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**Journal of Ethology**

ISSN 0289-0771

Volume 29

Number 3

J Ethol (2011) 29:493-497

DOI 10.1007/s10164-011-0278-9



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## Geographic variation in learning of escape behavior in the little brown skink (*Scincella lateralis*)

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Received: 24 June 2010 / Accepted: 18 March 2011 / Published online: 6 May 2011  
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**Abstract** A previous study showed that little brown skinks, *Scincella lateralis*, from a population in Louisiana, USA were poor at learning to escape to a specific retreat in a series of laboratory trials. The study was repeated on little brown skinks from a population in Oklahoma (650 km from the Louisiana population). The Oklahoma lizards were significantly better at learning to escape to a specific retreat than their Louisiana counterparts. This result demonstrates that geographic variation in the ability to learn an escape behavior exists in this species.

**Keywords** Lizard · Scincidae · Louisiana · Oklahoma · Escape behavior · Refuge

### Introduction

To be successful, every animal species must evolve traits that allow it to escape or avoid its predators. Reptiles are no exception and display a wide variety of morphological, chemical, and behavioral antipredator mechanisms (Greene 1994). Studies of squamate reptiles (lizards and snakes) have shown that the populations of geographically widespread species frequently display differences in the antipredator behaviors (Greene 1994; Burghardt and Schwartz 1999; Downes and Adams 2001; Husak and Rouse 2006; Stuart-Fox et al. 2006; Cooper et al. 2009). Experiments on naïve neonates originating from different populations have shown that geographic variation in antipredator behavior can have a strong heritable component (Burghardt and

Schwartz 1999; Downes and Adams 2001). However, complex behaviors can also be shaped by experience, and animals must be able to modify their antipredator behaviors through learning when environmental conditions change.

Studies of several species of small fishes have documented not only geographic variation in antipredator behaviors, but also geographic variation in the ability to learn certain behaviors (Magurran 1999; Kelley and Magurran 2003). Since squamate reptiles often exhibit geographic variation in antipredator behavior, it is possible that they also exhibit geographic variation in their ability to learn escape behaviors. I was recently presented with an opportunity to test this hypothesis by conducting a study with representatives of two disjunct populations of a small terrestrial lizard, *Scincella lateralis* (Scincidae), commonly known as the little brown skink. In an earlier study (Paulissen 2008), I showed that wild-caught adult little brown skinks from a population in Louisiana, southern USA, were very poor at learning to escape to a specific retreat. Subsequently, I repeated the study on wild-caught little brown skinks from a population in Oklahoma, central USA, 650 km from the Louisiana population. Herein I present the results from the Oklahoma lizards and compare them to the previous results from the Louisiana lizards to document geographic variation in learning ability in this species.

### Materials and methods

#### Study animals and captive maintenance

The little brown skink is a small lizard (snout-to-vent length, SVL, of adults 40–50 mm; mass 1–2 g) that has a large geographic range extending from the center of the continental USA south to near the border with Mexico,

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then east to the Atlantic coast (Conant and Collins 1998). It is commonly found in deciduous forests wherein it flees from predators by running under a pile of leaves, a log, a rock, or other retreat (Brooks 1967; Smith 1997; pers. observ.). The 12 adult little brown skinks (5 males and 7 females) used in the Louisiana study were captured in and near Sam Houston Jones State Park, Calcasieu Parish, LA, USA. The 15 adult little brown skinks (7 males and 8 females) used in the Oklahoma study were captured in and near Sparrowhawk Primitive Area, Cherokee County, OK, USA. The two sites are separated by approximately 650 km. The habitat at both sites was oak-hickory forest with a dense carpet of leaf litter and fallen logs that provided escape retreats for lizards. However, the Oklahoma site was hilly with deep ravines and many rocks large enough for little brown skinks to hide under, whereas the Louisiana site was flat with a substrate of sandy-loamy soil and no rocks. All lizards were collected between May and August (2002 and 2005 for Louisiana, 2008 for Oklahoma). There were no significant differences in the SVL or mass of lizards from the two populations [mean  $\pm$  SE SVL for Louisiana lizards:  $44.8 \pm 0.46$  mm, for Oklahoma lizards:  $43.5 \pm 1.06$  mm,  $t(25) = 0.963$ ,  $P = 0.34$ ; mean  $\pm$  SE mass for Louisiana lizards:  $1.4 \pm 0.042$  g, for Oklahoma lizards:  $1.6 \pm 0.112$  g,  $t(25) = -1.545$ ,  $P = 0.14$ ]. None of the lizards used in this study had prior experience with the test arena before testing.

Lizards were housed individually in  $300 \times 130 \times 70$  mm clear plastic boxes with paper towel flooring and a cardboard retreat made by cutting a toilet paper tube into four equal-sized arcs. Each lizard was fed 8–10 mealworms from a laboratory culture every other day; water was provided in a 30 mm diameter water dish that was filled daily. The boxes were placed in a room with lights on timers set to a 12:12 L:D photoperiod. Each box was equipped with a lamp with a 60 W standard bulb to provide heat during the 12 h “daytime” portion of the photoperiod. Lizards were tested within 1 week of being captured and were released near their original capture sites when testing was completed.

### Experimental design

The methods used are described in detail in Paulissen (2008). Data were collected from lizards placed in a  $900 \times 450 \times 550$  mm high glass-walled test chamber fitted with green poster board around the back and two sides leaving the front glass uncovered so lizards could use objects in the lab room to orient themselves. The substrate was a mix of potting soil and sand; light and heat were provided by a 125 W infrared heat lamp suspended 230 mm above the substrate in the center of the tank and by two 60 W lamps suspended 410 mm above the substrate on

either side of the infrared lamp. Two  $100 \times 100$  mm square cardboard retreats were placed on the substrate; each was halfway between the front and back walls of the test chamber, 120 mm from the side wall and 460 mm from each other. Each retreat was flanked on three sides by 30 mm diameter plastic Petri dish lid “markers” pushed flush into the substrate to prevent the lizards from using them as escape retreats [see Fig. 1 in Paulissen (2008) for a diagram of the test chamber]. I adopted this design to approximate the situation lizards encounter in the field, where there is typically more than one potential retreat available, but the retreats differ in how well they allow a fleeing lizard to escape.

Prior to the first trial, one of the two retreats was designated as the “correct” retreat by a coin-flip. Then a lizard was confined in an opaque plastic tube pushed into the substrate slightly behind the point upon which the infrared lamp was shining directly. The lizard was left within the tube for 90 s to allow it to warm up to a body temperature of  $32\text{--}33^\circ\text{C}$ , which is the body temperature at which *S. lateralis* attains maximum sprint speed (Smith 1997). A trial began when I moved behind the back of the test chamber, then lifted the tube to release the lizard. I then immediately began to tap the base of the lizard’s tail with a cotton swab to simulate a predatory attack. Tapping the base of the tail was used only to induce the lizard to run, not to guide the lizard toward either of the retreats, and the rate of tapping was maintained at approximately 1 tap/s throughout the course of the trial. The lizard could not see my eyes or body during the trials because of the poster board on the back of the chamber, though presumably it could see my hand as I tapped the base of its tail. The lizard always ran when tapped, and I continued to tap the base of its tail until either the lizard escaped under the correct retreat or until 90 s had elapsed. If the lizard ran under the correct retreat, I left it undisturbed for 5 min to facilitate its learning the correct escape behavior. If the lizard ran under the incorrect retreat, I immediately lifted up that retreat and resumed tapping the base of the lizard’s tail so the lizard did not receive positive reinforcement for choosing the incorrect retreat. The incorrect retreat was immediately dropped back into position; it was lifted up again if the lizard ran under it a second time. If the lizard did not escape to the correct retreat within 90 s, I stopped chasing it, caught it by hand, and placed it under the correct retreat after which time the lizard was treated the same way as if it had successfully “escaped” (I chose 90 s as the cutoff because little brown skinks tire and become reluctant to run after this time). After the lizard had been under the correct retreat for 5 min, it was removed from the test chamber and returned to its plastic box where it remained undisturbed until the next trial.

**Table 1** Number of trials (out of eight) that Louisiana and Oklahoma little brown skink lizards performed various behaviors

	Louisiana lizards ( $N = 12$ )	Oklahoma lizards ( $N = 15$ )	$P$ value
Number of trials lizard escaped to correct retreat	$3.3 \pm 2.2$	$5.4 \pm 1.9$	0.019
Number of trials lizard escaped to correct retreat in 35 s	$1.8 \pm 1.9$	$3.3 \pm 2.0$	0.070
Number of trials lizard failed to escape to either retreat	$3.2 \pm 2.0$	$1.5 \pm 1.9$	0.037

Values are mean  $\pm$  standard deviations. The  $P$  value of  $t$  tests to compare means is shown (a statistically significant difference is indicated by  $P < 0.05$ )

I conducted eight trials on each lizard, trials 1–4 on one day and trials 5–8 on a second day 48 h later. Consecutive trials were separated by at least 40 min to allow time for the lizard to recover. Between trials, the retreats and markers were removed and the substrate was turned to disperse odors. For each trial, I recorded whether or not the lizard escaped under the correct retreat, and if it did, how long it took to do so.

### Statistical analyses

For each lizard, I tallied (1) the number of trials (out of 8) that the lizard escaped to the correct retreat; (2) the number of trials it escaped to the correct retreat within 35 s (which is approximately the maximum amount of time I can chase little brown skinks in the field before they escape); and (3) the number of trials that the lizard failed to escape to either of the two retreats. I then computed the means of each of these variables for the Louisiana and Oklahoma populations and compared them using  $t$  tests. Finally I scored each lizard as having learned the escape behavior if it met the learning criterion of escaping to the correct retreat within 35 s in at least two of the trials after the first trial or as having not learned the escape behavior if it did not meet this learning criterion. Differences in the proportion of lizards from Louisiana and Oklahoma that did and did not meet the learning criterion were evaluated by a chi-squared test. Preliminary analyses showed no differences between males and females or between day 1 (trial 1–4) and day 2 (trial 5–8) results from the same population so all results from a single population were pooled. All statistical analyses were conducted using MYSTAT 12 for Windows (SYSTAT 2007).

### Results

Oklahoma little brown skinks escaped to the correct retreat in significantly more trials than Louisiana lizards (Table 1). The Oklahoma lizards also escaped to the correct retreat within 35 s in more trials than the Louisiana lizards, although the difference narrowly failed to reach statistical significance (Table 1). Much of the difference is attributable to Louisiana lizards failing to escape to either retreat

in significantly more trials than Oklahoma lizards (Table 1). A significantly greater proportion of the Oklahoma little brown skinks were scored as having met the learning criterion of escaping to the correct retreat within 35 s in at least two trials after the first trial [80% of Oklahoma lizards, 33% of Louisiana lizards:  $X^2(1) = 6.01$ ,  $P = 0.014$ ]. Taken together, these results demonstrate that Oklahoma little brown skinks are significantly better at learning to escape to a specific retreat than are Louisiana little brown skinks.

### Discussion

Traditionally ethologists had assumed that behaviors exhibited by members of a species from any part of the species' geographic range were representative of the species as a whole. Today abundant evidence has accumulated that this is not the case and that geographic variation in behavior is common (Foster and Endler 1999). Studies on several species of fish have documented that some of the geographic variation in behavior is due to geographic variation in learning ability (Magurran 1999; Kelley and Magurran 2003). By documenting geographic variation in learning of an escape behavior in a lizard, this study expands the list of taxa in which geographic variation in learning ability has been shown and suggests such differences may be found in other taxa as well.

The geographic variation in the ability of members of different populations of little brown skinks to learn a specific escape behavior may be a product of several factors. It is possible that the habitat in which members of each population lived prior to testing was an important influence. The habitats occupied by both the Oklahoma and Louisiana lizards were oak-hickory forests with widespread leaf litter and logs of various sizes, but the Oklahoma habitat also had many rocks large enough for little brown skinks to use as retreats. Regular use of several different types of objects as escape retreats may require Oklahoma lizards to develop greater behavioral flexibility than their Louisiana counterparts, perhaps making Oklahoma lizards more amenable to learning to use a novel escape retreat in laboratory trials. Even if the lizards from the two populations have the same degree of behavioral flexibility, the

Oklahoma lizards may have an advantage owing to their experience gained living in a habitat where rocks are available as retreats. If at least some of the Oklahoma lizards had learned to use rocks as escape retreats prior to being tested in the laboratory, they may perceive cardboard retreats as “rocks” and would therefore escape under them frequently. This would make it easier for the Oklahoma lizards to learn which retreat was the correct one. The Louisiana lizards’ habitat did not offer such experience, perhaps accounting for why Louisiana lizards escaped to cardboard retreats less frequently than Oklahoma lizards. This would make it more difficult for Louisiana lizards to learn to escape to the correct retreat. It should be noted that Louisiana little brown skinks that had been allowed to live in the test chamber for 48 h prior to testing learned to escape to the correct retreat significantly better than inexperienced lizards (Paulissen 2008) confirming that prior experience is very important in determining how well lizards learn escape behaviors.

Alternatively, there may be geographic differences in the cognitive abilities of lizards due to subtle differences in the structure of the parts of the brain involved in learning and memory. Differences in brain anatomy between groups of animals with differing learning abilities have been documented for active versus ambush foraging congeneric species of lizards (Day et al. 1999a) and migratory versus nonmigratory subspecies of sparrows (Pravosudov et al. 2006). Geographically disjunct, genetically isolated populations of conspecifics might also develop differences in brain structure that result in the differences in learning ability documented here.

It is possible that the difference between Oklahoma and Louisiana little brown skinks may not be due to differences in learning ability alone; other geographic differences may influence the results. In a laboratory study, Smith (1997) compared the response of adult little brown skinks from Oklahoma and Louisiana to a model predator moved slowly toward individual lizards confined in a transparent cylinder surrounded by leaf litter cover [the Oklahoma lizards used by Smith (1997) were collected from a population 210 km from those used in the present study; the Louisiana lizards were purchased from a reptile dealer]. He found that most of the Louisiana lizards attempted to flee as the model predator approached whereas most of the Oklahoma lizards remained immobile suggesting a geographic difference in flightiness. If such a difference existed among the lizards I studied, it might have influenced the results. However this seems unlikely; if Louisiana lizards were more flighty, their greater tendency to flee should have caused them to find and escape under retreats more often and more quickly than the Oklahoma lizards. This was not the case: Louisiana lizards failed to escape to either of the two retreats in significantly more trials than

did the Oklahoma lizards. This suggests that geographic differences documented here are not due to differences in flightiness but rather can be attributed to differences in learning ability.

Though as a group Louisiana little brown skinks performed poorly compared to their Oklahoma counterparts, one-third of the Louisiana lizards were scored as having learned the escape behavior. Individual variation in learning ability is common in squamates (Brattstrom 1978; Day et al. 1999b; Stone et al. 2000) and is likely due to differences in prior experience, brain structure, or both among individual animals. Teasing out the relative contributions of external experiential versus internal neural factors in producing individual variation in learning ability will require conducting studies like the one described in this report on inexperienced neonates from different populations exposed to a variety of environmental conditions in a systematic factorial design. Integration of the results of such studies with characterization of the environmental conditions to which animals are exposed in different parts of their geographic range is required before a complete understanding of geographic variation in learning ability can be achieved.

**Acknowledgments** I wish to thank the Department of Biological and Environmental Sciences, McNeese State University (MSU), for providing laboratory space for the Louisiana portion of the study. Funding for the Oklahoma portion of the study was provided by a Faculty Research Committee Grant from Northeastern State University (NSU); laboratory space was provided by the NSU Bioscience Research Facility. I wish to thank R. Murray, T. Hibbs, and T. Whychell for their assistance in catching or caring for lizards and T. Hibbs for providing constructive comments on a draft of this manuscript. The captive maintenance procedures and research protocols were approved by the MSU Institutional Animal Care and Use Committee and by the NSU Animal Welfare Committee. Little brown skinks were collected under the authority of Scientific Collecting Permits granted by the Louisiana Department of Wildlife and Fisheries (LNHP-05-004; LNHP-02-018) and by the Oklahoma Department of Wildlife Conservation (no. 4368).

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