Chapter 23
Chemical Communication in Archaic New Zealand Frogs

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23.1 Introduction

Studies of the social behavior of anuran amphibians traditionally have centered on the role of bioacoustic signaling in mate choice. Only in recent years have researchers begun to realize that frogs and toads communicate through multimodal channels including not only bioacoustic but also visual, seismic, and chemical signals (Starnberger et al. 2014a, b). Amphibians use chemical cues for detecting prey and predators, homing and navigation, territorial defense, alarm signaling, mate choice, and social recognition (reviewed in Waldman and Bishop 2004). By-products of physiological processes incidentally may inform conspecifics or heterospecifics of individuals' sex, diet, size, health, movements, reproductive state, or dominance status. When revealing this information benefits the sender, natural selection may favor the specialization of these cues into signals that foster social communication (Steiger et al. 2011).

While salamanders and caecilians are known to communicate through the chemosensory modality, the importance of chemical signaling in anurans only now is becoming appreciated (Belanger and Corkum 2009; Woodley 2010, 2014). Sex pheromones that may be important in male–male interactions and female mate choice have been identified in aquatic frog species (Wabnitz et al. 1999; Pearl et al. 2000). Some frogs secrete contact courtship pheromones, chemically similar to those produced by salamanders, from their nuptial pads (Willaert et al. 2013), while others release volatile pheromones to which conspecifics respond (Poth et al. 2012). Chemosignals modulate calling behavior in some terrestrial frogs (Byrne and Keogh 2007), and may be broadcast in concert with bioacoustic signals from vocal sac

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glands (Starnberger et al. 2014b). Even for species that communicate primarily through bioacoustic signaling, chemical communication probably influences social interactions.

Anurans that lack the ability to communicate through bioacoustic signaling, such as the archaic New Zealand frogs (family Leiopelmatidae) and the North American tailed frogs (family Ascaphidae), appear to rely on chemical cues as a primary means of social communication (Lee and Waldman 2002; Waldman and Bishop 2004; Asay et al. 2005). These taxa comprise the most basal lineage of extant anurans (Pyron and Wiens 2011), possessing characters found in Mesozoic fossils (Roček 2000). Although leiopelmatid frogs occasionally emit calls that may startle predators (Bell 1978), these vocalizations lack the structural properties characteristic of anuran advertisement or contact calls and thus are unlikely to serve as signals to conspecifics (Waldman, unpublished data). Furthermore, these frogs lack external eardrums that are characteristic of modern frogs (Lewis and Lombard 1988). Their hearing thus lacks acuity, but anatomical studies suggest that their vomeronasal and olfactory systems are functional (Stephenson 1951, 1955). Possibly the earliest anurans never evolved mechanisms to communicate by bioacoustic signaling (Bogert 1960). Thus, study of their abilities to use chemosignals may offer a window into the early evolution of anuran social systems.

Of the three extant species of leiopelmatid frogs, Hamilton's frog, *Leiopelma hamiltoni*, has the most limited distribution, living only on Maud and Stephens Islands in the Marlborough Sounds, New Zealand. The species is one of the rarest frogs in the world and access to its habitat is strictly regulated. Although frogs on Maud Island are considered by some to be a separate species (named *L. pakeka*) from those on Stephens Island (Bell et al. 1998), molecular analyses suggest that they are best considered different populations of the same species (Holyoake et al. 2001). The frogs are fully terrestrial, occupying small home ranges on the forest floor. They demonstrate site tenacity to rocks, logs, and litter, under which they find shelter during the day. At night, depending on environmental conditions, they emerge and travel over short distances, forage, and periodically interact with conspecifics including potential mates (Webster 2004). The frogs are extremely long-lived, and individuals have been repeatedly censused over 40 years or more in the same area, even under the very same rocks (Bell and Pledger 2010; B.D. Bell, personal communication).

23.2 Materials and Methods

23.2.1 Animal Collection and Odor Sampling

To investigate whether *L. hamiltoni* communicates by chemical cues, my research group conducted a series of field experiments over several years on the social recognition abilities of these frogs. We collected frogs that we found on or under rocks during evening hours. Sometimes several frogs co-occupied particular areas and

could be repeatedly found under the same rocks. In such cases, we collected all of the frogs. We mapped their locations and measured distances among them. Distances ranged from 0 to 90 m. We held the frogs for 72 h in plastic containers ($33 \times 20 \times 8$ cm) lined with clean, moist paper towels which we stored in a dark, quiet room in the field station on Maud Island. During this time, body secretions, urine, and feces from the subjects were collected on the paper substrates in each container. In this manner, subjects were allowed to "mark" the paper substrates. Different subjects were used in each experimental series, and no subject was tested more than once in any experiment.

23.2.2   Self/Nonself Recognition

First, we conducted tests to determine whether frogs could discriminate between chemical cues that they themselves had deposited on the substrate and those deposited by conspecific individuals. Three series of tests were run, using frogs collected in the same home range (under the same rock), nearby home ranges (less than 5 m apart), or distant home ranges (more than 5 m apart). Twenty frogs were tested in each series.

For all experiments, subjects were placed into a testing apparatus consisting of a plastic container ($33 \times 20 \times 8$ cm) divided by a line drawn in its center. On one side, we placed a paper towel that previously had been marked by the test subject itself; on the other side, we placed a paper towel that had been marked by another individual. We removed fecal matter from the towels prior to running the trials, but odors from feces, urine, as well as exocrine gland secretions may have persisted on the towels. Movements on either side of the testing apparatus were recorded for 60 min. Halfway through each trial, we switched sides by rotating the testing apparatus 180° to control for potential biases of subjects to move in particular directions.

23.2.3   Self Attraction vs. Conspecific Avoidance

Frogs might be attracted to odors of any conspecifics (e.g., Graves et al. 1993), but discrimination, if it occurs, may arise from attraction to one's own odor or avoidance of those of conspecifics. To distinguish between these possibilities, we placed frogs into containers and gave them a choice either between a paper towel with which they previously had had contact and an unmarked paper towel, or between an unmarked paper towel and one marked by a conspecific. Conspecifics were collected from a distant home range (more than 5 m away). Movements of subjects on either side of the apparatus were recorded for 60 min, following the same testing protocol as above. Twenty frogs were tested in each series.

23.2.4   Source of Chemical Cues

To determine the source of the chemical cues used in recognition, we next repeated these experiments but using paper towels marked only by specific cue sources. We tested subjects on paper substrate imbued with isolated samples of urine, feces, or skin secretions. Samples were collected from frogs from distant home ranges (more than 5 m apart). Urine was collected from frogs by gently inserting a blunt-ended cannula into each frog's cloaca until urine was released. The urine then was transferred onto the conspecific's side of the apparatus. Feces were collected from the containers in which subjects were housed and stored in sealed vials until needed. Feces were rubbed into the conspecific's substrate so that no tactile information was available to the experimental subject. Skin secretions were collected by swabbing the dorsal surface of a frog with a cotton bud. The contents of the cotton bud then were rubbed into the conspecific's substrate. Twenty-two frogs were tested in each series, following the same testing protocol as above.

23.2.5   Testing Conditions and Analysis

In all experiments, moisture levels were kept similar on both sides of the testing apparatus. Frogs respond differently to odors of larger and smaller frogs (Lee and Waldman 2002), so we matched sizes of frogs used in every test. This species cannot be reliably sexed by external traits, but adult females can be larger than adult males (Bell 1978). Thus, we were unable to break down results by sex. Times subjects spent on either side of the apparatus were summed over both halves of the experiment and, as data were normally distributed, compared by paired t-tests. Differences in preferences as a function of distance between home ranges were analyzed by one-way analysis of variance. Statistical analyses were conducted with Minitab 13.30. All statistical inferences were based on two-tailed probabilities.

23.3   Results

23.3.1   Self/Nonself Recognition

Frogs preferred the substrate that they themselves had marked to that marked by a conspecific but only if they had not previously shared a home range with them. The strength of the preference varied depending on the distance between the home ranges of the two individuals (Fig. 23.1). If frogs were collected together, under the same rock, they did not discriminate between the sides ($t_{19}=0.07$, $P=0.94$). Presumably the individuals had become familiar with each other, and their odors, prior to collecting them. However, frogs demonstrated preferences for their own side
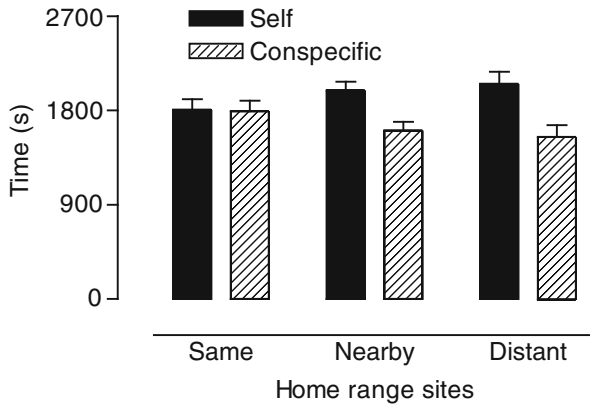
Fig. 23.1 Mean times (+SE) subjects spent on substrate that they had marked themselves and those marked by a conspecific collected in the same home range (under the same rock), nearby home ranges (less than 5 m apart), or distant home ranges (>5 m apart) (modified from Waldman and Bishop 2004)

if the conspecifics were from nearby (t_{19} = 2.37, P = 0.028) or distant home ranges (t_{19} = 2.30, P = 0.033). The further apart their home ranges, the stronger were their preferences for their own substrate to that of a conspecific ($F_{2,57}$ = 3.30, P = 0.044). Additional analyses on other behavioral measures confirm these results (Lee and Waldman 2002; Waldman and Bishop 2004).

23.3.2   Self Attraction vs. Conspecific Avoidance

Frogs preferred their own odor to that of an unmarked substrate (t_{19} = 2.53, P = 0.020) but preferred an unmarked substrate to one marked by a conspecific (t_{19} = 2.15, P = 0.045) (Fig. 23.2). Thus, individuals recognize and respond both to their own odors and those of conspecifics, moving toward their own marked areas but away from those of others (also see Waldman and Bishop 2004).

23.3.3   Source of Chemical Cues

Urine collected from frogs was sufficient to elicit discrimination (t_{21} = 2.11, P = 0.047) (Fig. 23.3). In contrast, we did not observe significant preferences for substrate marked with subjects' own feces to those marked by conspecifics (t_{21} = 0.96, P = 0.35). Discrimination between self and nonself markings was strongest in response to odors collected from swabs of frogs' skin (t_{21} = 3.04, P = 0.006) (Fig. 23.3). Thus, social discrimination in some contexts may be based on skin secretions or odorants in the urine rather than fecal cues (Waldman and Macfie 2005).
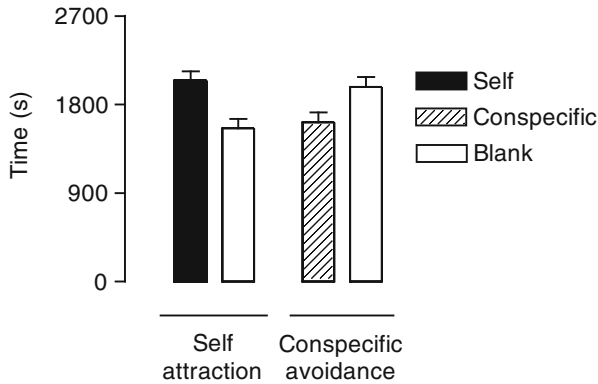
Fig. 23.2 Mean times (+SE) subjects spent on substrate that they had marked themselves and blank substrate (*left*), and on substrate marked by unfamiliar conspecifics (collected >5 m away) and blank substrate (*right*) (modified from Waldman and Bishop 2004)
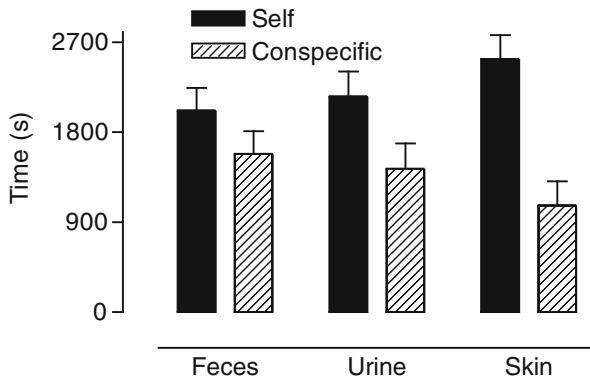
Fig. 23.3 Mean times (+SE) subjects spent on substrates marked with their own urine, feces, or skin secretions and those marked by conspecifics collected from distant home ranges (>5 m apart)

23.4   Discussion

Leiopelma hamiltoni is capable of chemosensory recognition of self, neighbors, and strangers, and maintains the ability to discriminate among these classes even after being held in separate containers for 72 h. Individuals from the same home range appear to tolerate one another and elicit no responses that result in assortative behavior. Individuals from distant home ranges elicit maximal levels of withdrawal behavior. This behavior corresponds with the known dispersal behavior of this species. Individuals travel slowly over small home ranges (<25 m^2) and return to their daytime refuges as morning approaches (Webster 2004). Frogs thus become familiar with the odors of their neighbors and learn to recognize chemical traces that mark their home ranges.

This social discrimination may be useful in a variety of contexts. The ability to discriminate between neighbors from the same or adjacent home ranges and strangers from afar, termed "dear enemy recognition", can be advantageous because it minimizes time and energy expended to maintain exclusive access to resources (Jaeger 1981). Furthermore, chemosignals may function to facilitate cooperation among close kin or selection of non relatives as mates (Madison 1975). The avoidance of close inbreeding, or optimal outbreeding (Bateson 1983), can be particularly important for species such as *L. hamiltoni* that live in highly structured genetic populations (Waldman and McKinnon 1993). The propensity of Hamilton's frogs to travel around fixed home ranges, apparently over their lifetimes, should enable them to recognize and respond appropriately to strangers that may be potential mates or competitors.

The source of the cues used by the frogs for social communication remains to be determined. Skin secretions and urine, and possibly to a lesser extent feces, all seem sufficient to elicit discrimination. In other studies, we found that fecal odors not only were sufficient to elicit discrimination between one's own substrate and those of conspecifics, but effectively conveyed information about individuals' body condition and physiological state. For example, frogs were able to effectively judge the size of conspecifics based on exposure just to their fecal cues, withdrawing from substrates marked by frogs larger than themselves but approaching those marked by subjects smaller than themselves (Lee and Waldman 2002). However, our most recent results suggest that the substrate used in those experiments may have been contaminated with skin secretions or urine.

The skin of *L. hamiltoni* contains mucous glands and two types of granular glands, each of which appears to secrete different peptide mixtures (Melzer et al. 2011). Although thought to be important in predator defense or as antimicrobial peptides that confer disease resistance, the secretions provide a rich source of chemosignals that might be used for social recognition. Further research is needed to analyze the chemical composition and biological properties of these secretions. Aside from information about sex, size, health, and genetic identity, chemical cues may reflect environmental factors, such as the frogs' diet which can vary among home ranges (Bell 1995).

Leiopelma hamiltoni never has been observed to breed in the wild. Because in many aspects of its ecology and behavior, this species resembles *L. archeyi* that lives in parts of New Zealand's North Island, researchers have assumed that, like *L. archeyi*, the frogs breed under rocks or leaf litter, with subsequent paternal care of young (Bell 1978). However, one night while conducting frog surveys on Maud Island, I was surprised to witness a *L. hamiltoni* frog adopting a posture, similar to those used by other species when making advertisement vocalizations, from a crevice in a tree trunk about 3 m above ground (Fig. 23.4). A few hours later, I found the frog amplexed with another less than 50 cm away (Fig. 23.5). These observations raise the possibility that *L. hamiltoni* climbs to find a suitable perch site from which it can more effectively broadcast chemosignals to attract potential mates.

Amphibians worldwide, including New Zealand *Leiopelma* frogs, are declining at precipitous rates as a result of numerous factors including habitat destruction,

Fig. 23.4 *Leiopelma hamiltoni* individual observed on a perch site within a tree crevice about 3 m above ground, possibly broadcasting chemosignals

Fig. 23.5 An amplectant *Leiopelma hamiltoni* pair observed near the perch site from which one had appeared to be signaling immediately before

introduced predators and competitors, chemical contaminants, and emerging infectious diseases. While communication by chemical signaling offers advantages for conveying information about home range boundaries, social status, reproductive condition, and individual identity, chemosignals have inherent properties that also may make frogs more vulnerable to predation (Hamer et al. 2011). Chemical communication systems are especially vulnerable to disruption by anthropogenic change (Park et al. 2001). Pesticides, herbicides, and industrial pollutants, even at low, sublethal concentrations, may have unpredictable effects on the stability of social systems based on communication with chemosignals. Ecotoxicological studies need to be broadened in scope to examine whether chemical noise makes social communication more difficult and interferes with normal reproductive behavior. Knowledge of the underpinnings of how frogs communicate may prove vital to the conservation of threatened and endangered species (Waldman and Tocher 1998).

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