Olfactory recognition of predators by nocturnal lizards: safety outweighs thermal benefits

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Many prey species are faced with multiple predators that differ in the degree of danger posed. The threat-sensitive predator avoidance hypothesis predicts that prey should assess the degree of threat posed by different predators and match their behavior according to current levels of risk. To test this prediction, we compared the behavioral responses of nocturnal velvet geckos, Oedura lesueurii, to chemicals from 2 snakes that pose different threats: the dangerous broad-headed snake Hoplocephalus bungaroides that eats geckos and the less dangerous small-eyed snake Cryptophis nigrescens that eats skinks (i.e., does not consume geckos). We also tested whether predator avoidance by prey was modulated by thermal costs associated with retreat-site selection. In both the presence and absence of thermal costs, velvet geckos avoided crevices scented by both dangerous and less dangerous snake species. When given the choice between a crevice scented by a broad-headed snake and a crevice scented by a small-eyed snake, most geckos avoided either retreat site. These results suggest that velvet geckos treat both snake predators as equally dangerous. To further explore these results, we quantified patterns of retreat-site selection by free-living velvet geckos on 2 sandstone plateaux. As in the laboratory, velvet geckos avoided thermally suitable rocks previously used by both snake species. Hence, a gecko’s choice of retreat site is influenced by the presence of snake chemicals but is independent of thermal costs or the level of danger posed by the predator. To minimize their risk of predation, geckos may use a simple rule of thumb: “all snakes are dangerous.”

Key words: chemical communication, predation risk, predator, reptile, snake.

PRedation is a powerful force that has shaped the behavior of prey. In response to predator cues, many prey species modify their behavior to reduce their risk of encountering predators or to increase their chances of surviving such encounters (Endler 1986; Lima 1998). Most experimental work on this topic has focused on responses of prey to single predator species (Lima and Dill 1990). However, in nature, most prey species occur with multiple predators (Andrewartha 1961; Sih et al. 1998). Because prey can show generalized or species-specific responses to single predators, their responses to multiple predators cannot always be predicted from experiments involving a single predator species (Sih et al. 1998; McIntosh and Peckarsky 1999). For example, in the presence of green sunfish (Lepomis cyanellus), male water striders (Aquarius remigis) shifted their microhabitat use to riffles and pool edges, but in the presence of fishing spiders (Dolomedes fimbriatus), the water striders avoided pool edges. In the presence of both predators, water striders used a generalized response and reduced the time spent active or searching for mates, thereby minimizing their risk of predation (Krupa and Sih 1998). Knowledge of prey responses to the relative salience of cues from multiple predators, therefore, may clarify predator–prey dynamics (Sih et al. 1998; McIntosh and Peckarsky 1999).

Many aquatic and terrestrial animals use chemical cues to detect predators (Weldon 1990; Chivers and Smith 1998; Kats and Dill 1998). Typical responses by prey to predator chemical cues include avoiding predator-scented areas, immobility, reducing activity, and hiding in refuges (Wisenden 2000; Apfelbach et al. 2005). Although these behaviors can decrease the risk of predation, they also represent a trade-off for foraging, reproduction, or courtship (Lima and Dill 1990; Lima 1998). If anti-predator behaviors impose high costs, natural selection should favor individuals that not only can identify potential predators but also can assess the degree of risk posed by different predators at different times (Helfman 1989). The threat-sensitive predator avoidance hypothesis posits that prey should assess the degree of threat posed by predators and match their behavior according to current levels of risk (Helfman 1989; Helfman and Winkelman 1997). Threat-sensitive responses to predator chemical cues have been demonstrated in invertebrates (Persons and Rypstra 2001), amphibians (Mirza et al. 2006), lizards (Thoen et al. 1986), and aquatic organisms (Chivers and Mirza 2001). For example, many fish species respond to chemical alarm cues released from injured conspecifics (Wisenden 2000), and several species show stronger anti-predator behaviors to higher concentrations of conspecific alarm chemicals (Ferrari et al. 2005; Zhao and Chivers 2005).

Lizards are good model organisms for studying threat-sensitive responses to predator chemical cues. Several lizard species exhibit anti-predator behaviors in response to chemicals from dangerous lizard-eating snakes but do not respond to chemicals from harmless snakes (Thoen et al. 1986; Dial et al. 1989; Downes and Shine 1998). However, other lizard species show similar behavioral responses to chemicals of dangerous and nondangerous snakes (Van Damme and Quick 2001; Amo et al. 2004; Webb et al. 2008a). Theory suggests that whether or not lizards display threat-sensitive behaviors to predator chemical cues will depend on the costs and benefits associated with those behaviors (Helfman 1989; Lima and Dill 1990). One potential cost of avoiding predator cues is the use of thermally suboptimal retreat sites (Downes 2001; Martin and Lopez 1999). Unlike diurnal lizards which bask, many nocturnal reptiles spend the entire day within the confines of a retreat site (Webb and Shine 1998b; Kearney and Predavec 2000). In these animals, choice of retreat site can affect body temperature, which in turn influences behavior (prey capture, locomotion) and physiological processes (digestion and growth), in ways that can reduce fitness (Huey 1991; Martin and Lopez 1999; Downes 2001).
In this article, we investigate retreat-site selection by the nocturnal velvet gecko *Oedura lesueurii*, a species that is syntopic with a dangerous predator that feeds on geckos (broad-headed snake *Hyplothelphus bungaroides*) and a less dangerous predator (*small-eyed snake Cryptophis nigrescens*) that feeds on skinks (Shine 1984; Webb and Shine 1998a). In the laboratory, velvet geckos display generalized anti-predator behaviors (freezing, tail waving, tail vibration) in the presence of chemical cues from both snake species (Webb et al. 2009a). These results support the multi-predator hypothesis, which predicts that when behaviors are not costly, prey can show generalized responses to different predators (Blumstein 2006). However, in nature, avoiding snake-scented retreat sites can involve thermal costs, and in these circumstances, geckos may modify their anti-predator behavior depending on the degree of risk posed by the predator (Downes and Shine 1998). To investigate this question, we offered velvet geckos the choice between 2 structurally identical shelters: one unscented and one containing chemical cues from either a dangerous or a less dangerous snake. Next, we gave geckos a choice between cold unscented sites and hot snake-scented retreat sites to test whether geckos would use hot sites in the presence of chemicals from the less dangerous predator. To determine whether geckos selectively avoid chemicals of the more dangerous predator when thermal costs were similar, we gave the lizards a choice between 2 hot retreat sites, one scented by a broad-headed snake and one scented by a small-eyed snake. To explore whether our laboratory findings were biologically relevant (Ylonen and Wolff 1999), we used data obtained from 2 independent field studies to test our predictions.

**MATERIALS AND METHODS**

### The prey and the predators

The velvet gecko inhabits sandstone rock outcrops in eastern Australia and is sympatric with the small-eyed snake throughout much of its geographic range (Cogger 2000). The broad-headed snake occurs within 200 km of Sydney, NSW, and is sympatric with both small-eyed snakes and velvet geckos (Cogger 2000). Small-eyed snakes feed mostly on sleeping skinks (Shine 1984), whereas juvenile broad-headed snakes feed on velvet geckos (70% of the numerical diet) and other lizards (Webb and Shine 1998a). In Morton National Park, all 3 species are syntopic, and during the cooler months, both predators and prey thermoregulate inside crevices or underneath rocks on sandstone escarpments (Webb and Shine 1998c).

### Collection and maintenance of study animals

We collected 51 adult velvet geckos (26 females: mean snout-vent length \(\text{SVL} = 63\) mm, range 58–68 mm; 25 males: mean \(\text{SVL} = 58\) mm, range 48–65 mm), 13 broad-headed snakes (8 females: mean \(\text{SVL} = 439\) mm, range 296–670 mm; 5 males: mean \(\text{SVL} = 465\) mm, range 262–640 mm), and 10 small-eyed snakes (3 females: mean \(\text{SVL} = 407\) mm, range 386–435 mm; 7 males: mean \(\text{SVL} = 529\) mm, range 375–635 mm) from Morton National Park. Each animal was placed in a numbered box (31 × 3 × 10 cm high, with clear lids and ventilation holes) with paper substrates, a plastic shelter, and a water dish. All snakes were fed skinks prior to behavioral trials. At the end of the study, we released the geckos and snakes at their original sites of capture.

### Retreat-site selection in the laboratory

We offered velvet geckos the choice between 2 structurally identical crevices that differed with respect to chemical and thermal cues. In the first experiment, we offered 14 geckos the choice between a cold retreat site scented by a small-eyed snake and a cold unscented control. A previous study found that velvet geckos did not avoid small-eyed snake-scented refuges (Downes and Shine 1998), so we predicted equal usage of both sites. In the second experiment, we offered geckos the choice between a cold unscented crevice and a hot crevice scented by either a broad-headed snake (\(n = 16\)) or a small-eyed snake (\(n = 16\)). In the third experiment, we offered geckos (\(n = 13\)) the choice between a hot retreat site scented by a small-eyed snake and a hot retreat site scented by a broad-headed snake. Each gecko was tested once, except for 2 males and 6 females that were used in 2 different experiments.

Ten small-eyed snakes and 13 broad-headed snakes were used as scent donors. To create a snake-scented crevice, we placed 2 clean concrete paving stones (230 × 180 × 40 mm) on top of each other, with a horizontal crevice between them (matched to suit snake body size) inside the donor snake’s cage for 24 h. Once the snakes had crawled into their crevices, the automated heating racks were either switched off (experiment 1) or left on (experiments 2 and 3). Thermal regimes of hot and cold crevices were recorded by placing miniature data loggers (Thermochron iButtons, Dallas, TX) under pavers. Temperatures in the warm snake-scented crevice were 24.5°C at dusk when the trials commenced, and thereafter, they slowly cooled to room temperature (17.0°C). In the morning, a 40-watt light globe positioned above the hot retreat site switched on, creating a hot retreat site which reached 29°C at midday. By contrast, temperatures under the cold rock ranged from 17.0°C (0900 h) to 21.4°C (1200 h). Thermal profiles of hot retreat sites were similar to those of natural sun-exposed rocks at our field sites during spring (Webb and Shine 1998c).

All experiments were carried out inside a controlled temperature room maintained at 18°C (range 17–19°C). The lights in the room were programmed to match the natural photoperiod, and they switched on and off gradually to simulate dawn and dusk. Test arenas consisted of 8 white plastic tubs (60 × 40 × 40 cm high, with ventilation holes) fitted with clear perspex lids. For each trial, we placed 2 structurally identical retreat sites at either end of the test arenas. Each retreat site consisted of 2 concrete paving stones (230 × 180 × 40 mm thick) placed one on top of the other, with a 6-mm crevice between them. New paving stones were used in all trials to avoid the possibility of scent contamination from previous experiments. In experiments involving temperature, a 40-watt light bulb was suspended above each retreat site, but only the bulb above the hot site switched on the next morning (at 0930 h), whereas the bulb above the cold site did not. Approximately 30 min before the experiments commenced, we removed the snake-scented retreat site from the snake’s cage, created a 6-mm crevice between the stones (using four steel nuts), and placed the retreat site at one end of the test arena. For all experiments, we randomly assigned retreat sites to either end of the test arenas to avoid problems that might arise if geckos consistently crawled in the same direction after entering the test arenas.
Ten minutes before dusk, we placed each gecko inside a plastic holding box (20 × 10 × 10 cm high, with ventilation holes) with a 3-cm plastic pipe (30 mm diameter) attached to one corner. We placed a plastic endcap over the plastic pipe to prevent geckos from leaving the holding box prematurely. At dusk, we attached the holding box to a 30-mm entry hole positioned in the middle of the test arena. The following morning, we held a mirror beside each retreat site and noted each gecko’s final choice of diurnal retreat site. In trials involving temperature, we noted the gecko’s choice of retreat site at 0900 h, before the light bulb switched on, and at midday, after the hot snake-scented rock had heated up. After each trial, all plastic tubes and lids were scrubbed with detergent and hot water, soaked for 20 min in bleach solution, thoroughly rinsed with freshwater, and dried with clean paper toweling before they were reused.

Retreat-site selection by geckos in the field

In nature, velvet geckos select rocks similar in size to those used by broad-headed snakes and small-eyed snakes (Shine et al. 1998; Webb and Shine 1998c). At our study sites, thermally suitable rocks are a limiting resource (Pringle et al. 2003); hence, velvet geckos may have to choose between using cold rocks or hot rocks scented by predatory snakes. If velvet geckos show threat-sensitive responses to snake chemicals, they may use rocks scented by small-eyed snakes but should avoid rocks scented by broad-headed snakes (Downes and Shine 1998). Because substrate-deposited snake chemicals can be detected by squamates for several weeks (Crews and Garstka 1982), we can test this prediction by comparing gecko usage of rocks previously used by small-eyed snakes and broad-headed snakes.

We analyzed data on rock usage by geckos and syntopic snake predators from field studies on 2 plateaux in Morton National Park, 160 km south of Sydney, NSW. The first study was carried out at 3 study sites on the western escarpment of a sandstone plateau (YN) of 400 m elevation (Webb et al. 2009b). From August 1992 to November 1995 one of us (J.K.W.) quantified rock usage by geckos and snakes by turning all rocks in exposed locations at least once each fortnight. Any snakes or velvet geckos sheltering underneath rocks were captured, measured, and permanently marked. Each rock used by a reptile was measured and given a unique number (with a paint pen), and its location was recorded on maps of the study sites. From 1996 onward, we surveyed all sites at least once each year, usually during spring. We turned all rocks and hand captured any snakes and processed them as described above and recorded whether any geckos were sheltering under rocks with snakes. The second study was carried out on a second plateau (MG) 5 km east of the YN plateau. Each month, from May 2007 to March 2009 inclusive, one of us (D.A.P.) turned all rocks on 75 experimental plots (each ~100 m² in area). All reptiles found under rocks were captured and processed as described above, and rocks were numbered, measured, and their locations recorded with a GPS. The vegetation on this plateau is similar to the YN plateau, but surrounding trees on 25% of the experimental plots had been removed as part of a manipulated landscape experiment.

RESULTS

Retreat-site selection in the laboratory

When offered the choice between 2 cold retreat sites (one unscented and one snake scented), all velvet geckos avoided the retreat site containing the scent of a small-eyed snake (G = 11.50, P < 0.001). When offered the choice between a hot snake-scented crevice and a cold unscented crevice, most velvet geckos selected the cold unscented crevice as their diurnal retreat site. Geckos avoided the hot crevice when it contained chemical cues from the ambush foraging broad-headed snake Hoplocephalus bungaroides (open bars) and the active foraging small-eyed snake Cryptophis nigrescens (filled bars).

Figure 1

When offered the choice between a hot snake-scented crevice and a cold unscented crevice, most velvet geckos selected the cold unscented crevice as their diurnal retreat site. Geckos avoided the hot crevice when it contained chemical cues from the ambush foraging broad-headed snake Hoplocephalus bungaroides (open bars) and the active foraging small-eyed snake Cryptophis nigrescens (filled bars).

Retreat-site selection in the field

During our field studies, we identified 168 and 70 rocks used by predatory snakes on the YN plateau and MG plateau, respectively. To see if velvet geckos avoid rocks containing chemical cues of the dangerous snake species but not the less dangerous one (as suggested by Downes and Shine 1998, but contrary to our laboratory results above), we calculated the proportion of rocks used by broad-headed snakes and small-eyed snakes that were subsequently used by velvet geckos. On both plateaux, geckos did not selectively avoid rocks previously used by broad-headed snakes compared with those previously used by small-eyed snakes (YN: 39 vs. 24%, G = 3.28, P = 0.07; MG: 60 vs. 36%, G = 2.68, P = 0.10).

Because substrate-deposited snake chemicals are long lasting (Crews and Garstka 1982), we expect that geckos will avoid using rocks recently vacated by snakes (i.e., that contain detectable snake scent). If geckos perceive both snake species as equally dangerous, they should avoid rocks scented by broad-headed snakes and small-eyed snakes for similar periods of time. To test this prediction, we compared the time period between vacation of the rock by a snake and its subsequent occupancy by a gecko. The duration between usage of a rock
by a snake and subsequent usage by a velvet gecko did not differ between snake species ($F_{1,52} = 0.13, P = 0.72$) nor between plateaux ($F_{1,52} = 0.04, P = 0.84$; interaction $F_{1,52} = 1.42, P = 0.24$). On both plateaux, velvet geckos did not use most “snake” rocks until several months after the snakes had vacated the rocks, irrespective of the degree of threat posed by the snake (Figure 3).

### DISCUSSION

In our laboratory trials, adult velvet geckos avoided retreat sites scented by 2 species of predatory snake. Importantly, the lizard’s avoidance of predator odors was independent of thermal costs or the degree of danger posed by the predator. Thus, lizards forfeited thermoregulation in the presence of chemicals from both the dangerous broad-headed snake and the less dangerous small-eyed snake. Although inconsistent with earlier work on the same study system (Downes and Shine 1998), the current result is consistent with a previous study on European common lizards (Lacerta vivipara), which found that juveniles avoided basking in the presence of chemical cues from both sympatric and allopatric snake predators (Van Damme et al. 1995). In the absence of other cues, velvet geckos avoided rocks scented by both snake species, suggesting that they show general rather than species-specific responses to snake odors (Webb et al. 2009a). Such generalized responses to multiple predators can be maintained, even if some predators are rarely encountered, provided that the costs of responding to each predator are equivalent (Blumstein 2006).

In lizards, avoidance of predator scent may enhance survival, but it can also impose longer term fitness consequences (Downes 2001). For example, garden skinks (Lampropholis guichenoti) raised in outdoor enclosures with snake chemicals grew more slowly than skinks raised in areas without snake chemicals (Downes 2001). Our field data show that velvet geckos avoided thermally suitable rocks that had recently been used by small-eyed snakes or broad-headed snakes. Geckos occasionally used rocks containing chemical cues from snakes but only several months after the snakes had vacated the rocks. Hence, gecko avoidance of thermoregulatory sites could impose longer term fitness costs, but further studies are necessary to investigate this possibility. Intriguingly, during 17 years of field work, we found geckos sharing a rock with a snake predator on 9 occasions (4 with small-eyed snakes and 5 with broad-headed snakes). Such cases could reflect resource limitation or the ability of geckos to exploit smaller crevices than most snakes. The complex 3-dimensional crevice structure beneath rocks (Croak et al. 2008) means that a gecko sharing a rock with a snake may not always detect the snake or its chemicals. Most of these cases involved hatchling geckos (6 of 9 records), suggesting that learning could also be involved in chemical avoidance of potential predators (Brown 2003). Future studies involving predator-naive hatchling geckos would be worthwhile.

Our laboratory and field results did not support the prediction that prey should respond to predator cues according to the degree of threat posed by the predator (Helfman 1989). However, one key assumption of the threat-sensitive hypothesis is that predators differ in the degree of threat they pose to prey, which may not be true for our gecko–snake system. Although small-eyed snakes feed mostly on sleeping skinks, they occasionally consume other lizards and snakes (Shine 1984; Webb et al. 2009b). Indeed, some adult small-eyed snakes will attack and consume juvenile velvet geckos but only in situations where the geckos cannot climb to escape from snakes (Webb JK and Shine R, unpublished data). Hence, small-eyed snakes pose a threat to velvet geckos, even though in nature these snakes rarely capture geckos. The cautionary tale here is that we cannot always predict the degree of threat posed by a predator based on the predator’s diet (as is often done in studies involving lizards and snakes; Head et al. 2002; Stapley 2003). Clearly, successful detection and avoidance of predator cues may result in a situation where the predator rarely poses a significant danger to the “prey” under field conditions; but this does not remove selection for continued recognition and
Avoidance of that predator (Endler 1986; Blumstein 2006). This issue is important for behavioral studies involving perceptions of risk; failure to observe predation on a prey species does not necessarily mean that the prey does not perceive the predator as dangerous (Lima and Dill 1990; Speakman and Rydell 2000).

Behavioral responses of prey to multiple predators are not always predictable from the results of experiments involving a single predator species (Sih et al. 1998). We found a similar phenomenon; when given the choice between 2 "risky" retreat sites (one scented by a broad-headed snake and one scented by a small-eyed snake), most velvet geckos abandoned retreat sites altogether. These geckos spent the night and the following morning clinging to the arena walls, a behavior that under field conditions likely would increase their vulnerability to mammalian or avian predators. In contrast, other studies have reported that lizards prefer to hide in a refuge containing predator chemicals rather than remain exposed (Head et al. 2002; Lloyd et al. 2009). For example, in tests involving a snake-scented and an unscented shelter, mountain log skinks Pseudemoia entrecasteauxii avoided the snake-scented shelters (irrespective of the risk posed by the snake). However, when the lizards had to choose between 2 shelters treated with odors from different snake species, the lizards always used a shelter and avoided refuges containing chemical cues from the more dangerous snake species (Stapley 2003).

In conclusion, our study suggests that 1) even when the costs of anti-predator behavior are significant, prey may respond similarly to cues from predators that differ in the level of danger they pose (i.e., even a small risk may outweigh such costs); and 2) field data on predator diets may be misleading in terms of risk prediction because even if a predator consumes that prey type rarely (or not at all) in the field, it may nonetheless try to consume that prey if given a chance to do so. That is, dietary composition of predators in the field will depend on several factors, such as the prey’s ability to detect and avoid predators, prey escape ability, and the ability of predators to capture prey. Even when predators differ in their degree of dangerousness, generalized rather than species-specific responses may evolve in some prey taxa (Blumstein 2006). For example, freshwater snails that were raised with molluscivorous and non-molluscivorous sunfish species responded to all sunfish species with a generalized response—reducing growth and producing relatively rotund shells (Langerhans and DeWitt 2002). When information from other sources is lacking, potential prey may benefit by treating chemical cues from potential predators as if they do indeed represent a threat.

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