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Pheromonal Discriminations of Sex, Reproductive Condition, and Species by the Lacertid Lizard *Podarcis hispanica*

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ABSTRACT In some vertebrate taxa, pheromones provide important information about species, sex, reproductive condition, kinship, and even individual identity. Because they possess highly developed nasal chemosensory systems, lizards are capable of many chemical discriminations, but many aspects of their pheromonal communication remain poorly understood even in major families. We report that males of a lacertid lizard, *Podarcis muralis*, are capable of differential response to surface chemical cues from conspecific males and females, from gravid and nongravid females, and from conspecific females and females of the closely related sympatric congener, *P. bocagei carbonelli*. In 60 sec trials in which stimuli from the femoral, cloacal, lateral, and upper body surfaces were presented to males on cotton swabs, males tongue-flicked at significantly higher rates to stimuli from conspecific females than males, from conspecific nongravid than gravid females, and from conspecific than heterospecific females. Responses to stimuli from conspecific males did not differ from those to distilled water. Together with previous findings that males can distinguish between chemical cues from familiar and unfamiliar males, these findings suggest that pheromones provide male *P. hispanica* important information regarding the presence of sexual rivals and the reproductive condition of potential mates without visually encountering other lizards. A growing body of literature indicates that lacertids are capable of sophisticated pheromonal discriminations that may play important roles in their social behavior. *J. Exp. Zool. 292:523–527, 2002.* © 2002 Wiley-Liss, Inc.

Chemical signals play important social roles in many vertebrates and are especially important in squamate reptiles, which have the most highly developed vomeronasal systems of all vertebrates (Halpern, '92; Mason, '92; Cooper, '94). Responses to chemical cues by squamates are exceptionally easy to study because tongue-flicking for chemical sampling for delivery to the vomeronasal organs provides an observable index of chemosensory investigation, making lizards and snakes ideal subjects for pheromone studies. Pheromonal communication occurs in several families of lizards (Mason, '92; Cooper, '94), including the large Old World family Lacertidae (Bauwens et al., '87; Lena and Fraipont, '98; López et al., '98; Aragon et al., 2000; Martín and López, 2000). Several aspects of pheromonal discrimination by lacertids remain equivocal or unstudied.

In some lizard families, pheromones allow males to discriminate between conspecific males and females, between potentially fertilizable and receptive females and nonreceptive females, and between conspecific females and females of closely related syntopic congeners. All of these abilities have been demonstrated in the broad-headed skink, *Eumeces laticeps* (Cooper and Vitt, '84, '87; Cooper et al., '86). Discrimination between male and female conspecifics using only chemical cues has been demonstrated in several families of lizards (Duvall, '79, '81; Brillet, '90; Cooper and Trauth, '92; Cooper et al., '94, '96; Cooper and Steele, '97), but not in Lacertidae.

In lacertids, Gómez et al. ('93) found no evi-

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dence that male *P. hispanica* tongue-flicked at different rates on substrates labeled (marked) by conspecific males and females, but noted that males appeared to locate females by scent-trailing and tongue-flick the tails of females during courtship. Bauwens et al. ('87) found no evidence for pheromonal discrimination of sex by male *Lacerta vivipara* in a study designed primarily to detect roles of chromatic cues in sex recognition. However, their results did not exclude pheromonal sex recognition because visual cues may have induced approach, which was the index of courtship, and differences in intensity of courtship that might have been induced by pheromones were not examined.

An ability to discriminate between conspecific females and females of other species is crucial for males to avoid wasting reproductive efforts. Ethological isolating mechanisms based on visual, auditory, chemical, and other sensory cues are widespread among animals to ensure that mating attempts are directed to conspecifics. Lacertids living in syntopy with congeners would appear to have the sensory capacity to discriminate pheromonally between conspecifics and syntopic congeners: The tongue-vomeronasal system, which mediates pheromonal responses (Kubie et al., '78), is more highly developed in lacertids (Gabe and Saint-Girons, '76; Schwenk, '93, '94; Cooper, '96, '97) than in skinks capable of interspecific pheromonal discriminations. That male *P. hispanica* tongue-flick substrates chemically labeled by conspecific females at higher rates than those labeled by females of the sympatric lacertid *Psammodromus algirus* (Gómez et al., '93) supports the possibility of discrimination among closer relatives.

Here we examine some abilities of adult male *Podarcis hispanica* to make reproductively important discriminations using only chemical stimuli. We tested abilities of males to use chemical cues from body surfaces to detect conspecific males and females and discriminate between the sexes and between gravid and nongravid females. We predicted that males would tongue-flick more frequently in response to cues from females than from males. No previous studies have been reported on response to pheromones from gravid females, but we predicted that males would tongue-flick more frequently in response to those from nongravid than gravid females because the former are likely to become sexually receptive sooner. We predicted that males would tongue-flick more frequently in response to chemical cues from conspecific females than from females of the syntopic *P. bocagei carbonelli*, a very closely related species (Harris and Arnold, '99).

MATERIALS AND METHODS

Adult *P. hispanica* and *P. b. carbonelli* were collected in the Sierra de Francia, Salamanca, Spain on 11 June 2000. They were transported to the laboratory at the University of Salamanca where they were housed one per cage in lidless transparent plastic terraria (40 cm × 26 cm × 26 cm or 46 cm × 26 cm × 26 cm). All cage sides were covered with white paper to minimize disturbance to the lizards by their neighbors and by the investigators. The natural light cycle for the region was maintained by light through a window. The temperature at the middle of each cage at floor level was 29°C–30°C. Incandescent bulbs adjacent to one end of each cage produced a thermal gradient that allowed the lizards to maintain higher body temperatures. The lizards readily ate mealworms in these conditions. Upon completion of the experiments, lizards were released at the sites of capture.

We conducted two experiments to assess the ability of adult male *P. hispanica* to use chemical cues to discriminate between sexes and reproductive conditions of conspecifics and between conspecific and heterospecific females. In both experiments we presented the stimuli to lizards on the cotton tips of 15 cm wooden applicators. To begin a trial, an experimenter approached a lizard's cage and slowly brought the swab from above into position ca. 1.5 cm anterior to the lizard's snout. Beginning with the first tongue-flick directed to the swab, we recorded the numbers of tongue-flicks directed to the swab for 60 sec. The experiments had repeated measures designs in which each male was tested in all stimulus conditions. We tested for homogeneity of variance using Hartley's Fmax tests (Winer, '62) in both experiments. Statistical tests were two-tailed, with $\alpha = 0.05$, except as noted below.

The experiment on the ability of males to discriminate between sexes and between reproductive condition of females was conducted between 1000 hr and 1130 hr on 14 June 2000. The chemical stimuli tested were distilled water, which served as an odorless control for responses to the experimental setting; male stimuli; gravid female stimuli; and nongravid female stimuli. The stimuli were prepared by dipping the cotton swab into distilled water and adding lizard stimuli if needed by rubbing the swab against the femoral and cloacal regions and on the lateral and dorsolateral skin of the type of lizard appropriate as the stimulus source. The sequence of stimulus presentation was randomized to avoid confounding effects of stimulus types with sequence of testing. When heterogeneity of variance was detected, we logarithmically transformed the data and verified that the variances for the transformed data were homogenous and then conducted a parametric analysis of variance for a single factor experiment having repeated measures (Winer, ’62). Paired comparisons among means were conducted using Newman-Keuls tests (Zar, ’96). A single one-tailed test was conducted of the difference in frequency of tongue-flicking between gravid female and male stimuli. That was justified by the prediction of greater response to female stimuli.

The ability of males to discriminate between conspecific females and female *P. b. carbonelli* was tested between 1600 hr and 1720 hr on the same day as the first experiment. The experimental stimuli were prepared as above, but there were only two conditions—conspecific and heterospecific female chemicals. The sequence of stimulus presentation was counterbalanced for six individuals and selected randomly for the seventh male to prevent any sequential biases. Following detection of heterogeneity of variance, we analyzed the data using a Wilcoxon signed ranks matched pairs test (Zar, ’96). In addition, we conducted a sign test for difference in the number of individuals that tongue-flicked more frequently in the two conditions (Zar, ’96).

RESULTS

Males performed more than three times as many tongue-flicks in response to chemical cues from nongravid females than to the any of the other stimuli (Fig. 1). All six males tongue-flicked in all conditions, and one individual bit the swabs bearing chemical cues from a gravid female and an adult male. Variances of raw numbers of tongue-flicks were significantly heterogeneous (Fmax = 13.28; df = 4, 15; $P < 0.01$), but the variances of the logarithmically transformed data were homogeneous (Fmax = 2.08; df = 4, 15; $P > 0.05$).

For transformed data, numbers of tongue-flicks differed significantly among conditions (F = 20.33; df = 3, 15; $P = 1.5 \times 10^{-5}$. Number of tongue-flicks in the nongravid female condition was significantly greater than in the gravid female ($P = 7.6 \times 10^{-4}$), male ($P = 2.0 \times 10^{-4}$), and distilled water ($P = 2.0 \times 10^{-4}$) conditions. Males responded significantly more strongly to stimuli from gravid females than from males ($P < 0.026$, one-tailed) or distilled water ($P < 0.038$). Numbers of tongue-flicks did differ between the male and distilled water conditions ($P > 0.10$).

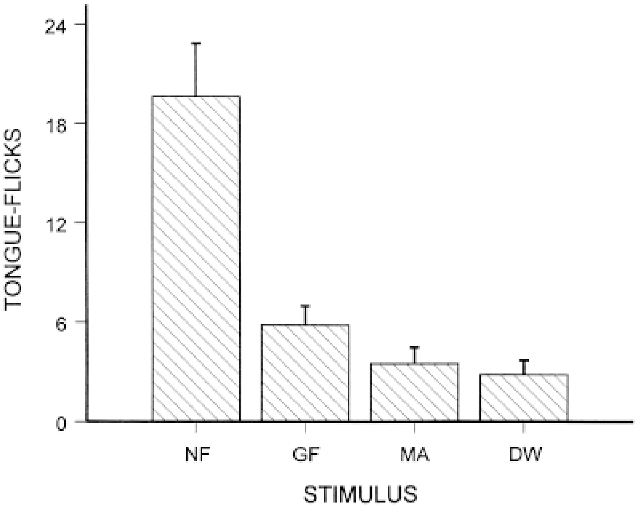

Fig. 1. Mean numbers of tongue-flicks directed by six adult male *Podarcis hispanica* in 60 sec to cotton swabs bearing surface chemical stimuli from nongravid females (NF), gravid females (GF), males (MA), and distilled water (DW). Error bars represent 1.0 SE.

In the study of interspecific discrimination, seven males responded in both experimental conditions, and all individuals tongue-flicked more frequently in response to conspecific than heterospecific females (sign test, $P < 0.016$). Males tongue-flicked 10.0 ± 2.6 times in response to chemical cues from female *P. hispanica* and 3.7 ± 0.7 times in response to cues from female *P. b. carbonelli*. The variance of number of tongue-flicks was significantly greater in the conspecific than heterospecific trials (Fmax = 6.31; df = 2, 12; $P < 0.018$). A nonparametric Wilcoxon signed ranks matched pairs test showed that the males tongue-flicked significantly more frequently in response to chemical cues from conspecific than heterospecific females (T = 0.0, n = 7, $P < 0.018$).

DISCUSSION

The lizards discriminated between sexes pheromonally, as shown by the higher tongue-flick rates in response to surface chemicals from nongravid and gravid females than to males. The elevated response rates to female cues presumably represents chemosensory investigation that is necessary for decisions about courtship, as suggested by the observation that males tongue-flick females when approaching them to court (Gómez et al., ’93). The finding of sex discrimination extends earlier work with several other families to

Lacertidae; the most closely related families previously known to be capable of pheromonal sex discrimination are Scincidae (Cooper and Vitt, '84) and possibly Amphisbaenidae (Cooper et al., '94), the latter having disputed taxonomic relationships (Estes et al., '88; Schwenk, '94). Pheromonal discrimination of sex is now known to occur in all three families of Scincoidea (Scincidae—Cooper and Vitt, '84; Gerrhosauridae—Cooper and Trauth, '92; Cordylidae—Cooper et al., '96). Together with the present findings, these data suggest that pheromonal sex discrimination may be universal in Scincomorpha, which consists of Scincoidea and Lacertoidea (Lacertidae + Teiidae + Gymnophthalmidae).

Males discriminated pheromonally between gravid and nongravid females. That nongravid females elicited higher tongue-flick rates suggests that males increase chemosensory investigation of females that may be or will soon become sexually receptive. Pheromonal discrimination between gravid and nongravid conspecific females has not been demonstrated previously in lizards, but may be widespread. It is unknown whether this discrimination is based on stronger responsiveness to pheromonal components found at higher concentrations in nongravid than gravid females, response to attractants produced only by nongravid females, on inhibitory properties of the pheromones of gravid females, or a combination of these. The only similar studies of discrimination between females based on their reproductive condition showed that male broad-headed skinks (*E. laticeps*) increase their tongue-flick rates and intensity of courtship to females that are producing a sex pheromone that is synthesized in response to estrogen (Cooper et al., '86). The possible production of female sex pheromones by lacertids should be investigated.

No evidence suggested that male *P. hispanica* detected conspecific male pheromones. However, *Lacerta monticola* discriminates between pheromonal cues from familiar males having overlapping territories and unfamiliar males (López et al., '98; Aragón et al., 2000; Martín and López, 2000). The conclusion that male *P. hispanica* cannot detect conspecific male pheromones is unwarranted because statistical power was low due to the small sample size and because the experimental design may have been inappropriate to show the ability. Males of other lacertid species respond to pheromones deposited on environmental substrates by altering the amount of time spent on them and tongue-flicks directed to them (López et al., '98; Aragón et al., 2000; Martín and López, 2000). Experiments using labeled tiles might be more realistic for *P. hispanica*.

The finding that male *P. hispanica* are capable of pheromonally mediated discrimination between syntopic congeners extends the previous finding that they can discriminate between conspecific pheromones and those of sympatric *P algirus*, which is a member of the same family (Gómez et al., '93), but differs substantially in being larger and using more arid habitats (Barbadillo et al., '99). Because *P. hispanica* and *P. b. carbonelli* are very closely related, pheromones permit discriminations previously unknown in lacertids. Their ability appears to be comparable to that ability by male broad-headed skinks (*Eumeces laticeps*), which discriminates pheromonally between female conspecifics and females of its two closest relatives, both of which occur syntopically (Cooper and Vitt, '87). That tongue-flick rates were lower in the second experiment than in the first is presumably a result of habituation to testing because responses by the same males had been tested previously in three experiments in two days.

An ability to distinguish conspecific from heterospecific pheromones could be important for lizards syntopic with closely related species. Male skinks (*E. laticeps*) court heterospecific females labeled with pheromones from conspecific females (Cooper and Vitt, '87). Pheromonal cues may take precedence over visual cues in species having similar color patterns, as do female *P. hispanica* and *P. b. carbonelli* (Barbadillo et al., '99). Failure to discriminate conspecific from heterospecific pheromones might lead to interspecific fighting, courtship, and mating, all of which may be costly due to energetic costs and increased risk of increased risk of predation due to decreased vigilance during social encounters. Fighting additionally increases risk of injury.

When a female is not present, pheromonal cues on environmental substrates, particularly those from fecal pellets and femoral gland secretions (López et al., '98; Aragon et al., 2000; Martín and López, 2000), may permit male *P. hispanica* to detect her presence and perhaps monitor her reproductive condition. Some lizards are able to follow scent trails (Garrett et al., '96), including those of conspecific females (Cooper and Vitt, '86), and behavioral observations suggest that lacertids may have this ability (Gómez et al., '93). Male *P. hispanica* might be able to locate conspecific females by following scent trails or searching the vicinity of pheromonal deposits. Discrimination between pheromones of conspecific and heterospecific females could be beneficial in avoiding time-consuming searches for inappropriate potential mates.

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