

Review of Aquatic Sex Pheromones and Chemical Communication in Anurans

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ABSTRACT.—Chemical communication is important in aquatic environments, particularly where visual and acoustical signals are limited. Both larval (tadpole) and adult anurans (frogs and toads) use waterborne chemical signals for many activities. Adult anurans commonly rely on acoustical communication for sex recognition and mating; however, a growing body of evidence suggests that anurans also may use aquatic sex pheromones for localization of potential mates. We provide an overview of how the anuran nasal cavity reorganizes during metamorphosis from the larval to the adult stage. Also, we focus on the behavior of reproductive anurans in response to chemical information detected by olfaction of waterborne chemical cues. Overall, we synthesize the current literature on anuran sex pheromones and chemical communication in the aquatic environment.

Biological communication has been defined as an action on the part of one organism (or cell) that alters the probability pattern of behavior in another (Wilson, 1970). Animals may use multiple types of sensory signals, delivered simultaneously to communicate with conspecifics, increasing the probability of the information being received (Cardé and Baker, 1984). Signals used for communication may be chemical, electrical, mechanical, visual, or acoustical in nature and may be used during agonistic interactions, courtship rituals, foraging, and for alarm signaling (Dusenbery, 1992; Reeb, 2001). Chemical signals are important for communication in animals, because all animals detect and react to chemicals in the external environment. Chemoreception has been shown to play an important role in guiding the behavior of many animals (Hildebrand, 1995).

Chemical communication can occur when both the originator (signaler) and one or more receivers possess specializations for the exchange of chemical information (Wyatt, 2003). This type of communication is important for many aspects of animal behavior, including aggregation, predator-prey interactions, and mate attraction (Wyatt, 2003). For animals, odorous chemical compounds (or chemosignals) are important for identifying conspecifics and for determining the gender and social or reproductive status of these individuals (Johnston, 1980; Wyatt, 2003). Thus, chemosignals play an important role in sexual communication, facilitating reproduction by attracting

potential partners, aiding in mate localization, and revealing when individuals are sexually receptive (Drickamer, 1999; Sorensen and Stacey, 1999).

Chemosignals that mediate interactions between either inter- or intraspecific organisms are called semiochemicals and may be subdivided into interactions between individuals of the same (pheromones) or different (allelochemicals) species (Nordlund, 1981). Pheromones have been defined as substances (or mixtures of substances) that an individual releases to elicit specific biological responses in conspecifics (Burghardt, 1970). Thus, chemosignals used for sexual communication between conspecifics are termed sex pheromones. Sex pheromones elicit various behaviors in the receiver, ranging from mate attraction to regulation of physiological processes, including ovulation (Johansson and Jones, 2007). To facilitate mating using sex pheromones, a signaler may convey its species, sex, and reproductive status (Johansson and Jones, 2007).

Sex pheromones are either single compounds or a mixture of different chemical compounds. Olfactory recognition of sex pheromones is mediated by a large ensemble of G-protein coupled receptors (important for signal transduction) located on the dendrites of olfactory sensory neurons (OSNs), situated within the olfactory epithelium of the nasal cavity (Firestein, 2001). Following the binding of an odorant to the receptor, a cascade of events is initiated that transforms the chemical energy of binding into a neuronal signal, changing the membrane potential of the receptor neuron and producing an action potential down the axon.

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This neural stimulation is then propagated to sequential neurons and ultimately to higher order brain sites where sensory information is integrated. Given sufficient stimulation, the initial neural reception may be transduced into behavioral or physiological responses by the receiving animal (for review, see Firestein, 2001).

Chemical signals can stimulate receivers in both terrestrial and aquatic environments. These signals may be especially important in aquatic environments where visual and auditory stimuli are limited (Burks and Lodge, 2002). Furthermore, aquatic chemical cues are easily transmitted and have the potential for high signal-to-noise ratios, increasing an animal's ability to detect these cues (Wisenden, 2000). For anurans (frogs and toads), both larvae (tadpoles) and adults can respond to chemical signals in the environment, including signals related to recognizing predators, kin, and injured conspecifics (Blaustein and O'Hara, 1982; Gallie et al., 2001). However, little information is available on the role of conspecific chemical communication, particularly in comparison with the large number of studies on acoustic communication, the primary mode of communication in anurans (Gerhardt and Huber, 2002). Nevertheless, in many anuran species, the males possess specialized breeding glands that release peptides (Thomas et al., 1993; Smith et al., 2003), suggesting that chemically mediated reproductive interactions in anurans may be widespread. Also, mate localization is not mediated acoustically in the anuran family Ascaphidae (tailed frogs), suggesting that these frogs may use either chemical or visual signals (or both) for mate localization (Stephenson and Verrell, 2003).

Our main points in this review are that anurans can generate chemosignals and also possess the neural circuitry to receive and process these chemical signals. First, we present a general overview of the organization of the nasal cavity in larval anurans, and then we review transformations of the nasal cavity during metamorphosis. We focus on changes in the nasal cavity that give adult anurans the ability to continue to perceive waterborne odorants, while also being able to sense airborne odorants. In this context, we examine the current literature on the use of aquatic sex pheromones by adult anurans, emphasizing the chemical exchange of information that facilitates mate attraction and courtship behavior.

OLFACTORY SYSTEM IN ANURANS

Most anurans are characterized by a larval period of the lifecycle that is exclusively aquatic,

whereas adults can be found in both aquatic and terrestrial environments. Undergoing the metamorphosis from an aquatic to a terrestrial form dramatically changes an animal's anatomy, physiology, ecology, and all aspects of the life history (Jermakowicz et al., 2004). One of the most striking changes that occur during metamorphosis is a rapid transformation of the olfactory region (Paterson, 1939; Hansen et al., 1998).

The olfactory system of anurans varies depending on the developmental stage (larval versus adult), developmental mode (aquatic versus terrestrial), and phylogenetic history (Jermakowicz et al., 2004). Anuran species having aquatic larval and semiaquatic adult stages typically have similar olfactory transformations during metamorphosis, changing from only being able to perceive odorants in the aqueous environment to being able to sample odorants in both the air and water. The aquatic larval olfactory morphology is designed for water moving through the external nares (EN) to the principal chamber (PC), then to the internal nares (IN), and finally into the buccal cavity (Fig. 1). In contrast, an adult semiaquatic anuran has two types of chemosensory arrangements, reflecting a nasal cavity designed for air flow into and out of the respiratory tract, as well as for sampling both airborne and water-soluble odorants (Døving et al., 1993). The nasal cavity consists of the structures comprising the nasal chambers, which have projections to both the olfactory and accessory olfactory bulbs. Taken together, these structures make up the "dual" olfactory system of adults.

Larval Anurans.—During the initial stages of anuran larval development, the PC of the olfactory organ is ovoid, extending along the longitudinal axis of the skull (Jermakowicz et al., 2004). The PC is lined medially with ciliated sensory epithelium and laterally with ciliated nonsensory epithelium. The dorsal and ventral olfactory epithelia of tadpoles detect waterborne chemicals (Duellman and Trueb, 1986). The EN is located rostral to the principal chamber and contains valvelike structures capable of blocking substances from entering the nasal cavity. At the early stages of larval development, there is a direct channel between the EN and IN to the buccal chamber. At this time, the inferior chamber (IC), vomeronasal (or Jacobson's) organ (VNO), and medial chamber (MC) have not formed. Later in the larval stage, the vomeronasal epithelium and vomeronasal organ appear, eventually branching off at the rostral end of the principal chamber (Fig. 1A). Afferent neurons from the sensory epithelium found in the PC project to the olfactory bulb, whereas those from the vomeronasal epithelium

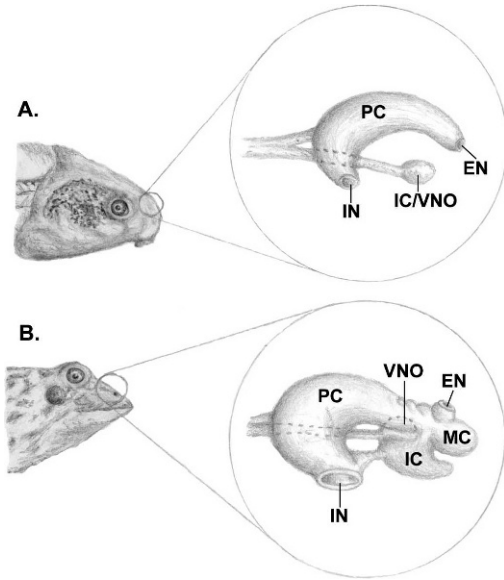


FIG. 1. Schematic representation of the olfactory region (or nose) of the frog at two developmental stages, the larval/tadpole stage (A) and adult (B). This diagram illustrates relative positions of the nasal cavities: principle chamber (PC), middle chamber (MC), inferior chamber (IC), and vomeronasal organ (VNO) as well as, the external (EN) and internal (IN) nares. Dendrites from olfactory sensory neurons in the PC project to the whole olfactory bulb during the larval stage and to the dorsal part of the olfactory bulb in adults. Projections from the VNO terminate in the accessory olfactory bulb. The MC and IC are absent in young larvae but are found in later stages of tadpole development. In adult anurans, the PC, MC, and IC are interconnected via a short channel common to all three chambers. The VNO is located in a medial recess of the IC. This is depicted by the dashed line attached to the IC. Waterborne or airborne odorants enter the olfactory chambers via the external nares. Waterborne odorants enter the MC and IC, whereas airborne odorants enter into the PC where they may stimulate olfactory receptor neurons located in the VNO (waterborne odorants) or PC (airborne odorants). This diagram was modified from Gaupp (1904), Rugh (1951), and Reiss and Burd (1997). Illustration by Yolanta Kita.

project to the accessory olfactory bulb (Scalia, 1976; Reiss and Burd, 1997). During subsequent larval development, the IC forms ventral to the PC and the PC, IC, and VNO chambers are all contiguous (Jermakowicz et al. 2004). Also, the VNO continues to increase in size. Little is known about the role of the VNO in larvae; however, the VNO presumably has a role in chemoreception (Elepfandt, 1996; Hansen et al., 1998).

At later stages of metamorphosis, the PC elongates, the ventral olfactory epithelium disappears, the IN migrates to a caudal position

in the buccal/oral cavity, and the buccal cavity transforms into the oral cavity. Some phylogenetic and ecological variations might occur in the olfactory system of larvae (Lannoo, 1999). Moreover, developmental comparisons of larvae from different species are difficult because authors may describe larval development using different developmental staging schemes when assessing changes in external morphology.

Adult Anuran.—At the conclusion of metamorphosis, the adult phenotype is present and the chemosensory organs are completely embedded in the olfactory capsule (Fig. 1B). The structure and function of the adult olfactory organ has been the subject of review by several authors (McCotter, 1917; Paterson, 1939; Scalia, 1976; Døving et al., 1993). The nasal cavity (see Fig. 1B) is described as being a large dorsally flattened chamber that is open anteriorly at the EN and posteriorly at the IN (or choana) leading to the oral cavity. The three chambers that form the nasal cavity are the PC, MC, and IC (McCotter, 1917). Occupying up to three-quarters of the nasal cavity, the PC is dorsoventrally flattened. The floor of this bean-shaped chamber is elevated by a ridge termed the eminentia olfactoria or olfactory eminence (Scalia, 1976). Jermakowicz et al. (2004) describe the PC as being lined with a thick layer of ciliated olfactory sensory neurons, which are found in all but the lateral portions of the chamber. The EN is located at the anterior end of the PC and is positioned on the dorsolateral side of the chamber, just above a vestibule (Døving et al., 1993). The IN is located posteriorly on the floor of the PC and on the lateral side of the olfactory eminence (Scalia, 1976).

Below the anterior end of the PC, set off somewhat laterally, is the MC. The MC is described as a small dorsoventrally flattened sac lined entirely by ciliated nonsensory epithelium (Scalia, 1976). The posterior end of the MC communicates both with the PC and IC through a short channel common to all three chambers (Fig. 1B). The IC is found below the middle of the PC, anterolateral to the olfactory eminence, and is located just below the posterior end of the MC. In this region, the IC communicates with both of the other chambers. The medial part of the IC, its medial recess, ends in a tubular sac lined by vomeronasal epithelium, which gives rise to the vomeronasal nerve. Therefore, the VNO is contiguous with the IC. The vomeronasal end of the medial recess is located below and somewhat medial to the anterior third of the PC. The IC communicates with the PC through a slitlike groove in the floor of the lateral corner

of the PC, and its posterior end opens alongside the latter into the IN. The IC is lined with ciliated nonsensory epithelium, except at the end of the medial recess, where the VNO is located (Scalia, 1976). The VNO is lined with ciliated nonsensory cells, as well as microvillar olfactory sensory neurons (Døving et al., 1993).

Entry of air or water into the nasal cavity occurs via the EN and is controlled by a movable lid. The lid fits into the medial part of the naris opening, and the position of the lid is controlled by the displacement of a muscle in the lower jaw. Partial opening of the lid allows water to enter into the vestibule via two fissures, one on each side of the lid (Gans and Pyles, 1983; Døving et al., 1993). Once in the vestibule, water enters the opening of the medial cavity and continues further down to the sensory epithelium of the IC (Døving et al., 1993). Water continues to be propelled to the VNO by ciliary action, visible as beats at the surface of the organ. The VNO is responsible for detection of waterborne odorants (Helling, 1938; Døving et al., 1993; Hansen et al., 1998; Halpern and Martínez-Marcos, 2003). A groove between the floor of the vestibule and the olfactory eminence prevents water from gaining access to the olfactory eminence (Døving et al., 1993).

Anurans also inspire air through the EN and force the air into their lungs by closing the EN. During inspiration, air passes the olfactory eminence of the PC and airborne odorants may then excite the ciliated olfactory receptor cells (Døving et al., 1993; Halpern and Martínez-Marcos, 2003). The olfactory eminence is well developed in anurans that live mainly terrestrial lives but may be absent in anurans that are primarily water-dwelling (Helling, 1938). Overall, the design of the adult anuran nasal cavity allows the animal to sample the chemical composition of the both terrestrial and aquatic environments. The PC is used for sampling airborne odorants, whereas the VNO samples waterborne substances.

ANURAN CHEMICAL COMMUNICATION

The olfactory systems of both larval and adult anurans respond to waterborne chemical cues. Larvae use chemical cues for mediating intraspecific competition (Glennemeier and Denver, 2002), female-offspring communication (Kam and Yang, 2002), detecting alarms (Birch, 1974; Hews, 1988; Summey and Mathis, 1998; Kiesecker et al., 1999), recognizing predators (Morin, 1986; Kiesecker et al., 1996; Kiesecker and Blaustein, 1997; Gallie et al., 2001), and recognizing conspecifics, heterospecifics, and kin (Waldman and Adler, 1979; Blaustein and O'Hara, 1982; Waldman, 1986; Mason et al.,

1998). Newly metamorphosed anurans continue to use sensitive chemoreceptors that become further developed after metamorphosis (Spaeti, 1978). Adult anurans use chemical cues for detecting prey (Altner, 1962), migrating to breeding sites (Oldham, 1967; Dole, 1968; Grubb, 1973; Sinsch, 1990), and recognizing predators (Flowers and Graves, 1997) and conspecifics (Graves et al., 1993), as well as for sex recognition and attraction (Wabnitz et al., 1999; Pearl et al., 2000; Asay et al., 2005; King et al., 2005; Table 1).

Many researchers (Rabb and Rabb, 1963a; Wabnitz et al., 1999; Pearl et al., 2000; Asay et al., 2005) have demonstrated that conditioned water, homogenized breeding glands, and pheromones (isolated from skin glands) attracted conspecifics (Table 1). Rabb and Rabb (1963b) speculated that the postaxillary skin gland of reproductive males is probably a breeding gland, that is, the source of substance(s) that may repel conspecific males or attract conspecific females. When water from a tank containing a mating pair (= "conditioned water") was presented to unmated male Surinam Toads (*Pipa pipa*), these males became visibly agitated and began calling and clasping (Rabb and Rabb, 1963a). A. E. Burns and E. O. Thomas (Behavioral evidence for mate attracting pheromones in the dwarf African Clawed Frog, *Hymenochirus curtipes*, abstract in Proceedings of the Combined Western/Southwestern Regional Conference on Comparative Endocrinology, Denver, CO, 1997) also showed that odorants from the male Western Dwarf Clawed Frog (*Hymenochirus curtipes*) were able to attract conspecific females, but these authors did not test whether the attraction was sex-specific.

The Tailed Frog (*Ascaphus truei*) is nocturnally active and does not communicate acoustically, likely because the frog lives in "noisy" (i.e., rushing water) stream habitats (Metter, 1967). Also, the Tailed Frog is likely to use chemical communication because this taxon is relatively basal and completely lacks acoustic communication (Stephenson and Verrell, 2003). Thus, without the use of visual or acoustic signals, these frogs may rely on chemical communication for localization of mates. Asay et al. (2005) collected conditioned water from reproductive males and females and used a three-way choice (male conditioned-water, female conditioned-water, and water/control) to examine behavioral responses. Results showed that both males and females jumped more frequently toward signals from the opposite sex than toward the other stimuli. Also, males spent significantly more time sitting in the "female-marked" area when compared to the other two stimulus areas. On the basis of these results, Asay et al. (2005)

TABLE 1. A summary of aquatic pheromone chemical communication in adult anurans.

Family	Genus species	Sex	Test substance	Behavioral response	Reference
Ascaphidae	<i>Ascaphus truei</i>	M	Female conditioned-water	Positive chemotaxis, increased time in female stimulus area, and increased jumping.	Asay et al., 2005
Ascaphidae	<i>Ascaphus truei</i>	F	Male conditioned-water	Increased jumping.	Asay et al., 2005
Pipidae	<i>Hymenochirus</i> sp.	F	Homogenized breeding gland aliquots (equivalent to half of one breeding gland)	Positive chemotaxis.	Pearl et al., 2000
Pipidae	<i>Hymenochirus</i> sp.	F	Water housing males	Positive chemotaxis.	Pearl et al., 2000
Pipidae	<i>Pipa pipa</i>	M	Water from the tank containing a mating pair	Unmated males became agitated and began calling and attempting to clasp.	Rabb and Rabb, 1963a
Hylidae	<i>Litoria splendida</i>	F	10^{-13} M splendipherin (male pheromone)	Change in posture and increased alertness. Female walked toward the odor source and sat on it until she was removed.	Wabnitz et al., 1999
Leptodactylidae	<i>Leptodactylus fallax</i>	M	Leptodactylus aggression-stimulating peptide	Attracts conspecific males and stimulates aggressive male-male interactions, leading to reproduction.	King et al., 2005

suggested that the signal for mate attraction or recognition in the Tailed Frog was most likely a waterborne chemical signal. However, the source of this signal has not been located, and the compound has not been identified.

Recently, the identification of chemical compounds has confirmed the existence of sex pheromones in frogs (Pearl et al., 2000; Wabnitz et al., 1999, 2000). Amines and peptides, which are secreted by specialized glands (Clarke, 1997; Erspamer, 1994), may function to deter predators (Williams et al., 2000) but may also serve a role in conspecific communication. Wabnitz et al. (1999) isolated and purified a peptide pheromone, splendipherin, from the parotoid and rostral glands of the male Magnificent Tree Frog, *Litoria splendida*. This is the first pheromone to be isolated from an anuran, and its concentration peaks during the breeding season (January to March). Wabnitz et al. (1999) showed that, in pheromone behavioral assays, female *L. splendida* displayed a distinct posture change and increased alertness when presented with the pheromone. Female frogs were attracted to a pad containing a concentration of 10^{-13} M splendipherin and remained on the odor source until they were removed.

Pheromones that stimulate aggressive behavior also have been identified in frogs. King et al. (2005) showed that males of the Mountain Chicken Frog (*Leptodactylus fallax*) secreted a pheromone (isolated from norepinephrine-stimulated skin secretions), called Leptodactylus Aggression-Stimulating Peptide (LASP). This pheromone, which is not present in skin secretions obtained from females, is believed to be released by dominant males. As in the Magnificent Tree Frog, the LASP pheromone is a peptide. Although LASP attracts males and stimulates aggressive behaviors (e.g., rearing and leaping), this peptide had no effect on females. Evidently, the pheromone plays a role in aggressive male behavior (e.g., male-male interactions associated with the onset of reproduction), a time when dominant males attract females to nesting sites (King et al., 2005).

A different source of sex pheromone(s) has been suggested for the African Dwarf Clawed Frog (*Hymenochirus* sp.). Pearl et al. (2000) proposed that the sexually dimorphic postaxillary breeding glands of male anurans may be a source for female-attracting sex pheromones because these glands develop during sexual maturation and enlarge during the breeding season. Thomas et al. (1993) found that most breeding glands in anurans are structurally and histochemically similar to the pheromone-secreting glands of other amphibians, specifically caudates. Also, Pearl et al. (2000) showed that water conditioned by African Dwarf Clawed

Frogs, and aliquots from homogenized breeding glands, were each sufficient to elicit positive chemotaxis in conspecific females. However, sex pheromones have not yet been purified and identified from these breeding glands.

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Recent studies provide strong evidence that waterborne chemical signals play a role in sexual communication in anurans. In one anuran (*L. splendida*), for example, sex pheromones that were released from distinct glands have been isolated and characterized (Wabnitz et al., 1999). Pearl et al. (2000) suggested that acoustical and chemical signals may have different efficacies, depending on a female's distance from the reproductive male. The use of acoustical and pheromone signals for reproduction should be evaluated to determine whether signal use is a function of habitat characteristics or phylogenetic history (or both). The growing awareness of a dual mate-attraction system in anurans (both acoustic and chemical signals) should lead to the examination of additional anuran species. This new information could lead to further insight into the potential use of multimodal sensory communication (i.e., acoustical, visual, and chemical) for mate recognition and localization in anurans. Despite the initial information on anuran pheromones (summarized in Table 1), the elicitation of behavioral response to chemical signals is still poorly understood in anurans. Whether chemical signals are commonly used by anurans to recognize and locate conspecifics for the purposes of mating requires additional study.

The detection of water and airborne odors occurs in anurans. Freitag et al. (1995) showed that anurans possess both fishlike receptor genes, specialized for detecting water-soluble odorants, and mammalian-like genes, responsible for the reception of volatile odors. Future neurophysiological studies may aid in (1) determining when sensory epithelia of the PC transitions from sampling waterborne odorants to responding to airborne odorants in metamorphosing anurans, and (2) identifying changes that occur at the level of the olfactory receptor during these developmental stages. Also, knowing which changes occur in the olfactory system during metamorphosis may lead to a better understanding of pheromone perception and communication during mating.

Knowledge of the structure and function of sex pheromones in anurans may be helpful to biologists because pheromones might be useful in both conservation and population control (Agosta, 1992; Corkum and Belanger, 2007). Understanding the specific mechanisms in-

volved in chemical communication in anurans during mate attraction and courtship behavior will have positive implications for captive breeding programs and conservation of endangered anuran species. Also, application of sex pheromones may serve to control population numbers in areas where anurans are considered pest species. This kind of control could be accomplished, for example, through trapping or by interrupting or confusing a female trying to locate a male for the purposes of mating. Overall, further understanding of chemical communication in anurans, including how the olfactory system changes developmentally to accommodate important new odors (i.e., sex pheromones), will broaden our understanding of how these animals integrate environmental cues when making behaviorally relevant decisions.

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