Sex recognition is based on color signals in many species of lizards. However, olfactory stimuli are also clearly involved, and many species might rely primarily on chemoreception. We aimed to examine whether color pattern or odors, or a combination of both factors, induce the aggressive response of males of the lizard *P. hispanicus*. We experimentally manipulated the coloration and odor of male *P. hispanicus*, thereby creating groups with all combinations between coloration and odor of males or females. Using data from staged encounters, we compared the responses of resident males to the experimental groups of manipulated males and their response to unmanipulated individuals (males and females). Responding males reacted significantly more aggressively to intruders with male odors independent of their coloration, whereas intruders with female odors did not elicit aggressive responses but were preferentially courted, irrespective of their actual sex and body coloration. In addition, intruders with female odors elicited a higher number of tongue-flick explorations than male odor ones. Comparisons with unmanipulated male and female intruders agreed with these expectations. Therefore, at least at close range, odoriferous cues seem to be more important than color patterns in sex recognition and intrasexual aggression by male *P. hispanicus*. We suggest that this might be a pattern commoner than expected in many species of reptiles. Aggr. Behav. 28:154–163, 2002. © 2002 Wiley-Liss, Inc.

Key words: agonistic behavior; coloration; chemical signals; lizards; sex recognition

**INTRODUCTION**

In vertebrates, males and females of many species use body coloration to convey information about the sender, such as fighting ability, sex recognition, or sexual stage [e.g., Andersson, 1994; Cooper and Greenberg, 1992; Harper, 1991]. Sex recognition is based on color signals in many species of lizards, especially in sexual dichromatic species where one sex, often males, display brighter colors than females [Cooper and Burns, 1987; for a review see Cooper and Greenberg, 1992]. Dull female coloration presumably allows sexual recognition, reducing the aggressive response of conspecific males.

The artificial manipulation of body colors by painting experiments has provided strong evidence that sex determination relies on visual cues in many lizards. For example, in lacertid lizard species with extreme sexual dichromatism, such as *Lacerta agilis*, the importance of sex-
related coloration has been demonstrated by experiments where lizards were artificially painted to resemble the body colors of the opposite sex. Thus, males attacked rather than courted females painted to resemble males and courted other males painted with the female-like pattern [Kitzler, 1941; Kramer, 1937]. However, olfactory stimuli were also clearly involved in sex recognition because male *L. agilis* that had responded aggressively to females painted with male-like colors stopped behaving aggressively after tongue-flicking them [Kitzler, 1941; Kramer, 1937]. In another experiment, male *L. vivipara* courted conspecific males painted as females, but females painted as males, or painted black, were also courted [Bauwens et al., 1987], suggesting that males can probably identify females by characteristics other than their color pattern.

Chemical cues are known to play an important role in the intraspecific communication of lizards [Cooper, 1994; Halpern, 1992; Mason, 1992], and several studies have shown pheromonal detection in different species [e.g., Alberts, 1989; Cooper and Vitt, 1984; Gómez et al., 1993]. For example, in other sexually dichromatic species, such as the skink *Eumeces laticeps*, males which tongue-flicked females painted as males ceased aggressive behavior [Cooper and Vitt, 1988]. In addition, male *E. laticeps* directed aggressive behavior primarily to conspecific males, whereas heterospecific males of the same genus, although visually quite similar, were ignored following chemosensory investigation [Cooper and Vitt, 1987]. Moreover, sex recognition seems to rely only on chemoreception, but not on coloration, for other species of reptiles. For example, gekkonids are known to use chemoreception of skin-derived chemicals in sex recognition [Greenberg, 1943; Mason and Gutzke, 1990]. Thus, we hypothesized that in most lizards pheromonal identification of sex might take precedence over the chromatic cue.

The Iberian wall lizard, *Podarcis hispanicus*, is a small (50–70-mm adult snout-to-vent length [SVL]) diurnal lacertid lizard of the Iberian Peninsula, common in rocky habitats or artificial walls [Martín-Vallejo et al., 1995]. The sexes differ in dorsal pattern and coloration (see below). Sexual dichromatism is more apparent in the belly, which can be orange in adult males during the mating season, whereas it is white in females and other males [Pérez-Mellado and Galindo, 1986]. On the other hand, *P. hispanicus* have well-developed chemosensory abilities and can discriminate between prey and non-prey odor-impregnated cotton swabs [Cooper, 1990] and, at least, between conspecífics and heterospecifics by chemical cues alone [Gómez et al., 1993].

In this paper we aimed to examine whether color pattern or odors, or a combination of both factors, are used by male *P. hispanicus* in sex recognition. We experimentally manipulated the coloration and odor of male and female *P. hispanicus*, thereby creating groups with all the combinations between coloration and odor of males or females. Using data from staged encounters, we compared the responses of resident unmanipulated males to the different groups of manipulated individuals and their response to unmanipulated males and females. We hypothesized that responding males should behave in the experiments as they normally did with unmanipulated individuals and in the field. Thus, they should respond to manipulated individuals that they recognize as males as if they were intruders in their home ranges and display aggressive behaviors toward them. Whereas individuals recognized as females should not elicit aggressive behavior but should be courted. The outcome of aggressive vs. courtship displays should indicate whether visual or odoriferous cues, or both, are used in sex discrimination by *P. hispanicus*.

**MATERIALS AND METHODS**

**Study Area and Subjects**

We captured adult *P. hispanicus* (males, *n* = 24; females, *n* = 12) on rocky outcrops in an oak forest near Cercedilla (40° 44′ N, 4° 02′ W; Madrid province, Spain). We captured lizards by
noosing in different places over a large area to ensure that individuals had not been in previous contact, which may affect the outcome of the interactions [Olsson, 1994b]. Lizards were housed individually at “El V entorrillo” Field Station (5 km from the capture site) in outdoor plastic cages (60×50×50 cm) containing sand substrate and rocks for cover. Cages were placed so that they received the same degree of sunlight. We provided mealworms dusted with a multivitamin powder as food and water ad libitum. The experiments were carried out during April and May 1998, which coincided with the mating season of lizards in their original natural population. All lizards were healthy during the trials and, at the end of the experiment, were released at their capture sites.

**Experimental Procedure**

We experimentally manipulated color and odor of male *P. hispanicus*. We painted individuals to resemble either male or female natural coloration and, then, we impregnated them with either male or female odors (taken from other individuals). Manipulated males (n = 8) were size matched by SVL within each sex and randomly assigned to each different treatment (four groups). Other control individuals were used as unmanipulated (four males and four females), and four males were left with normal unmanipulated color but were impregnated with other male odor. Each manipulated or control individual participated in four tests with different responding males (n = 8).

Before the treatment, lizards were cold anaesthetized in a refrigerator. We first attempted to eliminate odoriferous skin secretions with a treatment that has been effective in removing sexual pheromones of snakes [Noble, 1937; Ross and Crews, 1978] and lizards [Bauwens et al., 1987; Ferguson, 1966]. Lizards were first washed with 96° alcohol, devoting special attention to remove scents from the more odorous areas such as the cloacal and femoral regions. We then painted individuals and thereafter coated them with non-odoriferous Vaseline to eliminate odoriferous scents. Lizards were painted by using OcaldoR water colors (Calde Colors Ltd, Ashby-de-la-Zouch, UK), mixing them to achieve good visual matches with the natural colors and patterns of *P. hispanicus* in our study area [see, e.g., Arnold and Burton, 1978; Pérez-Mellado and Galindo, 1986]. The background (brownish-olive) was first painted covering the dorsum of the lizard’s body. The belly was then painted with orange (for male-like treatment) or white colors (for female-like treatment). The head, hands, and tail remained unpainted. After drying at room temperature, we painted with the tip of a pen small pale white spots forming a discontinuous and irregular longitudinal row along each side and a few small blue spots along the edge of the belly (male-like treatment) or two longitudinal white stripes along each side (female-like treatment). Lizards were replaced in the refrigerator until the paint had dried before using them in experiments.

Male and female odors were transferred to the experimental individuals immediately preceding each trial by rubbing with cotton swabs impregnated in distilled water, the head, neck, trunk, and tail skin and the cloacal area and femoral pores of a male or a female lizard, against the corresponding skin areas of the experimental individual. We made an effort to ensure odor transfer in those areas more frequently and intensely investigated by tongue-flicking during social encounters. This technique has been successfully employed to transfer odors between individuals in other lizard species [Cooper and Vitt, 1987].

We attempted to match natural male or female coloration and odor. Lizards might, however, respond to cues which are not in the spectrum visible for the human eye, for example, ultraviolet radiation [Fleishman et al., 1993], which might not be accurately imitated or concealed by the paint used. We are confident that this was not the case because we conducted observations of the
responses of males toward unmanipulated males and females. The results showed that responding males did not change their normal expected behavioral responses as a result of this manipulation. Thus, the number of aggressive responses to manipulated males painted as males and impregnated with male odor, to males with normal unmanipulated color but impregnated with other male odor, and to unmanipulated males were not significantly different (one-way repeated-measures ANOVA: \( F_{2,14} = 0.87, P = .43 \) (Fig. 1). Similarly, the number of aggressive responses to manipulated males painted as females and impregnated with female odor and to unmanipulated females were not significantly different (one-way repeated-measures ANOVA: \( F_{1,7} = 1.32, P = .29 \) (Fig. 1). Thus, the experimental treatments seemed to be effective in resembling the color and odor of males or females. Also, other species of lizards seem to respond normally to artificially painted individuals that resemble natural coloration [e.g., Cooper and Vitt, 1988; Olsson, 1994a; Martín and Forsman, 1999; Thompson and Moore, 1991].

**Staged Interactions**

We staged encounters between pairs of lizards in the home-cage of the responding male, and, thus, the responding male acted as the owner and the manipulated male or female acted as an intruder. With this design we tried to mimic a natural field situation where a resident male found...
a conspecific in his home range. The responding male had the initial advantage of being resident and should fight aggressively for maintaining its owner status against any intruding male, or be neutral and attempt to court a newcoming female. We planned a repeated-measures design in which each responding male (N = 8) encountered a different manipulated individual or an unmanipulated individual in a randomized sequence but participated in only one interaction per day to avoid stress. We also spaced staged encounters sufficiently (at least one day) so that fatigue resulting from one test did not affect subsequent tests. To avoid the effects of previous experience between individuals [Olsson, 1994b], in each test the two contestant lizards had never been together before the trials. All tests were made in outdoor sunny conditions between 0900–1200 hours GMT when lizards were fully active.

To begin a trial, we gently took one manipulated lizard from its cage and placed it gently in the middle of the responding male’s cage; from a blind, we recorded their behavior. In agonistic contests, we considered a “neutral response” when the two individuals were together but no response or nonaggressive interactions were observed or an “aggressive response” if the responding male approached another individual with aggressive display and made the other individual retreat or run away without contact, by touching him on the flanks, or, occasionally, by giving quick bites, especially on the snout or head. This fighting behavior was readily discernible from courtship behavior [Verbeek, 1972]. Approaching males employ threatening postures and strut toward an opponent on raised, stiff forelegs with their neck arched and the snout pointing slightly down. Whereas during courtship displays the male approaches the female slowly and begins to lick the tail or the surrounding substrate. He then grips and shakes the female’s tail with a gentle bite, which does not result in any discernible wounds. If the female is receptive she will allow mounting. Thus, the body posture of the male and the intensity of his approaching and bites unequivocally characterize the difference between courtship and aggressive behaviors. In our experiment with manipulated males, however, only the first phases of the courtship, including bites, were observed because experimental males responded by fleeing from the courting male.

We noted the number and characteristics of the agonistic interactions between individuals during each trial and the courtships or their attempts observed. We also noted, each time that the two lizards were together, whether the responding males explored by tongue-flicking the body of the experimental individuals or the surrounding substrate. Tongue-flicking presumably indicates chemosensory examination in lizards [Cooper, 1994; Cooper and Burghardt, 1990]. We noted the frequency of such tongue-flick exploration events, each exploration consisting of several tongue flicks emitted during sequences of variable duration.

A trial was interrupted after 15 min. Also, we decided to stop observations if persistent attacks or desperate attempts to escape were recorded. This was, however, not necessary as most interactions mostly consisted of threatened displays and short chases and only very rarely escalated to single quick bites that did not cause observable injury. None of the individuals suffered physical injuries or showed physical stress during or after the trials, and all of them had maintained or increased their original body mass at the end of the trials. The paint of experimental lizards was removed immediately with water after they had completed all the trials. No damage or necrosis of tissue due to the paint was ever recorded. Although agonistic interactions between lizards were not very aggressive, we used a minimal number of animals over the minimum time necessary to test our hypothesis.

To compare the aggressive levels and number of tongue-flick explorations of the same individual during all the trial duration across treatments, we used two-way repeated-measures ANOVAs, examining the effects of color and odor treatments (within-subjects factors). We included the interaction between color and odor treatments to determine whether responses to a
determined color treatment changed under different odor treatments [Sokal and Rohlf, 1995]. In addition, because coloration might be important in long distance sex recognition, we also analyzed with two-way repeated-measures ANOVAs the responses in the first 5 min of the trials. We used nonparametric Wilcoxon matched-pairs signed-ranks tests to compare the number of attempts of courtships between two treatments [Siegel and Castellan, 1988]. Significance level was .05 and all tests were two tailed.

RESULTS

The type of experimental treatment had a significant effect on the aggressive response of responding males; manipulated males impregnated with male odor received a significantly greater number of aggressive responses than those impregnated with female odor, whereas the effect of color treatment was not significant (two-way repeated-measures ANOVA: color effect, $F_{1,7} = 2.00, P = .20$; odor effect, $F_{1,7} = 9.77, P = .017$; interaction, $F_{1,7} = 0.11, P = .74$) (Fig. 1). Nevertheless, neither coloration nor odor seemed to influence the number of aggressive responses in the first 5 min of the trials (two-way repeated-measures ANOVA: color effect, $F_{1,7} = 0.24, P = .39$; odor effect, $F_{1,7} = 3.12, P = .12$; interaction, $F_{1,7} = 0.28, P = .61$).

Responding males never courted a manipulated male impregnated with male odor, even though he was painted as a female. However, the responding males attempted courtship of manipulated males impregnated with female odor, independent of the fact that they were painted as males ($X \pm SE = 0.5 \pm 0.3$ courtships/interaction) or as females ($1.1 \pm 0.7$ courtships/interaction; Wilcoxon signed-ranks test: $Z = 0.94, P = .34$). Manipulated individuals with female odor were courted by responding males at a rate similar to that of control unmanipulated females ($0.7 \pm 0.6$ courtships/interaction; painted as males: $Z = 0.13, P = .89$; painted as females: $Z = 0.40, P = .68$).

The number of tongue-flick explorations of responding males was also different among treatments. The number of explorations by tongue-flicking was significantly higher to manipulated males impregnated with female odor than to those with male odor, independent of their coloration (two-way repeated-measures ANOVA: color effect, $F_{1,7} = 0.23, P = .64$; odor effect, $F_{1,7} = 12.11, P = .01$; interaction, $F_{1,7} = 0.26, P = .62$) (Fig. 2).

DISCUSSION

The results of our experiment indicate that, at least at close range, odoriferous cues are more important than color patterns in sex recognition by male *P. hispanicus*. Thus, the aggressive response of males to manipulated individuals was mainly dependent on the scent, which they were manipulated, whereas color seemed to be less important. Moreover, when a conflict between the meaning of the visual and scent signals arose, males seemed to rely on the scent to identify the sex of the intruding conspecific. Therefore, pheromonal identification of sex seems to take precedence over the chromatic cue in the lizard *P. hispanicus*.

In lizards, the presence and relative concentration of pheromone components vary not only between sexes but also among individuals, which may convey information on the individual identity and serve a variety of functions [Alberts, 1992]. Therefore, discriminations based on pheromone components may be more reliable and also may provide more detailed information on the conspecific than might be obtained from color patterns alone. Nevertheless, the analysis of the first responses of male *P. hispanicus* to manipulated individuals suggests that coloration might be more important in long-distance communication, whereas pheromone identification may be the most important and confirmatory cue when two individuals are close together.
Other unrelated stimuli could have also influenced our results. For instance, individuals might be also identified by other morphological traits, such as sexual dimorphism in body size and head shape, or certain behavioral cues associated to their sex, and this presumably would explain some mixed responses. Thus, some experimental males with color and scent of females were courted, but they also received some aggressive responses, whereas unmanipulated females did not elicit any aggressive response from the males. When a male came nearby, females often exhibited submissive behavior, such as tail twitches and foot shaking, that seemed to deter the aggressive response of a male. Although these behaviors were also exhibited by young males toward adults, they were infrequent in encounters between two males with similar body size (López and Martín, unpublished data).

Responding males were less aggressive toward males impregnated with scent of females, suggesting that female-like scent indeed reduces the aggressive response of territorial males. Thus, females may inhibit aggression and remain in male territories by signaling their sex through chemosensory cues, which are reliable as they are probably dependent on female-specific hormones. In this context, that resident males responded aggressively toward intruders bearing odors of males, independent of their actual sex or coloration, could be interpreted in terms of acquired ability to recognize competitor males by chemosensory cues. By using a female-like dull coloration, a male with a
low resource-holding potential might evade aggression from a dominant male, adopting a satellite-sneaking mating strategy [Martín and Forsman, 1999]. Something similar occurs in passerine birds, where delayed plumage maturation in subadult dull-colored males reduces the attacks from dominant males and increases their breeding opportunities [Foster, 1987; Rohwer et al., 1980]. However, lizards could easily identify cheating individuals as males by chemosensory cues. Nevertheless, in the garter snake *Thamnophis sirtalis*, a small proportion of males, the so-called “she-males,” seem to be able to produce the female sex attractant pheromone, inhibiting aggression and eliciting courtship from normal males [Mason and Crews, 1985].

Male *P. hispanicus* attempted to court other males impregnated with female scent, independent of their coloration. This suggests that female pheromones are enough, and probably also necessary, to elicit male courtship in this lizard. The same pattern might be found in many other species of reptiles. For example, in an experiment with the snake *T. sirtalis parietalis*, male snakes receiving an application in the skin of serum from females were also courted [Garstka and Crews, 1981]. Similar results were obtained in another experiment in which tubes of female skin were placed on male *T. sirtalis sirtalis* [Gillingham and Dickinson, 1980]. Also, in the skink *Eumeces laticeps*, an urodeal pheromone from females induced male courtship of postreproductive females when transferred to the skin surfaces of the latter [Cooper et al., 1986]. Thus, chemosensory cues would reliably indicate not only the sex of the bearing but also the reproductive status.

In our experiment, tongue-flick explorations were more frequently directed to female-scent manipulated males. Although a previous experiment conducted outside the reproductive season provided no evidence of discrimination of substrates marked by male or female *P. hispanicus* [Gómez et al., 1993], our results suggest that, at least during the mating season and when a live individual is present, *P. hispanicus* is able to detect and discriminate between sexes by chemical cues. To support this view, also males of the gekkonid *Eublepharis macularis* responded with aggressive behavior, typical of their response to conspecific males, to females when these were shed of their skins [Mason and Gutzke, 1990], and this was based on detection of chemicals normally present in the skin of females that are absent when the female is shedding.

The results of our experiment raise the question why males of some lizard species have bright colors whereas others, even in the same genus, are more dull colored. Bright colors are useful in social interactions but also render lizards conspicuous to human observers and presumably to predators, especially avian and lacertilian predators [e.g., Forsman and Shine, 1995]. Therefore, they must be maintained by an adaptive benefit outweighing the effect of increased risk of predation [Zuk and Kolluru, 1998]. This would explain the existence of dull-colored species, which, however, should rely more on chemosensory cues than on coloration to identify sex.

We conclude that chemosensory cues seem to be more important than visual ones in sex recognition by *P. hispanicus*, at least at close range. We hypothesize that because most lizards seem to be able to detect pheromones from conspecifics [Mason, 1992], chemical recognition of sex taking precedence over chromatic cues might be more widespread than expected among lizards as well as among other animals with chemoreceptive capacities. Our findings point out the need for further laboratory and field studies of sex recognition, agonistic interactions, and intraspecific communication taking into account the different roles and the relative importance of color and chemical cues.

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