1 Animals in a chemical world

When two dogs meet and sniff, they gain a wealth of information from each other's smells. Each dog will discover the sex, maturity, and hormonal state of the other; some of these smells will be species-wide dog pheromone signals. Each dog also detects the individual smell of the other, which it learns as a "signature mixture" to remember in case they meet again.

When two ants meet and sweep antennae over each other, they have an olfactory exchange of information similar to that of the dogs, discovering age, sex, ovarian stage (reproductive or not), and caste (worker, soldier, queen), all signals from species-wide pheromones. They also detect the colony odor of the other ant, enabling them to decide by the "signature mixture" whether the other ant is a nestmate or not.

All animals produce a chemical profile, present on the body surface, released as volatile molecules, and from scent marks that they deposit (by dogs on lamp-posts for example) (Figures 1.1, 1.2, 13.2). As chemical senses are ancient and widespread, shared by all organisms including bacteria, animals are pre-adapted to detect chemical information in the environment (Box 1.1). Across the animal kingdom, animals of all kinds gain chemosensory information from other organisms. Chemical senses are used to locate potential food sources and detect predators. Chemical senses also mediate the social interactions that form the focus of this book, as illustrated by the dogs and ants above. We can probably say that more organisms use chemosensory communication than any other mode.

A chemical involved in the chemical interaction between organisms is called a semiochemical (Box 1.2). Some of the semiochemicals emitted by animals are pheromones, evolved as signals for communication. Other semiochemicals, such as the carbon dioxide in exhaled breath, did not evolve as a signal, but can be exploited as a cue by blood-sucking mosquitoes as a way of finding a host. Some of the other molecules emitted by animals, such as odors due to infections, may also be cues. The distinction between signals and cues is explored further in Section 1.3.

Pheromones and signature mixtures are semiochemicals used *within* a species. Semiochemicals acting between individuals from different species are called **allelochemicals** and are further divided depending on the costs and benefits to signaler and receiver (Box 1.2) (Chapter 11) (Nordlund & Lewis 1976; Wyatt 2011). Pheromone signals can be eavesdropped ("overheard") by unintended recipients: for example, specialist predatory beetles use the pheromones of their bark beetle prey to locate them. The predators are using the bark beetle pheromones as **kairomones**. Animals of one species can emit fake, counterfeit signals that benefit themselves at the cost of the receiving species. Chemical signals used in such deceit or propaganda are termed **allomones**: for example, bolas spiders synthesize particular moth pheromones to lure male moths of those species. Semiochemicals benefiting both signaler and receiver in mutualisms, such as those between sea anemones and anemone clownfish, are termed **synomones**. The multiplicity of terms is only useful as shorthand and the terms are clearly overlapping, not mutually exclusive (for example, a molecule used as a pheromone *within* a species can be used as a kairomone by its predator).

My aim in this book is to focus on patterns across the animal kingdom. I have tried to include examples from as many animal taxa as space allows, but for more detail see the suggestions in further reading and references in the text. This chapter introduces the ways in which animals use semiochemicals and many of the topics are explored at greater length in later chapters (see Preface for overview and rationale).

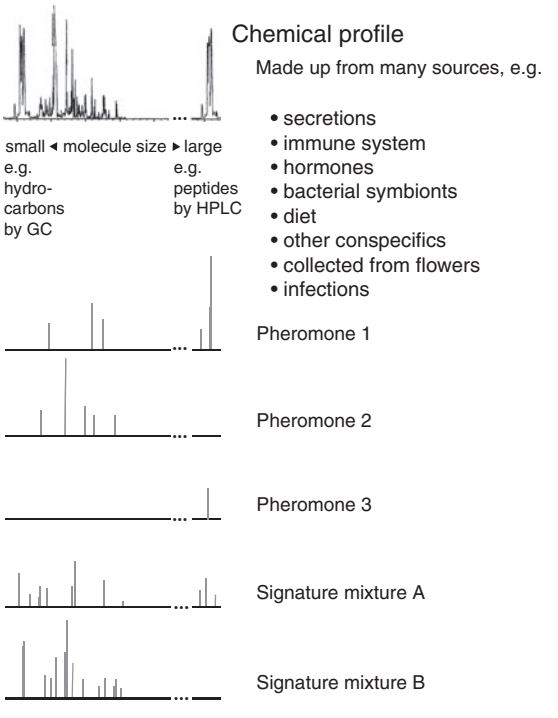

Figure 1.1 Pheromones occur in a background of molecules that make up the chemical profile consisting of all the molecules extractable from an individual. The chemical profile (top) is an imaginary trace from an imaginary column capable of analyzing all the molecules (at one side is high-performance liquid chromatography (HPLC) with large proteins, at the other is gas chromatography (GC) with small volatile molecules). Each peak represents at least one molecule.

Much of the chemical profile is highly variable from individual to individual. The sources of the molecules in the chemical profile include the animal itself as well as its environment, food, bacteria, and other individuals etc. It is this complex background that makes identifying pheromones so challenging in many organisms.

The pheromones could include sex pheromones or ones related to life stage or caste. The pheromones would be the same in all individuals of the same type in a species (dominant male, worker ant, forager, etc.); that is, they are anonymous, common across the species. As examples, I have included some possible kinds of pheromones that are known from organisms (not necessarily in the same species): a specific combination of large and small molecules (Pheromone 1), a combination of small molecules (Pheromone 2), or a particular large molecule by itself such as a peptide (Pheromone 3).

The signature mixtures (A and B) are subsets of variable molecules from the chemical profile that are learned as a template for distinguishing individuals or colonies. Different receivers might learn different signature mixtures of the same individual. For example, a male might learn a different signature mixture of his mate than the one her offspring might learn. Hypothetically it is conceivable that the male might learn different signature mixtures for the same female in different contexts, say immune-system associated molecules in one context and more diet influenced molecules in another. In other words, signature mixtures seem to be a "receiver-side" concept.

Adapted from Wyatt (2010). The layout is inspired by Figure 1 of Schaal (2009).

1.1 Intra-specific semiochemicals: pheromones and signature mixtures

Modern pheromone research could be said to date from 1959, when the chemist Adolf Butenandt and his team identified the first pheromone, the silk moth's sex pheromone bombykol, which prompted the coining of the word "pheromone," from the Greek *pherein*, to transfer; *hormōn*, to excite (Butenandt *et al.* 1959; Karlson & Lüscher 1959). Butenandt's discovery established that chemical signals between animals exist and can be identified (Chapter 2). From the start, Karlson and Lüscher (1959) anticipated pheromones would be used by every kind of animal, from insects and crustaceans to fish and mammals. Since then, pheromones have been found across the animal kingdom, in every habitat on land and underwater, carrying messages between courting lobsters, alarmed aphids, suckling rabbit pups, mound-building termites, and trail-following ants (Wyatt 2009). They are also used by algae, yeast, ciliates, and bacteria. It is likely that the majority of species across the animal kingdom use them for communication of various kinds. Much is known about the pheromones of

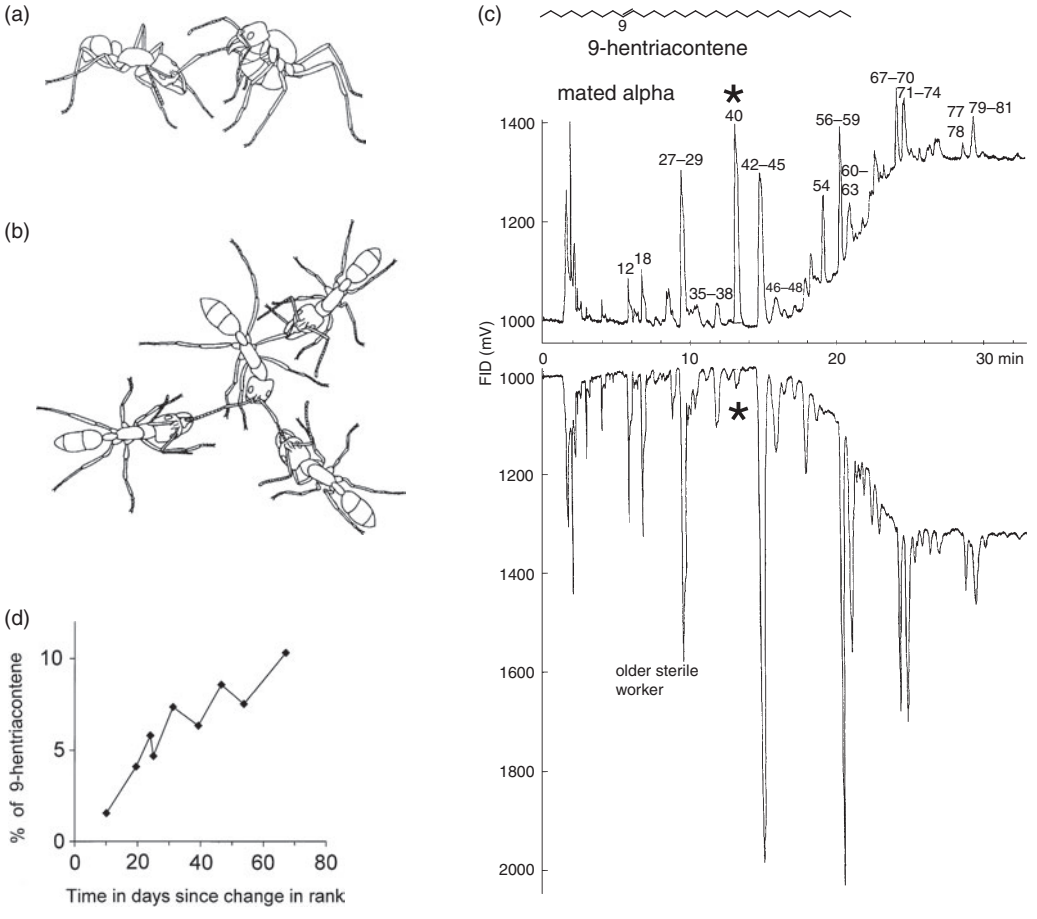

Figure 1.2 The "queenless" ant, *Dinoponera quadriceps*, lives in small groups headed by an alpha female, the only egg-laying individual in the colony. The hierarchy is maintained by physical aggression. This can include gaster rubbing (a) in which the alpha female rubs the antenna of the subordinate on the cuticular hydrocarbons, which include the alpha's pheromone "badge" of dominance, 9-hentriacontene (c, top). This molecule is characteristic of alpha females in all colonies of the species.

(b) If a subordinate female becomes reproductive and starts to produce the molecules characteristic of an alpha female, other ants in the colony detect this and immobilize her (an example of an honest signal maintained by punishment, Section 1.6).

(c) The colony profile of ants in the colony can be shown in a solid phase micro extraction (SPME) gas chromatographic analysis of their cuticular hydrocarbons (Monnin *et al.* 1998) (Chapter 2). As well as the many-peaked hydrocarbon chemical profile shared by the other ants in the colony, the alpha female also has the additional peak #40 (indicated by the asterisk) which is the pheromone 9-hentriacontene. Below, her fellow colony members have the same colony profile as her but lack this peak.

(d) Non-destructive SPME sampling allowed changes in the percentage of 9-hentriacontene in the cuticular hydrocarbons of an individual ant to be followed in the days after she became the alpha female. In a larger sample of ants undergoing the transition, the significant difference was between the quantities at 15 and 30 days.

(a) and (b) from Monnin and Peeters (1999), (c) chromatograph from Monnin *et al.* (1998), (d) from Peeters *et al.* (1999).

insects, fish, and mammals, but some other taxa have not been well studied. For example, crabs and other Crustacea make extensive use of pheromones but relatively few of these have been chemically identified (Breithaupt & Thiel 2011). Birds, too, have now been shown to have a rich olfactory life though we are only

Box 1.1 Chemical and other senses compared

Chemical senses are shared by all organisms including bacteria. However, while the general way that molecules interact with chemosensory receptor proteins in a "lock and key" manner is shared, the chemosensory receptor proteins are highly variable across the animal kingdom and even within animal taxa. This is because the chemosensory system, like the immune system, tracks a changing world of molecules generated by other organisms. Over evolutionary time, the chemosensory systems of organisms co-opt, test, and discard chemosensory receptor genes and neural coding strategies, leading to great divergences in receptors (Bargmann 2006b; Bendesky & Bargmann 2011). Chemosensory receptor genes turn over rapidly, in a birth-and-death process of gene duplication and loss (see Chapter 9). The rapid evolution of chemosensory receptor proteins, evolved independently in insects and vertebrates, made chemoreception much harder to investigate than vision (Chapter 9). The key proteins (opsins) for light-detection in eyes do vary considerably and insect and vertebrate opsins have diverged. However, unlike chemosensory receptor proteins, they form a large monophyletic group within the G-protein-coupled receptor (GPCR) superfamily (Porter *et al.* 2012).

At the level of the individual, variation in olfaction is much greater than in the opsin genes. For humans, mutations in the four genes for opsin receptor proteins sensitive to different wavelengths of light give us a small number of different kinds of color vision deficiency or "color blindness." By contrast, we have more than 400 olfactory receptor genes, each of which can be mutated, so each of us smells a unique world (Chapter 13) (Olender *et al.* 2012). For this reason too, we might each remember different mixtures of molecules as signature mixtures to recognize the odors of other people.

The chemical senses of olfaction and taste are very different from vision and hearing, which detect the energy of different wavelengths in the form of light and sound: chemical senses rely on the physical movement of molecules from the signaler to the sense organ of the receiving animal. This requires either diffusion, only likely to be important for small organisms at the scale of millimeters, or flow of currents (Chapter 10). Either way, the time taken for molecules to travel to the receiver means that chemical signals are rarely instantaneous in the way that visual and acoustic signals can be.

Challenges remain for studying chemical communication (Chapter 2). We can record and play back the sound signals of an animal easily enough, but we do not have devices to do the same for chemical signals. Each molecule needs to be correctly synthesized, in every detail (see Section 1.4.3 and Appendix), before it can be "played back" to the animal. This can be challenging for a team of biologists and makes chemist partners invaluable. For example, methyl-branched alkanes, important components of ant CHCs, are not commercially available and synthesizing these is a costly and time-consuming process (van Zweden & d'Ettorre 2010).

Yet, perhaps more than other modalities such as sound or vision, chemosensory systems are amenable to molecular manipulation: in model systems we can now study communication at the

Box 1.1 (cont.)

level of the genes involved in signal production (e.g., enzyme pathways) and signal reception (genetics of receptors, brain, and behavior) especially in model animals such as *Caenorhabditis elegans*, moths, *Drosophila*, and the mouse.

just beginning to discover what molecules their pheromones might be (Campagna *et al.* 2012; Caro & Balthazart 2010; Hagelin & Jones 2007; Zhang *et al.* 2010). Research on human semiochemicals is at a similarly early stage; I review our current state of knowledge in Chapter 13.

The idea of chemical communication was not new in 1959. The ancient Greeks knew that the secretions of a female dog attracted males. Charles Butler (1623) warned in *The Feminine Monarchie* that if a beekeeper accidentally crushes a honeybee, the bees "presently finding it by the ranke smell of the poisonous humor, will be so angry, that he shall have work enough to defend himself." In *The Descent of Man, and Selection in Relation to Sex* (1871), Charles Darwin included chemical signals alongside visual and auditory signals as outcomes of sexual selection, describing the strong smells of breeding males in moths, pythons, crocodiles, musk ducks, goats, and elephants. Jean-Henri Fabre (1911), also writing in the 1870s, described how male great peacock moths, *Saturnia pyri*, flocked around a female moth hidden behind wire-gauze, but ignored visible females sealed under glass. A female moth's smell could be collected on a cloth and males would flock to that too. Many other scientists in the nineteenth century and first half of the twentieth century, including Niko Tinbergen, had worked on phenomena we would recognize as being mediated by pheromones (some are mentioned in Karlson & Lüscher 1959). However, because the quantities emitted by an individual animal were so small, the chemistry of the day could not identify them, until the inspired idea of using domesticated silk moths, which could be reared in the hundreds of thousands necessary to collect enough material for analysis using the techniques available at that time (Chapter 2).

The enormous variety of organic molecules identified as pheromones since the first, bombykol, in 1959 is as diverse as the animal kingdom, and offers an ongoing challenge for chemists interested in the identification, synthesis, and exploration of natural functions of novel compounds (Cummins & Bowie 2012; El-Sayed 2013; Francke & Schulz 2010). The likely explanation for the diversity of pheromone chemistry is that these signals have evolved from chemical cues naturally released by organisms, facilitated by the broad tuning of olfactory receptors (Chapter 9) (Section 1.3).

Invertebrates and vertebrates, in a wide range of habitats, use chemical communication in similar ways. Animals as different as moths and elephants may share the same molecule(s) as part of their pheromones. However, there are more fundamental parallels in sensory processes, even if we are not always sure whether this has occurred by convergence or via shared ancestors. The parallels include the combinatorial way that the sense of smell is organized in the brain: olfactory sensory neurons with the same olfactory receptor all collect at the same spot (glomerulus) in the brain; the information from different glomeruli is combined to identify the molecule (the combinatorial mechanism) (Chapter 9).

1.1.1 Pheromones

Pheromones are molecules that have evolved as a signal between organisms of the same species. The

signal elicits a specific reaction, for example, a stereotyped behavior (releaser effect) and/or a developmental process (primer effect) from a conspecific (member of the same species) (Box 1.2) (Section 1.9) (Wyatt 2010). Many, probably most, pheromones (including the sex pheromones of most moths and some mammal pheromones) are *not* single compounds, but rather a species-specific combination of molecules in a precise ratio. This combination *is* the pheromone (though sometimes called a multicomponent pheromone or pheromone blend). A pheromone can elicit a variety of effects, depending on the context and the receiver (Section 1.8). Responses to pheromones usually seem to be innate (though this is not a part of the definition). In the few instances where learning is first required for a pheromone to act, all animals normally learn the same molecule(s), which is what defines it as a pheromone (Section 1.2).

Box 1.2 Definitions of chemical mediators

Pheromones are signals. The other categories of semiochemicals in this box are cues that can be used for information but did not evolve for that function (Section 1.3). Adapted from Wyatt (2010, 2011) based on Nordlund and Lewis (1976).

See Wyatt (2011) for a discussion of the origins and usage of these terms. I discuss interspecific interactions mediated by allelochemicals in Chapter 11. "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.

A. **Hormone:** a chemical agent, produced by tissue or endocrine glands, that controls various physiological processes **within** an organism. (Nordlund & Lewis 1976).
B. **Semiochemical:** a chemical involved in the chemical interaction between organisms. (Nordlund & Lewis 1976) (from the Greek: *semeion*, mark or signal).
    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). (From the Greek: *pherein*, to carry or transfer, and *hormōn*, to excite or stimulate).
    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, mice) or as a member of a particular social group such as a family, clan, or colony (e.g., ants, bees, badgers). (Wyatt 2010; derived from Johnston's "mosaic signal" *sensu* 2003, 2005; Hölldobler and Carlin's, 1987 ideas; and Wyatt's, 2005 "signature odor").
    3. **Allelochemical:** chemical significant to organisms of a species different from their source, for reasons other than food as such. (Nordlund & Lewis 1976).

Box 1.2 **(cont.)**

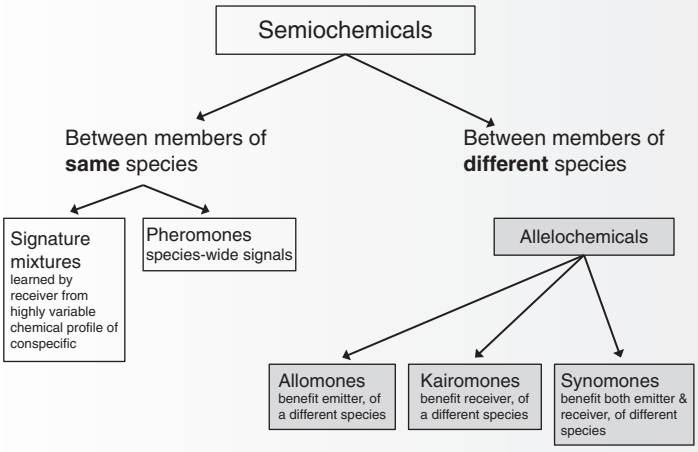

Diagram showing the relationships between different kinds of semiochemicals. Inspired by Box 7.1 in de Brito-Sanchez *et al.* (2008) and other sources.

Karlson and Lüscher (1959) predicted that most pheromones would act via the conventional senses of olfaction or taste, but that some pheromones might be ingested and act directly on the brain or other tissues. We would call these allohormone pheromones (Section 1.11). They speculated that royal jelly in honeybees might contain such a pheromone, and indeed an active molecule (royalactin) has been identified, which causes larvae receiving it to develop into queens rather than workers (Chapter 9) (Kamakura 2011).

Pheromones include the familiar sex attractant pheromones, and numerous others that serve a wide variety of functions. Some pheromones are specific to different life stages or castes. One key feature of pheromones is that they are "anonymous," that is, a given pheromone is the same in all individuals within a species of the same type (e.g., male or female) or physiological state, and it conveys a stereotyped message that is independent of the individual producing it (Hölldobler & Carlin 1987).

However, quantities of pheromone can differ between individuals or in the same individual over time. Some male mouse pheromones, the farnesenes, are produced only by dominant male territory holders, not subordinates (Hurst & Beynon 2004). In the ant *Dinoponera quadriceps*, when an ant becomes the top (alpha) female, she starts to produce the standard chemical badge of a "top female" in her species, 9-hentriacontene (Figure 1.2) (Peeters *et al.* 1999). However, in the male mouse and the top female ant of these examples, the pheromones are still anonymous (Hölldobler & Carlin 1987; Hölldobler & Wilson 2009, p. 270). They indicate the presence of, for example, a dominant male mouse or an alpha female ant, not a particular individual.

Some of our expectations of pheromones have been heavily influenced by the well studied response of male moths to the sex attractant pheromones of conspecific females. For example, the antennae of male moths have thousands of highly specialized receptors

for the pheromone and specific areas of the brain dedicated to processing the pheromonal signal. However, other pheromone processing in insects may involve less specific receptors, without dedicated brain areas (glomeruli) (see Chapter 9). Thus, we now know that narrowly tuned and highly specialized receptors and dedicated glomeruli are not a prerequisite for pheromone use. For example, honeybee alarm pheromone components seem to be processed by receptors and glomeruli that also process other, non-pheromone molecules (Chapter 9) (Wang *et al.* 2008b).

Similarly, male moths' enormously enlarged antennae, covered with thousands of olfactory sensilla that are tuned specifically to the pheromone, reflect selection for extreme sensitivity to low concentrations of female pheromone, necessitated by the scramble competition to be the first to reach the female (Chapters 3, 9, and 10). Based on the great body of work on male moths, we tend to expect all receivers of pheromones to be very sensitive to them and to respond at great distances. However, other animals may not use attractant pheromones at all, although they may still use sex-specific contact pheromones for sex and species recognition when in close proximity to each other (for example the contact sex pheromones used by some copepods; Snell 2011b). The stimulus concentration on contact can be high and thus exquisite sensitivity in the olfactory or gustatory receptors that perceive contact pheromones is unnecessary. A small number of specialized chemosensory neurons may be sufficient. This seems to be the case for short range species recognition mediated by contact chemicals during the courtship of *Drosophila* males and females (Chapters 3 and 9).

When the original definition of pheromone was proposed in 1959, only a single pheromone had been chemically identified: bombykol of the silk moth female (Karlson & Lüscher 1959). It is a tribute to Karlson and Lüscher, and their wide consultation, that the definition has held up so well (Wyatt 2009). It is not surprising that the definition has needed to be updated slightly since then (Box 1.2) (Wyatt 2010). (See Box 1.3 and Box 1.4 for why words matter and how distinguishing the concepts can be helpful).

Box 1.3 Pheromones and signature mixtures: why words matter

Definitions matter because they can provide useful generalizations and predictions. My purpose in separating pheromones from signature mixtures is pragmatic and based on the heuristic (rule of thumb) value of separating these kinds of chemical information. When we say something is a pheromone, the reader can anticipate that it is a molecule (or a particular combination and ratio of molecules for a multicomponent pheromone) that will be found, for example, in all sexually mature females. Quantities of the pheromone may differ between individuals, and this may be important in mate choice (Chapter 3), but not in ways that allow an individual female to be recognized as an individual. In Hölldobler and Carlin's (1987) terms, the pheromone signal is "anonymous," it could be any female (see also Hölldobler & Wilson 2009, p. 270). (See also Box 1.4 Operational definition of pheromone.)

In contrast, if a phenomenon, such as a male distinguishing his mate from other females, relies on a learned signature mixture, it would be fruitless to search for a single combination of molecules eliciting individual mate recognition across the species: it is precisely the great

Box 1.3 (cont.)

differences *between* females' chemical profiles that makes learning signature mixtures by males possible.

In the first edition of this book, I included signature mixtures within the definition of "pheromones" (Wyatt 2003, pp. 2–4). I now think it is more helpful to explicitly separate signature mixtures as it is emerging that their characteristics are different, in particular the variability of signature mixtures and the need for learning (Tables 1.1 and 1.2) (Wyatt 2010). It seems to be a useful distinction, which has helped understand phenomena best explained by species-specific pheromone molecules appearing on a background of variable chemical profiles from which signature mixtures are learned, in situations as varied as the male effect in sheep (Hawken & Martin 2012) and trail pheromones in stingless bees (Reichle *et al.* 2013).

So, to be clear, not all molecules included in this book are pheromones. I will discuss many molecules that are not pheromones (Section 1.3), including the highly variable signature mixtures used to avoid mating with kin (Chapter 3) and learned by ants to distinguish nestmates from non-nestmates (Chapter 6), as well as chemical cues such as barnacle settlement cues (Chapter 4) and fish alarm cues (Chapter 8).

Box 1.4 Operational definition of pheromone

The formal definition of a pheromone includes both evolved emission and reception of the signal for that function (Section 1.3) (Table 1.1) (Maynard Smith & Harper 2003, p. 3). However, for many otherwise respectable pheromones, we do not know enough about the ways in which production and/or reception may have evolved. So, I propose we formalize an operational definition of pheromone, which most people already use in practice, as "fully identified molecule(s), the same across a species, in all lactating mature females for example, which when synthesized elicit the same characteristic response in the conspecific receiver as the natural stimulus."

To legitimately assert that a molecule or specific combination of molecules qualifies as a pheromone for a species (or in a genetically defined subpopulation within a species):

1. The synthesized molecule/combination of molecules (combination) should elicit the same response as the natural stimulus in the bioassay.
2. It should act in this way at realistic concentrations similar to the natural stimulus.

Box 1.4 (cont.)

3. For multicomponent pheromones, experiments should demonstrate that all compounds in the combination are necessary and sufficient.

4. Only this molecule or the proposed combination of molecules elicits the effect (and other similar molecules or combinations that the animal would encounter do not).

5. There should be a credible pathway for the pheromone signal to have evolved by direct or kin selection.

6. Quantities may vary between individuals (e.g., subordinate and dominant males).

The requirements follow those explored in Chapter 2. They are the equivalent of "Koch's postulates" for establishing causal relationships for pheromones: initial demonstration of an effect mediated by a pheromone, then identification and synthesis of the bioactive molecule(s), followed by bioassay confirmation of activity of the synthesized molecules. It can be equally important to show that other similar molecules do *not* have the effect of the proposed pheromone.

How the response develops (ontogeny) in an individual is a separate question (Section 1.2). Normally we do not know the details. Fish alarm substances are thought to be cues rather than pheromones (Chapter 8) as they fail to satisfy criterion #5.

Sadly, the experimental literature on humans, and other mammals, includes many unidentified extracts or molecules that have never been rigorously demonstrated to be biologically active by the full bioassay evidence and synthesis process. It is misleading to call them even "putative pheromones" (Chapter 13).

1.1.2 Signature mixtures

Returning to the dogs and ants that opened this chapter, the individually distinctive mixture of molecules that allows dogs to tell each other apart by smell and allows ants, at a colony level, to distinguish nestmate from non-nestmate, are *not* pheromones and were not included in the original definition.

We need a different term for the molecules that animals learn and use to distinguish other individuals or colonies. I have proposed "signature mixture" (Wyatt (2010) inspired by Johnston's (2003, 2005) "mosaic signal," Hölldobler and Carlin's (1987) ideas, and based on Wyatt's (2005) "signature odor"). I think some of the early doubts about mammal pheromones (Box 1.5) came from treating signature mixtures as if they were pheromones. Be aware when reading the past and current literature that the term "pheromone" is still used ambiguously and may be used in contexts where "signature mixture" or "chemosensory cues" would be more accurate or helpful.

Signature mixtures are the subsets of variable molecules from the chemical profile of an individual (Figure 1.1) that are learned as templates by members of the same species (conspecifics) and used to recognize an organism as an individual or as a member of a particular social group such as a family, clan, or colony