

Differential Avoidance Responses to Chemical Cues from Familiar and Unfamiliar Conspecifics by Male Iberian Rock Lizards (*Lacerta monticola*)

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ABSTRACT.—We conducted a field study to analyze spatial relationships among male Iberian Rock Lizards, *Lacerta monticola*. We then used the same individuals in a laboratory experiment to test whether avoidance responses of a male lizard in a previously unknown area is affected by presence of scents of familiar and unfamiliar resident males. Time spent attempting to escape in presence of unfamiliar odors was significantly higher than in presence of familiar odors suggesting avoidance of unfamiliar scent marks, which may lower the costs of aggressive interactions. Our results are concordant with previous studies in which differential tongue-flick rates showed discrimination between odors of familiar and unfamiliar males of *L. monticola*.

In lizards, chemical systems play an important role in intraspecific communication (Halpern, 1992; Mason, 1992; Cooper, 1994). Discrimination between familiar and unfamiliar individual males has been suggested in several lizard species (Glinski and Krekorian, 1985; Alberts and Werner, 1993; Cooper 1996; Aragón et al., 2001a). The capability of territorial lizards to discriminate between neighbors and nonneighbors might help to stabilize social systems by reducing frequency and intensity of aggressive encounters (Glinski and Krekorian, 1985). Most studies on discrimination of familiar individuals were conducted by keeping animals jointly until habituation occurred to obtain familiar individuals (e.g., Cooper, 1996; Guffey et al., 1998). However, there is little direct empirical support for chemosensory discrimination among individuals whose actual spatial relationships have been previously determined in the animal's natural environment (Aragón et al., 2000, 2001b). By creating artificially familiar individuals, the experimenter might choose pairs of males that would not be neighbors in natural conditions. For example, in free-living juvenile *Anolis aeneus* there were fewer than the expected number of first encounters involving dyads in which one member was disadvantaged (Stamps, 1994).

Lacerta monticola is a small diurnal lacertid lizard found mainly in rocky habitats of some high mountains of the Iberian Peninsula. Males of this species defend territories against other males, but overlap between home ranges is extensive and agonistic encounters occur during the mating season (Martín and Salvador, 1993, 1997). However, taking into account the high spatial overlap between males, the frequency of agonistic interactions might be expected to be higher than we have observed in the field (Aragón et al., 2001b). A high rate of agonistic interactions in males may provoke energetic and survival costs derived from higher activity (Marler and Moore, 1988, 1989). Therefore, mechanisms for reducing the frequency of aggressive encounters would be advantageous. Previous studies with *L. monticola* showed that males are

able to discriminate, through chemosensory investigation of fecal pellets and femoral secretions, between odors of familiar and unfamiliar conspecific males (Aragón et al., 2000, 2001a). Interestingly, the direction of the differences in chemosensory investigation (i.e., tongue-flick rates) was dependent on the intruder-resident status (Aragón et al., 2000, 2001a,b), suggesting that the balance between costs and benefits might differ between residents and intruders. The aim of these previous studies was to test for discrimination between familiar and unfamiliar conspecifics; however the function of this recognition remains unclear.

We studied spatial relationships in the field between male *L. monticola* by determining degree of familiarity based on percentage of overlap in their home ranges. We then used the same individuals in a laboratory study to assess their behavioral responses to scent marked areas. The goal of this experiment was to emulate a natural situation in which experimental lizards were intruders in a conspecific's area. We specifically tested whether avoidance of an area by an intruder male was affected by presence of chemical cues from familiar or unfamiliar conspecific males.

MATERIALS AND METHODS

We conducted fieldwork from May to June at "Alto del Telégrafo" (Guadarrama Mountains, Central Spain) at an elevation of 1900 m. Patches of large granite rocks and scree interspersed by shrubs (*Cytisus oromediterraneus* and *Juniperus communis*) were dominant at the study site, together with meadows of *Festuca* and other grasses (Martín and Salvador, 1992). In the study area, *L. monticola* is found between 1750 and 2350 m elevation. Mature individuals are approximately 61–90 mm snout-vent length (SVL). Lizards are active from May to October, mating in May and June and producing a single clutch in July (Elvira and Vigil, 1985).

We conducted fieldwork in a 0.3-ha plot (80 × 40 m), which was divided into 32 quadrats of 10 m² each to form a grid. Male lizards were captured by noosing and individually marked with paint marks on the back and remarked when necessary. Censuses were performed each day during May to June from 0800 to 1500 h GMT. The home range of each male was determined by recording its positions on a map with respect to the grid

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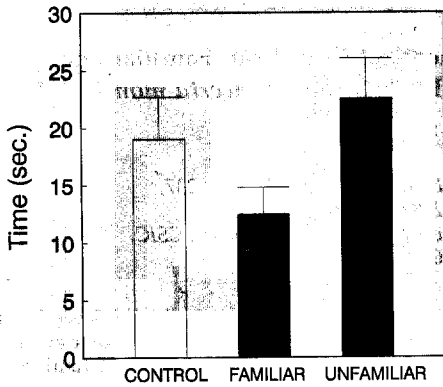


FIG. 1. Time (sec; means \pm 1 SE) spent trying to avoid an area (i.e., attempting to escape from the cage) by male *Lacerta monticola* during a period of 10 min in a clean control cage and in cages with scents from a familiar male or an unfamiliar male.

marks and calculating the area of the convex polygon surrounding the points on the map (Rose, 1982). We used the computer program RANGES V (Larkin and Halkin, 1994) to obtain home ranges and the degree of overlap among males. We considered familiar males those with overlapping home ranges, and unfamiliar males those with nonoverlapping home ranges, and home range centers separated by at least 50 m. We calculated the home range centers with the kernel fix estimator included in the RANGES V computer program, which is the equivalent Gaussian Kernel estimator (Worton, 1989) and is more robust than the simple arithmetic mean.

On 21 June, we captured by noosing in the study plot 20 adult male *L. monticola* for which we knew the relationships (familiar or unfamiliar). Males were weighed (mean \pm SE = 8.1 ± 0.2 g, range = 6–10 g) and measured (SVL: 75 ± 1 mm, range = 67–80 mm). They were individually housed at "El Ventorrillo" Field Station (Navacerrada, Madrid Province) 5 km from the capture site in outdoor plastic cages (60 \times 40 cm) containing sand substrate and rocks for cover. Food (mealworms and crickets) dusted with a multivitamin powder was provided daily and water was provided ad libitum. Males were held in their home cages for at least one week before testing for familiarization with the novel environment. All the animals were healthy during the trials and, at the end of the experiments, were released to their initial sighting locations prior to the last capture.

To begin a trial, we took one individual male lizard from his cage, placed him gently in the middle of a cage previously occupied by either a familiar male, an unfamiliar male, or in an empty clean cage as a control, in a random order of presentation for each test male. We performed 60 trials (20 individuals, each tested for three treatments). In each trial, the donor lizard was drawn out of its home cage a few seconds before the beginning of the trial and returned again at the end. To ensure that the odors of lizards (fecal pellets, femoral secretion, etc.) were present in each test cage, the trials began after at least one week of captivity. After each trial, the cages were cleaned thoroughly with water, and the sand and

rocks were replaced to avoid odor contamination in successive trials. We waited for another week with the donor lizard inside before another test in that cage. All trials were made in outdoor conditions during June and early July in sunny days between 0900–1200 h GMT. Before trials, lizards were allowed to bask for at least two hours in their cages, and no lizard was tested more than once per day.

Experiments were recorded on videotape (Hi-8 format, 25 frames s^{-1}) using a video camera aligned perpendicularly over the center of the terraria. We recorded lizard behavior for 10 min and later calculated from the tapes the time that lizards spent attempting to escape. Time attempting to escape was defined as the time that males were trying to climb the cage walls. Because of the shape of the walls, no lizard was successful in climbing the walls effectively.

Pearson's correlations were calculated between the time spent attempting to escape in each condition and the body size of the experimental males. To examine differences in time attempting to escape among conditions, we used repeated measures one-way ANCOVA controlling for body size (Sokal and Rohlf, 1995). We included in the model the body size as a covariate because it was related with time attempting to escape (see Results). The dependent variable and covariate were log-transformed to meet assumptions of ANCOVA, and we tested for homogeneity of slopes prior to the significance test. Pairwise comparisons of central tendency were assessed using Tukey's honestly significant difference tests.

RESULTS

Male size (SVL) and escape time in the control cages were significantly correlated ($r = 0.51$, $F = 6.31$, $df = 1,18$, $P = 0.02$). However, the correlation between SVL and escape time was not significant in the familiar male cages ($r = 0.52$, $F = 1.21$, $df = 1,18$, $P = 0.28$) or in the unfamiliar male cages ($r = 0.054$, $F = 0.05$, $df = 1,18$, $P = 0.81$). The slopes of these regression lines did not differ significantly ($r = 0.054$, $F = 1.79$, $df = 1,54$, $P = 0.17$).

There were significant differences among conditions in the time attempting to escape ($F = 4.41$, $df = 2,38$, $P = 0.01$; Fig 1). Pairwise comparisons indicated that time attempting to escape was significantly higher in the unfamiliar than in the familiar male cage condition (Tukey's HSD test: $P = 0.01$). Time escaping in the control cage was intermediate (unfamiliar vs. control: $P = 0.16$; familiar vs. control: $P = 0.52$).

DISCUSSION

Ability to discriminate between neighbors and non-neighbors is considered adaptive ("dear enemy" hypothesis; Fisher, 1954) because it minimizes energy expended on aggressive acts and may prevent escalated contests between neighbors (Jaeger, 1981; Glinski and Kerkorian, 1985). The selective forces that have led to the evolution of the dear enemy behavior as an evolutionarily stable strategy must have affected the response of individuals not only when they are residents but also when they are intruders.

An intruding male can become a rival because he could access to sources included in the resident's home range. However, social relationships between neighbors may have been established through repeated

encounters. Thus, the aggressive response of a resident male should be higher toward nonneighbors because social relationships have not been established. Therefore, from the point of view of an intruder male, the potential costs of finding an unfamiliar resident male might be higher than when encountering a familiar resident male. When a male enters an unknown area and detects the scent of an unfamiliar male through chemical cues, he will possibly attempt to avoid this area because the probability of success in an agonistic encounter with the resident male is low. Other studies showed that territorial male desert iguanas, *Dipsosaurus dorsalis*, quickly reacted aggressively to unfamiliar males, whereas neighbors were ignored, and as the distance in the field increased between two males, the likelihood of mutual challenge displays increased and the latency to mutual challenge display decreased (Glinski and Krekorian, 1985). A field study with juvenile *Anolis aeneus* demonstrated that new arrivals were more quickly attacked than were previous residents (Stamps, 1987). Moreover, the aggressive response of male *Podarcis hispanica* toward another male decreased through repeated encounters (López and Martín, 2001), and this rival recognition was based on chemical cues (López and Martín, 2002). The ability of male *L. monticola* to discriminate among neighbors and nonneighbors, and to respond quickly by more avoiding the areas scent-marked by unfamiliar males may reduce costs of aggression (Marler and Moore, 1988, 1989) and play an important role in the organization of their social system.

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