mammals. For aquatic animals, solubility of signal molecules is the equivalent of volatility for airborne messages.

The division of chemical senses in vertebrates into taste and smell does not work well for crustaceans and other invertebrates. Like vertebrates, crustaceans have olfactory receptor neurons (ORNs) (these are also known as olfactory sensory neurons) with one end exposed to the chemical world outside the animal and the other leading to the brain. These ORNs in the aesthetasc sensilla of crustaceans approximate to vertebrate “olfaction” as the ORNs project to glomeruli in the crustacean olfactory lobe, analogous to the organization of the brain in vertebrates (Caprio and Derby 2008; Derby and Sorensen 2008; Schmidt and Mellon, Chap. 7). Long distance sex pheromones and chemical cues for social interactions tend to be processed by the olfactory/aesthetasc pathways, for example, spiny lobster responses to conspecific urine signals (Horner et al. 2008). However, crustaceans also have “distributed chemoreception” (Schmidt and Mellon, Chap. 7) which goes beyond vertebrate taste. Distributed chemoreceptors are typically packaged with mechanosensors into sensilla over other parts of the body and have a nonglomerular organization in the brain/ganglia. While distributed chemoreception does include the equivalent of taste, with contact chemoreception for food for example, it also includes other chemical senses such as control of antennular grooming and the coordination of mating and copulation (Schmidt and Mellon, Chap. 7). Schmidt and Mellon point out that integrated inputs from sensilla in both the olfactory and distributed chemoreceptor systems may participate together in the control of complex behaviors, including associative odor learning.

As you will see in the rest of this book there is already an impressive understanding of crustacean chemical communication – but really we are just at the beginning of the exploration. In the coming years, as demonstrated in the chapters

of this book, chemical communication by crustaceans will be seen to be just as spectacular as the currently well-known examples of moth males flying upwind to find the female moth releasing pheromone.

What is surprising is that only a small number of pheromones have been chemically identified for crustaceans compared with the thousands for insects. Part of the difference may be the importance of moths as agricultural pests, which has prompted governments to provide funding, but I wonder if the disparity also suggests that crustaceans are harder to study. Is it the type of molecules used by crustaceans or the current difficulties of working with the chemical communication of aquatic animals? Hardege and Terschak (Chap. 19) discuss the importance of discriminating bioassays for identifying pheromones. It may be that further work needs to be done on bioassays that yield discriminating data for crustaceans.

In this chapter I would like to introduce concepts of chemical ecology, to discuss pheromones and signature mixtures, and contrast the research on terrestrial pheromones with research on aquatic pheromones.

## 2.2 Definition of Pheromones

Pheromones are chemical signals used to communicate within a species. Pheromones are “molecules that are evolved signals, in defined ratios in the case of multiple component pheromones, which are emitted by an individual and received by a second individual of the same species, in which they cause a specific reaction, for example, a stereotyped behavior or a developmental process” (Wyatt 2010, modified after Karlson and Lüscher 1959). The word is derived from the Greek *pherein*, to transfer and *hormon*, to excite. Karlson and Lüscher intended that pheromones should include chemical signals in animals of all kinds and they included Crustacea (the prawn *Palaemon serratus*), as well as fish, mammals, and insects, among their examples.

Karlson and Lüscher anticipated that species would not necessarily have exclusive use of a particular molecule as their pheromone. We now know many examples of shared use of molecules (Kelly 1996; Wyatt 2003, 2010). One of the most spectacular is the way that the female Asian elephant shares its small molecule sex pheromone, (*Z*)-7-dodecen-1-yl acetate, with some 140 species of moth (Rasmussen et al. 1996). Because the moths use defined combinations of molecules, of which (*Z*)-7-dodecen-1-yl acetate is just one, there is not likely to be confusion between elephants and moths. Because the defined combination is different for each moth species there is no confusion between the moth species: in cases where the combination is the same, the species “call” with their pheromones at different times or have nonoverlapping habitats.

Mice and some bark beetle species also share some terpene compounds as pheromones. There are a number of reasons why these overlaps occur. The first is that all animals share a common heritage and so have basically the same metabolic

pathways and molecular capabilities. Second, the number of potential small volatile molecules, which are also chemically stable and nontoxic, is limited. Incidentally, this may be less of a problem for underwater communication as even very large molecules can be soluble. We might expect some aquatic species to have unique large pheromone molecules used singly, for example species-specific peptides with different amino acid sequences, rather than blends of common small molecules as in moth sex pheromones.

### 2.2.1 *Signals vs. Cues*

Pheromones are signals as defined by Maynard Smith and Harper (2003, p3) in their book *Animal Signals*: “any act or structure which alters the behavior of other organisms, which evolved because of that effect, and which is effective because the receiver’s response has also evolved.” The definition is further discussed by Scott-Phillips (2008) in a thoughtful paper which makes explicit that the response itself has to have evolved to be affected by the act or structure concerned (not just evolved to respond to something else). For something to be a signal, both the emission and response need to have evolved.

This distinguishes signals from cues. Cues are things such as the CO<sub>2</sub> released by an animal as it breathes, and used as a cue by a blood-sucking insect to find its host. The mosquito’s response is certainly evolved (and indeed it has highly specialized receptors to detect CO<sub>2</sub>), but the release of CO<sub>2</sub> by the host does not evolve to have the effect of attracting mosquitoes so CO<sub>2</sub> release does not count as a signal. The odors used by mammals and crustaceans when recognizing kin or familiar animals are also probably best seen as cues rather than signals, in my opinion (see below).

How do we know that a chemical signal has evolved for an effect? In moth females we can see the specialized pheromone glands evolved for secreting and releasing the female sex pheromones – for the effect of signaling – and in the male, the highly evolved feathery antennae and specialized olfactory sensory cells on those antennae for detecting the pheromones, combined with the specialized sex-specific brain structures, the macroglomerular complex (MGC), in the male brain for processing the data (Hansson 2002; Wyatt 2010). Often the evidence of evolved structures and processes for signal reception is more subtle. For example, male and female *Drosophila* antennae are morphologically very similar. The evolved effects of pheromones in *Drosophila* are at the receptor level and circuits of the brain of the male and female (Vosshall 2008). In lobsters, pheromones may be added to the urine before release rather than there being a special gland visible. Pheromone components can be acquired as well as synthesized by the emitter. For example, to attract females, male euglossine bees in the American tropics must collect perfume oils such as mono- and sesqui-terpenes from orchid flowers (see Wyatt 2010).

### 2.2.2 *Pheromone Types by Function*

Pheromones are often described by function, by the effect they have. For example, sex pheromones describe those involved in mate-finding or attraction. Others include aggregation, alarm, and trail pheromones. Some responses are context-specific – for example in some ant species alarm pheromones cause ants to disperse if released far from the nest but to attack if released close to the nest. In a way, these descriptors allow us simply to describe the range of behaviors mediated by pheromones and then generalize from these to describe patterns in use, say, of sex pheromones across taxa.

Our definitions reflect our attempts to make sense of the world and identify patterns. Useful definitions enable us to generalize and predict characteristics. However, no definition is going to be a perfect match to the range of observed scenarios. There will always be fuzzy areas and difficult cases. Part of the problem may be trying to include all phenomena in the same definition. Another problem is that we may be describing pheromones at different stages of evolution – it is likely that many pheromones start as chemical cues and only later evolve into signals, an evolutionary process known as ritualization (Maynard Smith and Harper 2003, p. 15).

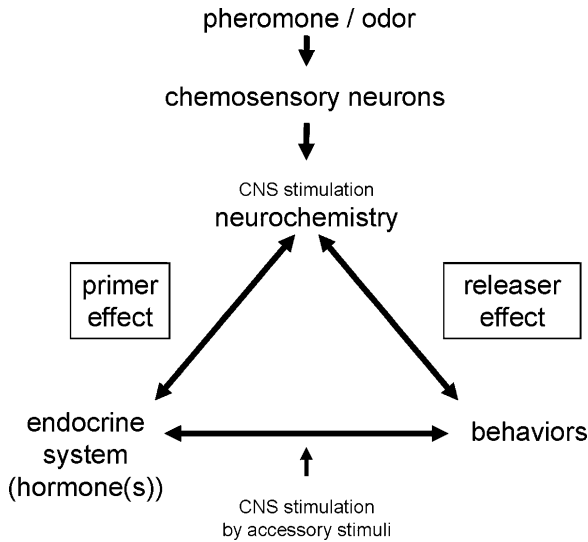
### 2.2.3 *Releaser vs. Primer Effects*

Pheromones may elicit a behavioral response (releaser effects), longer-lasting developmental (primer effects) mediated via hormones, or both (see Fig. 2.2). So for example, prior exposure for some days to the female pheromone(s) in the premolt urine of the shore crab *Carcinus maenas* primes his sexual behavioral responses such as cradling when he is later exposed to the female premolt urine (Ekerholm and Hallberg 2005).

### 2.2.4 *Semiochemicals vs. Infochemicals*

As the variety of chemical interactions between organisms became better understood during the 1960s and 1970s, many authors attempted to classify the interactions and the chemical agents involved. If pheromones describe intraspecific signals, what terms should be used for interspecific chemical interactions? In the recent aquatic chemosensory literature, authors seem to be almost equally divided between using “semiochemicals” and “infochemicals.”

*Semiochemicals* (from the Greek *semeion* mark or signal) are the chemicals, acquired or produced by the sender, involved in the chemical interactions between organisms (Nordlund and Lewis 1976) (Table 2.1). In their original definition, Law and Regnier (1971) used the phrase “chemical *signals* for transmitting information between individuals” [my italics], which is fine for pheromones but causes a



**Fig. 2.2** Pheromones can be a stimulus leading to a prompt behavioral response by nerve impulses from the brain (CNS, central nervous system) (*releaser effects*) or can act indirectly by stimulation of hormone secretion resulting in physiological changes, “priming” the animal for a different behavioral repertoire (*primer effects*). A given pheromone can of course have primer or releaser effects at the same time or in different contexts. The distinction between primer and releaser effects has become blurred as we understand more about the links, interactions, and feedback loops in the sequence from odor to behavioral and endocrine effects. Hormonal effects can be rapid, and memories, sometimes facilitated by local neurochemistry changes, can be long-lasting. The diagram brings together ideas from figures in Wilson and Bossert (1963) and Sachs (1999). Text and diagram adapted from Wyatt (2003, Fig 1.10, p. 18)

problem for many interspecific interactions as we now reserve the terms “communication” and “signals” for mutually evolved signals. Nordlund and Lewis (1976) recognized the problem and reworked their definition of semiochemicals to remove “signals.”

Pheromones are thus semiochemicals used within a species. Interspecific semiochemicals are *allelochemicals*. They are further subdivided depending on the costs and benefits to the sender and the receiver (Nordlund and Lewis 1976). If the sender benefits at the expense of the receiver, for example a bolas spider releasing moth-identical pheromones to lure a male moth, the spider’s chemical message is an *allomone*. If a prey species such as the gammarid *Gammarus roeselii* avoids its predators’ odors, then the gammarid is using those odors as a *kairomone* (Hesselschwerdt et al. 2009). If both partners benefit, as in the case of mutualisms between sea anemones and anemone clown-fish, the chemicals involved are termed *synomones*. The multiplicity of terms is only useful as shorthand and they are clearly overlapping, not mutually exclusive (a molecule used as a pheromone within a species can be used as a kairomone by its predator, for example).

In proposing the word kairomone, Brown et al. (1970) had in mind the word’s Greek root *kairos*, especially in its senses of “opportunistic” and

**Table 2.1** Definitions of chemical mediators important in chemical interactions among organisms, based on Nordlund and Lewis (1976) and Wyatt (2010)

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- A. Hormone: A chemical agent, produced by tissue or endocrine glands, which controls various physiological processes within an organism (Nordlund and Lewis 1976)
- B. Semiochemical: A chemical involved in the chemical interaction between organisms (Nordlund and Lewis 1976)
1. Pheromone: Molecules that are evolved signals, in defined ratios in the case of multiple component pheromones, which are emitted by an individual and received by a second individual of the same species, in which they cause a specific reaction, for example, a stereotyped behavior or a developmental process (Wyatt 2010, modified after Karlson and Lüscher 1959)
  2. Signature Mixture: A variable chemical mixture (a subset of the molecules in an animal's chemical profile) learned by other conspecifics and used to recognize an animal as an individual (e.g., lobsters, mammals) or as a member of a particular social group such as a family, clan, or colony (e.g., *Hemilepistus reaumuri*, ants, bees) (Wyatt 2010, derived from Wyatt 2005 "signature odor" and Johnston's "mosaic signal" 2003)
  3. Allelochemical: Chemical significant to organisms of a species different from their source, for reasons other than food as such (Nordlund and Lewis 1976)
    - a. Allomone: A substance, produced or acquired by an organism, which, when it contacts an individual of another species in the natural context, evokes in the receiver a behavioral or physiological reaction adaptively favorable to the emitter but not to the receiver (Nordlund and Lewis 1976)
    - b. Kairomone: A substance, produced, acquired by, or released as a result of the activities of an organism, which, when it contacts an individual of another species in the natural context, evokes in the receiver a behavioral or physiological reaction adaptively favorable to the receiver but not to the emitter (Nordlund and Lewis 1976)
    - c. Synomone: A substance produced or acquired by an organism, which, when it contacts an individual of another species in the natural context, evokes in the receiver a behavioral or physiological reaction adaptively favorable to both emitter and receiver (Nordlund and Lewis 1976)
    - d. Apneumone: A substance emitted by a nonliving material that evokes a behavioral or physiological reaction adaptively favorable to a receiving organism, but detrimental to an organism, of another species, which may be found in or on the nonliving material (Nordlund and Lewis 1976)
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"Infochemical" as an alternative to "semiochemical" was proposed by Dicke and Sabelis (1988) though its main change was to replace "produced or acquired by" with "pertinent to biology of" in each case for allelochemicals. Apneumone is rarely used. Paragraphs from Nordlund and Lewis (1976) with permission

"exploitative" – which is just how a predator uses a kairomone. Kairomones have not evolved as signals and this is reflected in my usage of kairomone usually in the context of "using [molecule x] as a kairomone" rather than defining a particular molecule itself as a kairomone.

"Infochemical" was proposed by Dicke and Sabelis (1988) as a term to replace "semiochemical" at the head of the classification. They argued that the cost-benefit of the interaction should be the only criterion, not the origin of the chemicals concerned. This was prompted perhaps by their work on tritrophic interactions, which have volatiles produced by the herbivores themselves and also induced in their host plants. However, indirectly produced or induced volatiles were already

included by Nordlund and Lewis (1976) in their definition for kairomone which includes substances “released as a result of the activities of an organism” (Table 2.1).

On balance I feel the best solution is to stick with “semiochemicals,” but with a relaxation of the definition of pheromone to include, where needed, compounds produced by symbionts or other associated organisms (Wyatt 2010).

### 2.2.5 Pheromones, Signature Mixtures, and Recognition

One of the most contentious problems in chemical communication has been what to name the variable odors used by mammals to distinguish individuals of their own species and used by social insects to distinguish fellow colony members from “foreigners” (and probably used by lobsters and hermit crabs to recognize individuals in fights, see Atema and Steinbach 2007; Gherardi and Tricarico, Chap. 15). These chemical cues do not fit the pheromone definition of a “defined combination of molecules causing a specific behavior or response” (Table 2.1; Wyatt 2010). Instead, the variation in chemical mixtures between individuals or colonies *is* the message. I propose that we use the term “signature mixtures” to describe these (Wyatt 2010) (Table 2.1). These signature mixtures are learnt (in contrast to pheromones, which tend not to need learning for response) (Table 2.2).

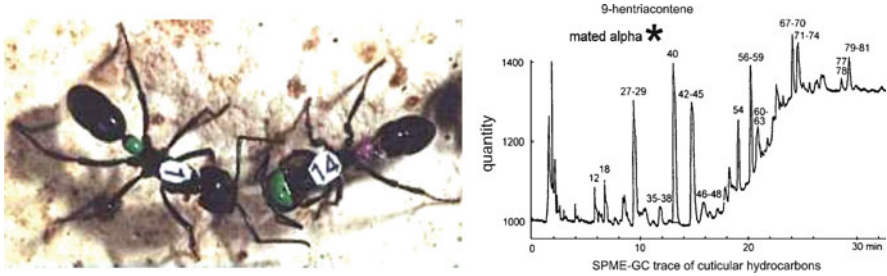
For kin recognition, animals learn any cues (signature mixtures) that give a statistical probability allowing recognition (Sherman et al. 1997). Sometimes these signature mixtures are produced by the animal itself but in some species they may be acquired from the shared local environment instead, for example in wood frog tadpoles *Rana sylvatica* (Sherman et al. 1997). Desert woodlice (*Hemilepistus reaumuri*) show family recognition by cuticular chemical signatures, which appear to be a mixture of compounds from all family members; signature mixtures have to be reacquired after each molting event (Linsenmair 1987, 2007). The recognition by guards in *Hemilepistus* is clearly highly evolved (as it is in honey bees). However, the cuticular molecules in *Hemilepistus*, perhaps with a waterproofing

**Table 2.2** Contrasting pheromones with signature mixtures

	Molecule(s)	Receiver system/perception
Pheromones	Species specific	“Hardwired,” learning not usually required
<i>Anonymous</i>	e.g., Sex pheromones e.g., “Category” pheromones (such as immature, adult)	
Signature Mixtures	Individual, family, colony odors	Learning required
<i>Variable</i>		

Hölldobler and Carlin (1987) introduced the idea of anonymous signals (pheromones) contrasted with variable signature mixtures (though their terminology was different). The anonymous pheromone signals are uniform throughout a category (e.g., species, male, female, and perhaps molt state and dominance status). In contrast, signature mixtures vary and can be used to recognize the organism as an individual or member of a particular social group such as a family or colony. A more extensive comparison is given in Wyatt (2010)





**Fig. 2.3** Most animals have both anonymous pheromone signals and signature mixtures (Wyatt 2010). This is demonstrated in the cuticular hydrocarbons of the “queenless” ant *Dinoponera quadriceps* (Monnin et al. 1998). A gas chromatographic analysis of the cuticular hydrocarbons on the alpha female, the only fertilized and egg-laying individual in the colony, shows the anonymous hydrocarbon pheromone 9-hentriacontene (indicated by the asterisk) characteristic of alpha females in all colonies, together with the diverse range of other hydrocarbons which make up the colony odor. Her fellow colony members have the colony odor but lack the 9-hentriacontene. Ants photograph, courtesy Thibaud Monnin. Gas chromatogram, Monnin et al. (1998), with permission

function, might have been coopted for a signature function. It is possible that complexity of signatures might later be selected, under natural selection, for ease of recognition (Tibbetts and Dale 2007). Do the eusocial sponge-living shrimps use odor in colony recognition in a similar way to *Hemilepistus*, ants and bees?

A given animal will produce both pheromones and signature mixtures (Fig. 2.3). In addition to its sex pheromone(s), each lobster has its own highly individual odor mixture and this complex bouquet can be used by other lobsters for recognition (see Atema and Steinbach 2007).

It is likely that signature mixtures, as they involve learning, are processed differently from pheromones though this is still not fully understood (Wyatt 2010). The neurobiology of the olfactory imprinting of signature mixtures by adult mammals is well studied in mice (for the Bruce effect, the odors of her mate are learnt in her accessory olfactory lobe) and sheep (the mother learns the odors of her lamb in her main olfactory lobe) (Brennan and Kendrick 2006). Individual recognition in lobsters is mediated by the esthetic/olfactory pathways (Johnson and Atema 2005) though currently not enough is known about olfactory processing in lobsters to know if pheromones are processed differently.

## 2.3 Evolution of Pheromones

Pheromones are used right across the animal kingdom because any molecule that gives a selective advantage can potentially evolve into a pheromone. This is in part a consequence of the combinatorial mechanisms of olfaction. Invertebrate and vertebrate olfaction, despite all the superficial differences between crustacean aesthetascs and mammalian noses, works in roughly the same way. Crustaceans, with an impermeable exoskeleton, have evolved olfactory “windows” in the exoskeleton.

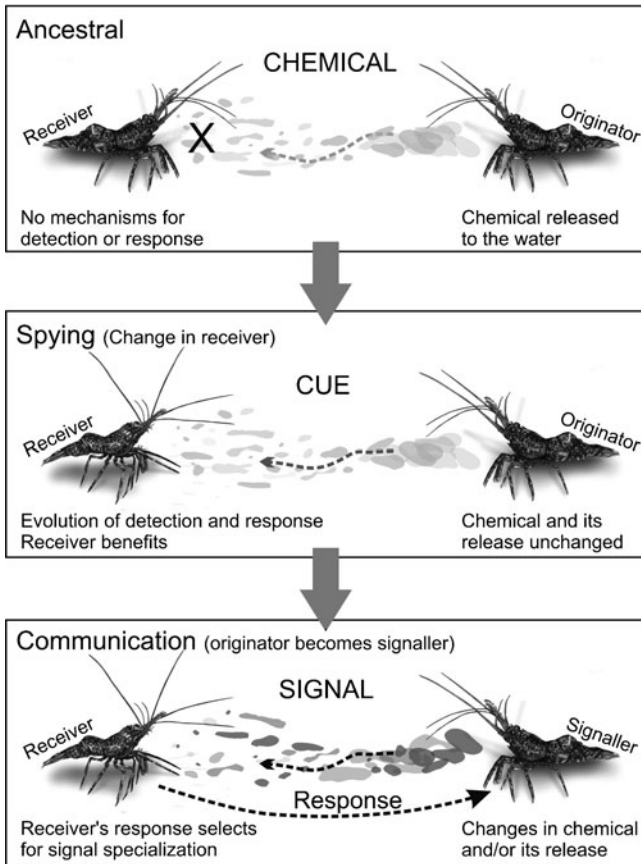
Odor molecules interact with olfactory receptor proteins on the surface of each ORN. In arthropods there are hundreds of different olfactory receptor proteins depending on species, each with a broad sensitivity/range of molecules which will interact with (Hallem and Carlson 2006). Each receptor's sensitivities overlap with those of other receptor proteins, so that a huge "olfactory world" can be covered. Each ORN presents just one of the different olfactory receptor proteins. Chemosensory systems are discussed in chapters by Hallberg and Skog (Chap. 9), and Schmidt and Mellon (Chap. 7). Derby and Sorensen (2008) remind us that detectors of pheromones and olfactory social cues are not exclusively located in the aesthetasc/olfactory pathway, giving as an example male crayfish which have sensors on their claws that detect female odors.

All the ORNs carrying the receptor of a particular type feed to the same glomerulus (neuropil) on each side of the olfactory lobe (Caprio and Derby 2008). The number of glomeruli (and by implication the number of different ORs) in Crustacea ranges from about 150 to 1,300 (Caprio and Derby 2008). The brain builds up an olfactory picture of the world from these, integrating the responses across the different glomeruli. So for example, hypothetically a molecule might stimulate ORN types 1, 3, and 25. A different molecule might stimulate ORN types 2 and 91. This combinatorial processing allows organisms to discriminate and distinguish innumerable molecules, including ones never encountered before.

Thus the olfactory system is primed to respond to any odor chemical(s) in the environment that give a selective advantage, for example detecting females about to produce eggs. Selection can start on the receiver to become more sensitive, with more selective olfactory receptor proteins, more expressed on each ORN and more of the increasingly specific ORNs in the animal's chemosensory organ(s). In this way we can evolve the sensitive pheromone detection system of the male crab. There will be corresponding selection on the emitter to release more of the molecules, now becoming an evolved signal or pheromone. A hypothetical scenario, from "spying" to an evolved signal, is shown in Fig. 2.4.

Pheromones can often be related to their likely original source or function, though they may have been modified somewhat to give specificity. For example, many fish sex pheromones are or have evolved from steroid and prostaglandin hormones (Stacey and Sorensen 2006). Alarm pheromones in ants often appear to have evolved from defensive compounds used by that species – and presumably evolved the pheromone function as these compounds would be released during combat. The Settlement-Inducing Protein Complex in barnacles appears to have evolved from  $\alpha$ 2-macroglobulin-like proteins which occur in the barnacle cuticle (perhaps through duplication of an ancestral barnacle A2M gene) (Dreanno et al. 2006; Clare, Chap. 22).

In aquatic systems, solubility takes the place of volatility. Aquatic pheromones can be large molecules so long as they are soluble in water (though contact pheromones, such as those used in mate recognition in shrimp and copepods are presumably almost insoluble – see e.g., Bauer, Chap. 14 and Snell, Chap. 23). Many aquatic organisms use polypeptides as pheromones, for example the crab "pumping pheromone" (Rittschof and Cohen 2004). Pheromone specificity can be gained by



**Fig. 2.4** Proposed stages in the evolution of a communication function for molecules released by an individual. The X in the upper panel indicates that the receiving individual has no adaptations to receive the cue. In the “spying phase,” only the receiver benefits. The transition to bilateral benefit to both sender and receiver could occur later if there is a selective advantage to the sender. In the “spying phase” there need not be changes in the molecules released by the sender. An original figure by Ivan Hinojosa, inspired by and with text from Stacey and Sorensen (2006, Fig. 9.1, p. 363) (with permission from the authors)

unique amino acid sequences. Peptides have short half-lives due to rapid consumption by microbes and this can be a useful property for some signals (Rittschof and Cohen 2004) though it may be one reason why territorial marking by scent marks does not seem to be found in aquatic organisms (Thiel and Duffy 2007).

As the pheromones of fish and crustaceans become better understood, species specificity through multicomponent blends may be revealed (Stacey and Sorensen 2006). Multicomponent synergy, with two or more pheromone components needed together, has been demonstrated to stimulate a gonadotropin surge in male goldfish.

### **2.3.1 Pheromones and Speciation**

For moths we know enough about the pheromones across species and families to analyze the way pheromones change in speciation. Closely related sympatric moth species have multicomponent pheromone blends that differ by added, lost, or changed components (Wyatt 2010). In some species we can see the changes at the gene level (e.g., Xue et al. 2007). Sex pheromones are used widely by crustaceans but copepod cuticular pheromones aside (Snell, Chap. 23) they have so far resisted identification (though we are close, see Hardege and Terschak, Chap. 19; Kamio and Derby, Chap. 20). We therefore do not yet know enough about the pheromones of any group of Crustacea to look at the variation between species. Over time I am confident we will be able to do this.

## **2.4 Peculiarities of Chemical Communication as Compared with Visual and Acoustic Communication**

The key difference between chemical communication and visual and acoustic communication is the need for molecules to travel from signaler to receiver. This requires either diffusion, likely to be important only for small organisms at the scale of millimeters (see Yen and Lasley, Chap. 9) or flow of currents (see Koehl, Chap. 5). Chemical signals are thus rarely almost instantaneous in the way that visual and acoustic signals can be. On the other hand, chemical signals can be long-lasting. The chemical cues from barnacle cuticular proteins, for example, resist a variety of chemical attacks including acids and caustic alkalis (Knight-Jones 1953; Clare, Chap. 22).

Specificity is achieved in different ways in chemical communication compared with visual and acoustic routes, which are a continuous range of spectra, differing by wavelength and temporal structure. Chemical communication offers ways of differing in many dimensions, and in particular the opportunity to combine different molecules to give specificity. A further difference may be the honesty of chemical signals. Deception in signaling, and contrasts between visual, acoustic, and chemical signals, are discussed by Christy and Rittschof (Chap. 16).

## **2.5 Novel Techniques and Questions in Chemical Communication Research**

For research on pheromones, the bioassay is crucial and is the first step to establish that there is a chemically mediated effect to be studied. The bioassay test for

activity can be behavioral or physiological depending on the pheromone being studied. The gold standard or pheromone equivalent of “Koch’s postulates” requires the isolation, identification, synthesis, and bioassay confirmation of activity (Wyatt 2009). Demonstration of activity at the biologically relevant concentration is also important.

Chemical communication is more difficult to investigate than sound and visual signals because molecules are involved rather than being phenomena amenable to spectral analysis. First there is the challenge of identifying the molecules involved (which may be at low concentrations, near the limits of instrumentation) and second there is the problem of playback. Whereas video or an MP3 player can reproduce visual or sound signals, and many signals can be created by software and then replayed, for studies of chemical communication the exact molecules have to be offered. Part of the difficulty is that the olfactory system is sensitive to every aspect of the precise identity of molecules, including their chirality.

Simple bioassay-guided fractionation approaches separate a biological sample into fractions based on a number of properties, including solubility in solvents of different polarity, molecular mass, and molecular charge (Derby and Sorensen 2008; Hardege and Terschak, Chap. 19; Kamio and Derby, Chap. 20). However, many pheromones are multicomponent, requiring a number of molecules to be present in the correct ratio and concentration for activity, so simple fractionation may not work as the components may end up in different fractions, inactive by themselves (we are very lucky that the first pheromone identified, bombykol, had a main component that was active by itself, otherwise Butenandt’s bioassays would not have revealed the molecule). This problem is made more difficult because the active molecules may not be the ones present in the largest quantities. One approach to tackle the problem of bioassaying multicomponent pheromones is to use a subtractive-combination bioassay in which a complete putative mixture is tested with successively more fractions or molecules missing, to see which ones can be removed without extinguishing the signal (Byers 1992; Kamio and Derby, Chap. 20).

Progress in chemical techniques of extraction, analysis, and synthesis are likely to enable many more pheromones to be identified. The history of pheromone research, like many other areas of science, shows the importance of new technologies and methods including gas chromatography (so helpful for work on insect pheromones), mass spectroscopy, and, more recently, the use of molecular biological techniques. The new technique of metabolomics has promise to identify biomarkers, molecules enriched in a particular context and thus candidate pheromone components (Derby and Sorensen 2008; Kamio and Derby, Chap. 20). The problem of the highest peaks, not necessarily being the pheromones, still remains though.

Recording from the animals’ own sensors as a pheromone-identifying tool shows promise though the antennogram is problematic in aquatic crustaceans because of the technical difficulty of isolating the recording electrodes from the stimulus water. There has, however, been extensive single cell recording of chemosensory organs of crustaceans (Caprio and Derby 2008).

## 2.6 The Promise of Crustaceans as Model Systems for Chemical Communication Research

Crustaceans could provide a new range of model organisms contributing to an understanding of chemical communication and chemoreception in general. However, currently a key challenge to using crustaceans as model systems in chemical communication is the lack of knowledge about the chemical identity of the pheromones involved. As more crustacean pheromones are identified, a rich range of research questions will come within reach. Nonetheless there is much that can be done in the meantime even if the chemical identities of the molecules have not been confirmed. Behavioral work in particular can go ahead using active biological samples or extracts as the chemical stimuli.

With the *Daphnia pulex* water flea genome now available (wFleaBase), genetic dissection of chemoreception in Crustacea becomes possible (e.g., Peñalva-Arana, et al. 2009). As wFleaBase's introduction points out: "*Daphnia* shares with *C. elegans* an interesting and rich gene structure and wealth of gene duplications. It has closest gene homology to the insects, *Apis* and *Drosophila*. However, it has nearly as many unique or strong gene homologies to Mouse as to insects" (wFleaBase 2009).

Many crustaceans can be cultured in controlled laboratory environments (Barki, Jones, and Karplus, Chap. 25). While many large decapods have planktonic larval stages, the peracarids (which include isopods and amphipods) are direct developers, with the eggs brooded by the female, facilitating lab culture (Thiel, Chap. 10). One possible area of research could use such direct developing species to generate particular strains, for example that have grown in particular selective environments.

One topic which is already showing the important contribution of work on crustaceans is the orientation behavior of animals to chemical stimuli. How do animals follow an odor plume to its source? Orientation behavior by crustaceans is explored in Weissberg (Chap. 4). One advantage of decapods is that they are large enough to carry tracking devices. There seem to be intriguing differences between the mechanisms used by different crustacean groups, even within the decapods.

## 2.7 Conclusions and Outlook

Chemical communication is the commonest method of communication across the animal kingdom. Crustaceans provide some of the most interesting examples of this, from sex to settlement, but with few exceptions, we do not know the molecules that are being used. Crustaceans have the potential to be among the key model organisms for work on chemoreception in the future, in particular if their pheromones can be identified and synthesized.

One area of debate, for chemical communication in all animals, is the definition of pheromone and whether the signature mixtures used for recognition should count as pheromone signals or cues (Wyatt 2010). While this is partly of semantic

interest, the question is also important because of the generalizations that come from definitions.

A key priority for the future is the continued collaboration between crustacean researchers and chemists – for both pheromone identification and synthesis. There are good signs of this happening. The next decades may be among the most exciting yet.

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