# Biological Responsiveness to Pheromones Provides Fundamental and Unique Insight into Olfactory Function

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## Abstract

When exposed to the odor of conspecifics, most organisms exhibit an adaptive behavioral response, particularly if the individuals are sexually mature. Evidence increasingly suggests that behavioral responsiveness to these odors, which are termed 'pheromones', reflects neuroethological mechanisms associated with olfactory function. Reproductive pheromones, which are the best understood, are commonly used by both invertebrates and vertebrates. In both instances they are generally comprised of mixtures of compounds and behavioral responsiveness to them is largely instinctual, sexually-dimorphic, and attributable to a specialized component(s) of the olfactory system. While pheromonal responsiveness in some systems (e.g. moths) appears highly stereotypic and symptomatic of a relatively simple 'labeled line', behavioral responsiveness of other animals (e.g. rodents) can be modified by experience, suggesting a more complex underlying central mechanism. In any case, our understanding of these fascinating systems is progressing only because of an active dialogue between behavioral and neurological investigations. This review briefly examines how behavioral studies have provided fundamental insight into the neuroethology of olfactory function by drawing comparisons between some of the better understood sex pheromone systems which have been described in heliothine moths, the goldfish, and the pig. Many similarities between invertebrate and vertebrate pheromone systems are noted. **Chem. Senses 21, 245–256, 1996.**

## General introduction

Many organisms exhibit specific behavior patterns when exposed to odors released by conspecifics. Karlson and Luscher (1959) coined the term 'pheromone' to describe these odors, and although their definition is notably vague and somewhat confusing (not a single pheromone had been identified at the time the term was coined), it remains in common usage (see Christensen and Sorensen, 1996, this issue). Notably, the definition of a pheromone is functional in nature: it implies that particular odors have specific and instinctual effects on the behavior and/or physiology of particular organisms. This, of course, implies the existence of at least somewhat specialized neural pathways mediating responsiveness to these cues. Nevertheless, demonstrating behavioral responsiveness remains the bottom line in pheromonal investigations. Several hundred pheromones have now been clearly identified in insects and several dozen in vertebrates, including a few in mammals. This review attempts to shed light on commonalties in the neuroetho-

logical basis of pheromonal function in invertebrates and vertebrates by selectively examining behavioral actions of some relatively well understood reproductive pheromones. Aspects of pheromonal function which will be considered include:

(i) the importance of pheromonal signals to animal behavior;
(ii) the role of mixtures in behavioral responsiveness;
(iii) instinctual responsiveness and the influence of experience and physiological state on this process;
(iv) the importance of temporal patterning to an oriented behavioral response.

These topics will be briefly reviewed in a variety of insects, teleost fish and several species of mammals for which we have at least some understanding of the chemical nature of the signal involved, the physiology of the chemosensory response to it and the nature of the behavior evoked. Readers are encouraged to read this article in conjunction with those of Christensen and Sorensen (1996), Kaissling (1996) and Mustaparta (1996; all this issue), where other details may be found.

## Importance of pheromones to the behavior of insects, fish and terrestrial vertebrates

Most insect species rely heavily on chemical cues to mediate their everyday behaviors and to synchronize their activity cycles. Behavioral effects of pheromones include: attraction to sexually mature conspecifics, repulsion by competing males or different species, and dispersion associated with high population densities (see Payne et al., 1986). Several characteristics of insect biology are notable because of the manner in which they influence pheromone usage. First, winged insects are relatively small and light-weight, which makes oriented movement quite a challenge. Second, insect nervous systems are comparatively simple, although their behaviors may be quite complex. Third, insects are specious and closely-related species may frequently be sympatric, making hybridization a danger. All of these factors are particularly important in understanding how male moths locate conspecific females using pheromonal cues (see below). We will pay particular attention to the heliothine moths because of the great detail with which their behavior patterns and physiologies are understood.

Like insects, many species of fish face enormous problems finding their way around their environment, which is relatively large, dense, and often dark and devoid of visual cues. Furthermore, the aquatic medium contains innumerable dissolved compounds. Notably, fish are also highly specious, and like insects, may be expected to frequently encounter closely-related species so hybridization can be a problem. It is not surprising then that teleost fish have also evolved sophisticated and varied chemical signaling systems. These systems have a variety of important functions including: recognition of injured conspecifics (Smith, 1992), attraction to conspecifics (Li et al., 1995), kin recognition (Brown and Brown, 1992), synchronization of reproductive physiologies (Dulka et al., 1987) and synchronization of reproductive behavior patterns (Sorensen et al., 1988, 1989; Stacey and Sorensen, 1991). As has been the case with insects, reproductive pheromones have received much attention in fish and it is now well established that fish olfactory systems are acutely and specifically sensitive to a variety of hormonally-derived compounds and that some of these compounds have behavioral (pheromonal) activity. This review will focus on the goldfish, Carassius auratus, because it is the only species for which pheromone release, neural sensitivity, and behavioral responsiveness have all been characterized.

Finally, although pheromonal function is less understood in terrestrial vertebrates, it is clear that pheromonal cues play essential roles in synchronizing endocrine cycles and reproductive behaviors in a wide variety of reptiles (Mason et al., 1989) and mammals (Vandenbergh, 1988). This review will focus on those few mammals for which both the chemical signal and neural basis of responsiveness have been characterized. It should be noted that our poor understanding of mammalian pheromones is not for lack of effort—in fact, quite the contrary—and many attribute this situation to the likelihood that mammals are using these cues in a much more complex and sophisticated manner than 'lower' organisms (Beauchamp et al., 1976). Nevertheless, several conspecific compounds which exert potent effects on the reproductive behavior and physiology of naive conspecifics have now been clearly identified. The pig's pheromone is particularly well understood and serves as a focus in this paper. It is our assumption that while the pig and the goldfish may represent relatively simple models, they are representative of a much larger group of vertebrates.

## Behavioral recognition of complex pheromonal odors

Contrary to the thinking of many early workers, the preponderance of evidence now suggests that insect sex pheromones

are generally not singular, highly-specialized biochemical products, but rather specific mixtures of relatively unspecialized metabolites. Frequently, individual components of a pheromonal blend either lack behavioral activity or may even be repulsive. In fact, it was this discovery which lead to the realization that pheromones are likely to be mixtures. Thus, the discovery of bombykol, the first insect pheromone to be identified, which turned out to be a unique compound, has set a misleading precedent (see Kaissling, 1996, this issue). Bombykol is now known to be accompanied by another cue, its aldehyde, bombykal, which appears to reduce behavioral and olfactory sensitivity to bombykol. Additionally, several elegant examples now exist which explicitly demonstrate that moths use mixtures of up to seven compounds that function as female sex attractants. Some of the best examples of this are found in the Lepidoptera (see Linn and Roelofs, 1989), for which at least two components of a pheromonal blend must generally be present to elicit significant behavioral activity: tests of the major component alone may totally lack activity. Furthermore, behavioral activity is generally contingent upon the insect in question being exposed to precise blends of pheromonal components. For instance, the noctuid moths, *Pelosi pisi* and *P. flammea*, have the same two principal components in their blends, but use different relative concentrations (see Kaissling and Kramer, 1990; Kaissling, 1996, this issue). The noctuid moths are also interesting because some of them are sensitive to compounds released by related species which function as behavioral inhibitors (Priesner 1980; Priesner *et al.*, 1984; Kaissling, 1996, this issue). Thus, both intraspecific and interspecific pheromones may exist, and there often is little direct relationship between what a particular insect species produces and what its olfactory system detects (Van der Pers and Löfstedt, 1986).

A particularly interesting example of the importance of pheromonal mixtures comes from behavioral studies of two sympatric heliothine moths, *Heliothis virescens*, the tobacco budworm, and *Heliocoverpa* (formerly *Heliothis*) *zea*, the corn earworm moth. Neural correlates of this behavior are reviewed by Mustaparta (this issue). (Z)-11-hexadecanal (Z11–16:Ald) and (Z)-9-tetradecanal (Z9–14:Ald) were first identified as sex pheromones in *H. virescens* by Roelofs *et al.* (1974) using a simple laboratory behavioral assay and gas chromatography. Soon thereafter, Tumlinson *et al.* (1975) suggested that there might be other active components because crude extracts of female pheromone glands had greater behavioral activity. Subsequently, Klun *et al.* (1980a,b,c) demonstrated that this species releases five

additional compounds: tetradecanal (14:Ald), hexadecanal (16:Ald), (Z)-7-hexadecenal (Z7–16:Ald), (Z)-9-hexadecanal (Z9–16:Ald), and (Z)-11-hexadecenol (Z11–16:OH). Klun *et al.* (1980a,b,c) then demonstrated that *H. virescens*'s congener, *H. zea*, also produces four of the components found in *H. virescens* (Z11–16:Ald, Z9–16:Ald, Z7–16:Ald, 16:Ald), but in different ratios. Behavioral assays demonstrated that *H. virescens* responded best to a mixture of Z11–16:Ald with a moderate amount of Z9–14:Ald, while *H. zea* responded best to Z11–16:Ald with a small quantity of Z9–16:Ald (Vickers *et al.*, 1991). Furthermore, when male *H. zea* are exposed to the *H. virescens* blend they are actually repelled. Exposure to the primary component alone is also unable to effect oriented behavior. Thus, insects may use pheromonal interspecific signals as behavioral inhibitors and/or repellents to prevent hybridization. These behavioral observations have stimulated electrophysiological examinations of olfactory function which have now demonstrated that recognition of these cues is extremely precise: different populations of specific receptor neurons (Almaas *et al.*, 1991) project to different regions of the antennal lobe (Christensen *et al.*, 1991; Mustaparta, 1996, this issue). Interestingly, the latter advances have in turn stimulated further behavioral studies which have now demonstrated that all principal components of the blend must be present simultaneously, and present in the 'correct' temporal pattern, to stimulate upwind flying behavior (Vickers and Baker, 1992; see the last Section).

Evidence increasingly suggests that fish also use pheromonal mixtures. Although hormonal compounds have long been hypothesized to serve as pheromones in teleost fish because of their close association with the fish's physiological state and their solubility, the first clear demonstration of this possibility came with the serendipitous discovery that the maturation-inducing steroid 17,20β-dihydroxy-4-pregnen-3-one (17,20βP) also functions as a priming pheromone in the goldfish. This finding was the consequence of a study investigating the behavioral and physiological effects of this steroidal hormone, which is associated with final gonadal maturation in both males and females (see Sorensen, 1992). 17,20βP was found to 'escape' to the water when injected into fish, apparently mimicking natural release rates by ovulatory females, and stimulating spermatogenesis in conspecifics with intact olfactory systems (Stacey and Sorensen, 1986). This finding has since been extended and it is now clear that goldfish use at least five 'hormonal pheromones' and they are released as blends (Scott and Sorensen 1994; Sorensen *et al.*, 1995, 1996). Additionally, it is now clear

that at least several dozen other species of fish are also sensitive to a variety of hormonal compounds—apparently hormonal pheromones are widely used (Stacey *et al.*, 1994). Interestingly, as with insects, closely related groups of fish often share similar peripheral sensitivities (as measured by electro-olfactogram or 'EOG' recording), with differences usually only evident at the level of the genus or family. Also, most species detect more than a single compound. Unfortunately, little is known about behavioral responsiveness to these cues or relative release rates, so the importance of blends can not presently be ascertained. However, it is notable that behavioral studies using crude odors suggest that at least some fish sex pheromones can be species-specific (McKinnon and Liley, 1987), suggesting this possibility should be considered in future neuroethological studies.

In spite of our rudimentary understanding of pheromonal specificity for teleost fish, several analogies can be drawn between goldfish and insect pheromone systems. It is striking that, as was the case with bombykol, the original supposition that 17,20βP might represent a specialized cue which functions on its own has proven unfounded. This steroid is now known to be a relatively minor component of a rather complex mixture of over a dozen steroids. At least two other steriods are also specifically detected by the goldfish olfactory system: 17,20β-dihydroxy-4-pregnen-3-one-sulfate (17,20βP-S) and androstenedione (AD) (Sorensen *et al.*, 1995). Thus, as with insect pheromones there is little correlation between what goldfish release and what they detect (Sorensen and Scott, 1994). Bioassays also demonstrate that AD has inhibitory effects on biological activity of 17,20βP; however, the importance of precise ratios has not been investigated. The possibility that the goldfish pheromonal mixture may include some non-hormonal components is also suggested by the observation that responsiveness to 17,20βP is enhanced in odor plumes containing the odor of juveniles (Bjerselius *et al.*, 1995a). Direct comparisons between the pheromonal efficacy of steroidal mixtures and crude odor extracts of goldfish have yet to be made.

In addition to releasing steroidal derivatives during their pre-ovulatory period, ovulated (i.e. sexually active) female goldfish release a suite of pheromones derived from circulating prostaglandin F2α (PGF2α). EOG recording, in conjunction with descriptions of behavioral responses of groups of male goldfish, suggests that this cue may be comprised of two components, unmodified PGF2α and its metabolite 15Keto-PGF2α (Sorensen *et al.*, 1988); however, studies of

actual release rates demonstrate that only 15Keto-PGF2α is released in appreciable quantities (Sorensen *et al.*, 1996). Other studies now demonstrate that goldfish release three other F prostaglandin (PGF) metabolites which they neither detect nor exhibit behavioral responses to (Sorensen and Goetz, 1993; unpublished). Intriguingly, a close relative of the goldfish, the common carp, (*Cyprinus carpio*) releases a different suite of PGF2α metabolites when injected with PGF2α (P.W. Sorensen, unpublished). Whether or not the goldfish prostaglandin pheromone is a specific mixture is unknown. Interestingly, many other species of fish are also now known to detect PGF2α and its metabolites but unfortunately little is so far known about what fish release or exhibit behavioral responses towards (Stacey *et al.* 1994). Also lacking is single-cell recording from either olfactory receptor neurons or the olfactory bulb to determine how pheromonal mixtures are distinguished. In conclusion, while very little is understood about fish hormonal pheromones, it appears likely that they are mixtures of rather common hormonal metabolites, the nature of which varies between broad taxonomic groups.

The search for mammalian pheromones has been long and fraught with dead-ends. However, there have been a few success stories, all guided by behavioral assays (Singer, 1991). Melrose *et al.* (1971) discovered that estrous female pigs, *Sus scrofa*, assume a copulatory stance when exposed to the odor of several metabolites of testosterone found in the boar's saliva. Furthermore, one compound, 5α-androstenone ('boar taint'), a reduced androgenic steroid, was found to function on its own. Although subsequent investigations have revealed that this steroid is likely part of a steroidal mixture, the importance of the mixture has unfortunately yet to be discerned. All other known mammalian pheromones appear to be mixtures, and there are a great many examples of pheromones losing efficacy upon fractionation (ex. Crump *et al.*, 1984). One example of the few instances in which the fractions were successfully reassembled is that of the beaver, *Castor canadensis*, which builds large mud piles that it scents with extracts of its castoreum gland, apparently to mark territorial boundaries. Using a behavioral assay, Müller-Schwarze (1992) clearly demonstrates that, of the eight compounds found in the beaver castoreum gland, six evoke behavioral responses, and all of these must be present to elicit a normal response. In another study, Cohen-Tannooudji *et al.* (1994) discovered that three specific fractions of the male sheep (*Ovis canadensis*) primer pheromone could be isolated from their wool, and that while all lacked the ability to stimulate

hormonal surges in ewes when tested on their own, they were active when tested as a mixture. In the one other clearly identified mammalian pheromone system, there is clear evidence that the pheromone associated with puberty enhancement in the mouse (*Mus domesticus*), is comprised of at least three adrenal metabolites; however, the precise manner in which this mixture functions remains untested (Novotny *et al.*, 1990).

## Behavioral responsiveness to pheromones is instinctual, but can be influenced by experience and physiological state

In their definition of a pheromone Karlson and Luscher (1959) strongly imply that one of the defining features of these cues is that behavioral responses to them should be instinctual. This assumption has been readily accepted by most workers in the field (Beauchamp *et al.*, 1976). For the most part, studies of insect reproductive behavior support this hypothesis, suggesting the existence of well-defined neuroethological mechanisms. For instance, not only is responsiveness of giant sphinx moths (*Manduca sexta*) generally restricted to males but only males have olfactory receptor cells which respond to pheromones (Hildebrand, 1995). A similar situation has also been described for *Bombyx* and several other species of moth (see Kaissling, 1996, this issue). Furthermore, in many species of moth it has been clearly shown that male-specific olfactory receptor cells project to specific sexually-dimorphic glomeruli (see Mustaparta, 1996, this issue). Thus, behavioral and neuro-physiological studies both suggest that the responsiveness of moths to sex pheromones is likely mediated by a 'labeled line' (Shepherd, 1994; Hildebrand, 1995).

A more complex, but equally fascinating scenario has presented itself with respect to the honeybee, *Apis mellifera*. Using an associative learning paradigm, Smith (1993) has found that honeybees can be readily trained to extend their proboscis (a feeding response) when exposed to a variety of odorants including several components of their alarm and Nasonov pheromone. This is an 'inappropriate' response because the Nasonov pheromone is normally used by bees to guide conspecifics to unscented food sources, water and nests during swarming. Even more remarkably, Smith and colleagues (1991) have also been able to demonstrate that honeybees can be trained not to respond to the presence of sucrose (a very appealing feeding stimulus) when exposed

to citral, a primary component of the Nasonov pheromone. Indeed, in these learning paradigms, the only distinguishing characteristic of the responsiveness of honeybees to phero-mones versus 'generalist' odorants was that they seemed to be slightly more difficult for honeybees to learn to recognize. Thus, at least for the honeybee and these particular phero-mones, behavioral responsiveness to pheromones appears rather 'plastic', and not what one might expect from a system which utilizes a simple labeled line. Unfortunately, electrophysiological recording has not yet been conducted to determine whether the honeybee olfactory system has specialized olfactory neurons for these pheromones. Another important question is whether similar results would be expected from sexual pheromones in this species; however, mammals have been trained to modify their behavioral responsiveness to sexual cues (see below).

There are indications that responsiveness to sex phero-mones is largely instinctual but modified by endocrine status is teleost fishes, but clear tests of this are lacking. The possibility that responsiveness may be modified by experi-ence remains. For instance, while rudimentary experiments demonstrate that naive male goldfish will respond to the PGF pheromone and that mature females won't, males pre-exposed to the pre-ovulatory pheromone exhibit a much enhanced .level of behavioral responsiveness to spawning females (DeFraipont and Sorensen, 1993). Behavioral experi-ments also clearly demonstrate that pheromonal function can be sexually dimorphic in fishes. For instance, only ovulated female gobies (*Gobius jozo*, Colombo *et al.*, 1982) and ovulated female African catfish, *Clarius gariepensis* (Resink *et al.*, 1987) respond to the odor of males—suggesting rather sophisticated and specific endo-crine modulation of neural responsiveness. Conversely, in many other species, only mature males will respond to pheromonal cues. In goldfish, crucian carp (*Carassius carassius*) and Atlantic salmon (Salmo salar), only mature males respond behaviorally to pheromonal odorants and, as measured by EOG recording, their olfactory epithelium is most sensitive to PGFs and selected other hormonal com-pounds (Moore and Scott, 1991; Sorensen and Goetz, 1993; Bjerselius *et al.*, 1993, 1995b). Furthermore, in another group of fishes, *Punctius sp.*, it has now been shown that androgen treatment can induce both behavioral and enhanced EOG sensitivity to the PGF pheromones (Cardwell *et al.*, 1995). Interestingly, androgen treatment does not appear to affect sensitivity to amino acids (putative feeding cues), suggesting that neural olfactory sensitivity to pheromones is regulated in a specific manner. Recent behavioral studies

of goldfish behavior also suggest that central processes differ between males and females because even when female goldfish are exposed to extremely high concentrations of PGF pheromones (which they can detect by EOG), they fail to respond behaviorally (P.W. Sorensen, unpublished) and Hara (1967) has noted that steroid exposure elicits changes in the spontaneous EEG of the goldfish olfactory bulb. Behavioral studies which have investigated the effects of ablating or stimulating specific portions of the olfactory bulbs and tracts of several species of fish have clearly demonstrated that, as in moths, specific portions of the fish olfactory system are specialized for the detection of pheromonal cues (see Mustaparta, 1996, this issue). Notably, however, lesioning studies conducted to date on fish have been at a rather gross level—individual olfactory glomeruli/ nerve pathways have not been targeted.

Although the manner in which mammals respond to pheromones is clearly more complex than that of insects or fish, there is compelling evidence that responsiveness also reflects a large degree of genetic programming (Beauchamp et al., 1976; Vandenbergh, 1988). A complicating factor is that tetrapods generally have two distinct olfactory systems: an accessory (or vomeronasal system) and a main olfactory system (see Mustaparta, 1996 this issue). Although the functional relationship between these systems is not totally clear, a variety of behavioral studies strongly suggest that the vomeronasal system (which also has a specific class of olfactory receptor cells) is responsible for instinctual recognition of pheromonal cues. This statement must be interpreted carefully however because the main olfactory system also appears capable of detecting pheromones: sexually experienced animals may continue to respond to pheromones after their vomeronasal system is removed (see Wysocki and Meredith, 1987). In golden hamsters, *Mesocricetus auratus*, removal of the vomeronasal organ completely obliterates the androgen surges typically elicited by exposure to female urine in sexually inexperienced animals, while removal of the main olfactory system is without effect (Pfeiffer and Johnston, 1994). However, lesions of either system can lead to deficits in reproductive behavior, particularly in naive animals. In another salient example, lesioning and behavioral investigations have demonstrated that the neural substrates responsible for mediating responses to nipple odor (the suckling pheromone) in rats (*Rattus norvegicus*) and rabbits (*Oryctolagus cuniculus*), are not restricted to any particular region of the olfactory bulb (Risser and Slotnick, 1987; Hudson and Distel, 1987). This finding contrasts with studies using 2-deoxyglucose which suggest that a specific region of the rat main olfactory bulb (termed the 'modified glomerular complex' or MGC) is specifically stimulated by this pheromonal odor (Teicher et al., 1980). The functional significance of the MGC and this non-reproductive cue thus remain unresolved.

A behavioral attribute suggesting a role for the vomeronasal system mediating responses to pheromones is that, when exposed to conspecific odors, many species will exhibit a characteristic (innate) lip-curl, or 'flehmen' response, which is thought to draw odor into this system (see Wysocki and Meredith, 1987). Flehmen behavior is routinely monitored as an index of pheromonally-stimulated activity, and the persistence of this response in naive and sexually-experienced animals is often regarded as strong evidence that the odor being assayed is a pheromone and not a novel odor which tends to elicit investigation (ex. Rasmussen et al., 1993). This point is an important one because mammals detect a broader range of odorants than non-mammalian vertebrates and will tend to investigate 'novel' odors. Notably, as mentioned above, there is at least one clear example of a pheromone, the suckling pheromone, which is not exclusively associated with vomeronasal function (Hudson and Distel, 1986, 1987). It is quite possible that responsiveness to sexual pheromones is mediated by a different mechanism(s) than that responsible for regulating responses to non-sexual pheromones.

Behavioral studies have demonstrated that responsiveness to sex pheromones can be sexually dimorphic in mammals, suggesting that responsiveness to these cues is mediated by a specialized system(s). Early observations of pigs demonstrated that only ovulated sows responded behaviorally to boar taint. To determine whether this is related to olfactory sensitivity, Dorries et al. (1995) attempted to train sows, boars, and castrated animals to androstenone as well as control cues. They found that only sows could easily be trained to androstenone, suggesting sexual dimorphism in peripheral sensitivity. Notably, however, they were able to train boars to respond to this pheromone so the dimorphism can not be absolute. An interesting physiological correlate of differential responsiveness to androstenone has emerged from a recent study by Wang et al. (1993), who found that groups of mice that were previously insensitive to this steroid developed a sensitivity to it with repeated exposure as measured by both behavioral conditioning and EOG recording.

Responsiveness to pheromones also appears robust and thus largely instinctual in mammals. As one might expect, this is particularly evident for priming pheromones: mice

and sheep may be repeatedly exposed to pheromones and will continue to respond with increased hormone secretion rates (Novotny *et al.*, 1990; Cohen-Tannooudji *et al.*, 1994). However, this is not to say that responsiveness to pheromonal cues can not be modified by experience. For instance, Johnson *et al.* (1978) have also found that the behavioral responses to male golden hamster pheromone (female vaginal fluid) can be modified by experience as animals which are poisoned after their first adult exposure to females become extremely hesitant to approach and investigate females associated with this odor. Similarly, as discussed above, Dorries *et al.* (1995) has shown that normally unresponsive boars can be trained to respond to the male pheromone, to which they would not normally exhibit a response. Training experiments have also shown that artificial odors can assume pheromone-like properties. For example, exposing rat pups to citral in association with their mother's odors causes this compound to elicit sexual arousal in adult males exposed to it later in life (Fillion and Blaas, 1986). In conclusion, although little is understood about the physiological control of responsiveness to pheromones in mammals, behavioral studies indicate that these behaviors are largely instinctual but can be modified in adaptive manners, suggesting that while components of their olfactory systems are specialized, they do not constitute a simple labeled line.

## Behavioral orientation to pheromones is associated with temporal patterning

An interesting and well-understood model of how pheromones can affect precise, complex behavioral responses is that of pheromone-mediated upwind orientation by male moths (Vickers and Baker, 1994; Mafra-Neto and Cardé, 1994). This system has demonstrated how both odor composition and the temporal pattern of exposure are critical to behavioral responsiveness. Although the principal components of several moth sex pheromones had been known for some time, not until the early 1980's was it realized that minor components can make a substantial contribution to the behavioral efficacy of the blend (Linn *et al.*, 1986; Linn and Roelofs *et al.*, 1989). It was also noted that temporal pattern of odor exposure influenced the ability of flying moths to locate the source of the pheromone. For instance, Kennedy and co-workers (1980) noted that males did not sustain upwind orientation in homogenous plumes and instead behaved as if the odor had been lost by casting from side-to-side. Subsequently, Baker *et al.* (1985) noted that if the pheromonal cloud were pulsed by intermittently

introducing swaths of clean air, male would maintain constant upwind progress. Electrophysiological recording from single sinsilla of the moth antennae then confirmed that sensory adaptation could explain the lack of responsiveness to homogenous pheromone clouds and that sensory cells can respond quickly enough to odor pulses to account for the more effective upwind orientation of moths in heterogeneous plumes (Baker *et al.*, 1988; Rumbo and Kaissling, 1989). This behavioral work corroborated an earlier observation that odor plumes are not homogenous but, owing to the shearing and eddying of turbulent air masses, are heterogenous in nature and characterized by pheromonal 'filaments' (i.e. transient wisps) interspersed with pockets of 'clean' air (Murlis and Jones, 1981).

The behavioral mechanisms underlying upwind flight by male moths and pheromonal source location have been the focus of intensive investigation. Relatively soon after the discovery of moth sex pheromones it was realized that one of the mechanisms likely to be used by males to find the pheromone source was optomotor anemotaxis (Kennedy and Marsh, 1974; Marsh *et al.*, 1978). This mechanism, which had presciently been proposed to explain upwind orientation of flying mosquitoes in carbon dioxide plumes (Kennedy, 1940), postulates that flying males visually monitor their wind-induced drift, correcting the bias in their displacement. However, although optomotor anemotaxis can explain how a moth located in a heterogeneous pheromone plume can displace itself towards the odor source in an upwind direction, it can not explain how a moth might relocate an odor plume if there is a shift in wind direction or its own movements take it out of contact with the plume. Earlier observations that moths exhibit turning patterns when initially exposed to a pheromone suggested an answer—there seemed to be another mechanism that acted in concert with optomotor anemotaxis. This second mechanism was termed 'counterturning' by Kennedy (1983), who noted that moths exhibit temporally regular reversals in direction flown whether they are flying directly upwind or flying across the wind. Thus, moths 'zig-zag' in a manner which tends to give them a bias toward an odor source while limiting the distance they might fly should they diverge from it.

Realizing that counterturning alone can not explain how moths manage to orient in a discontinuous pheromone plume, Baker (1990) has recently proposed the existence of a complementary neuroethological mechanism to affect upwind orientation. Noting that moths typically make erratic progress when flying upwind, he suggests that when males first encounter a pheromonal filament they respond by flying

forward or 'surging' towards the source, but if they should then fall out of contact with it, they revert to flying across the wind or 'casting'. Should a surging male intercept another pheromone filament prior to reverting to casting flight, Baker (1990) suggests that the surge will be extended. Hence, by reiteratively surging in response to contacts with individual filaments the male could sustain progress towards an upwind source. Counterturning would also be modulated by odor contacts such that, when filaments are intercepted at a high rate, turns would be executed more frequently. In situations where the rate of filament contact decreases, the time interval would increase as it does, for instance, when a moth is casting in clean air. Reasoning that neural substrates should underlie this behavioral repertoire, Baker (1990) hypothesized that different types of olfactory neurons might be responsible for each behavior: phasic olfactory neurons could explain the short duration surge associated with the initial contact with a single filament, whereas tonic olfactory neurons could account for the long-lasting counterturning that occurs as casting flight in clean air following pheromone exposure. Interestingly, this hypothesis is now supported by both behavioral and neurophysiological investigations. Independent behavioral experiments on two different moths, *H. virescens* (Vickers and Baker, 1994) and *Cadra cautella* (Mafra-Neto and Cardé, 1994), have clearly demonstrated that males in casting flight make a brief upwind surge after intercepting a single pheromonal filament. Additionally, electrophysiological investigations have now identified potential candidates for neurons and neural circuits that underlie phasic surging and tonic counterturning behaviors in several species of moth (see Mustaparta, 1996, this issue).

Fish species found in flowing and/or dark waters which contain low densities of conspecifics appear to face many of the same challenges of flying moths. Interestingly, recent studies of the goldfish have shown they release conjugated steroids and F prostaglandins (i.e. pheromones) exclusively via their urine (Scott and Sorensen, 1994; Sorensen unpublished data; Appelt *et al.*, 1996), and that urinary release can be pulsed (Curtis and Wood, 1991; Appelt *et al.*, 1996). How goldfish recognize and use these discontinuous cues has not yet been studied. However, several studies have used mazes with homogeneous laminar flows to demonstrate that fish can locate crude pheromonal mixtures (Sorensen *et al.*, 1986; Resink *et al.*, 1987). Interestingly, one such study used this technique to examine behavioral responses of goldfish in a homogenous mixture of a single component of their preovulatory steroidal blend (17,20βP; Bjerselius

*et al.*, 1995a) and describes a curious finding: behavioral avoidance. Could goldfish, like moths, require a pulsed, multi-component signal to locate plumes correctly? It is certainly intriguing that migrating adult fish have been commonly observed to 'zig-zag' in-and-out of odor plumes (Johnsen and Hasler, 1980; Døving *et al.*, 1985), in a manner similar to that of male moths. Clearly, a close examination of how fish orient to pheromone plumes and the neural basis of such behavior is strongly warranted.

To the best of my knowledge, the behavior of mammals searching for the source of an identified pheromone has yet to be systematically examined. However, because mammalian pheromones can be relatively non-volatile (ex. hamster flank gland smears) and pheromonal cues are sometimes used as stationary territorial markers, it is not clear that this question is always relevant. Nevertheless, it is clear that some mammals can track air-borne trails for some distance (e.g. dogs). In these instances, it is also notable that sniffing, flehmening, and zig-zagging across odor trails are characteristic of pheromonal sampling—temporal patterning of pheromonal exposure is likely important in at least some instances. In conclusion, although one might expect the specific behaviors elicited by pheromone exposure in mammals to be more subtle than in lower organisms, and likely influenced by experience and other environmental variables, it seems highly likely that these behaviors too are associated with specific temporal patterns which are detected by neuroethological mechanisms.

## Summary

Portions of the olfactory system of many species of animals, from insects to mammals, are specialized for the perception of complex pheromonal cues. Insight into these systems has only come about because of an active dialogue between behavioral and neurophysiological investigation. Although our understanding of these systems is for the most part still rudimentary, it is clear that these systems can function in elegant manners, reflecting sophisticated neuroethological mechanisms closely associated with olfactory function. In part this likely reflects the behavioral challenges intrinsic to using a chemical signal with considerable social importance: it must be specifically and instinctually recognized, and capable of being followed in a complex, three-dimensional and chemically-noisy environment. There is also strong behavioral and neurophysiological evidence that at least

at the level of olfactory receptors, responsiveness to sex pheromones is likely mediated by a labeled line of some kind. However, it also now appears that at least some animals have the ability to modulate behavioral responsiveness to particular pheromones as a result of learning processes, suggesting that 'higher' levels of control may be more complex. In any case, because of their well defined and

fundamental actions on organismal biology/behavior, pheromones serve as powerful tools with which to investigate olfactory function. Although our understanding of moths pheromones is far better than our understanding of any vertebrate, many similarities are evident, and it seems certain that many exciting findings await us as our understanding of pheromone function improves.

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