Chemical cues from both dangerous and nondangerous snakes elicit antipredator behaviours from a nocturnal lizard

Jonathan K. Webb [a,*], Wei Guo Du [a,b,1], David A. Pike [a], Richard Shine [a]

[a] School of Biological Sciences, University of Sydney
[b] Department of Environmental Sciences, School of Life Sciences, Hangzhou Normal College

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Many prey species use chemical cues to detect predators. According to the threat sensitivity hypothesis, prey should match the intensity of their antipredator behaviour to the degree of threat posed by the predator. Several species of lizards display antipredator behaviours in the presence of snake chemical cues, but how species specific are these responses? In Australia, most snake species eat lizards, and are therefore potentially dangerous. Hence, we predicted that lizards should display generalized rather than species-specific antipredator behaviours. To test this prediction, we quantified the behavioural responses of velvet geckos, Oedura lesueurii, to chemical cues from five species of elapid snakes that are syntopic with velvet geckos but differ in their degree of danger. These five snake species included two nocturnal ambush foragers that eat geckos (broad-headed snake Hoplocephalus bungaroides, and death adder, Acanthophis antarcticus), two active foragers that eat skinks (but rarely eat geckos) and that differ in their activity times (nocturnal small-eyed snake, Cryptophis nigrescens, and diurnal whip snake, Demansia psammophis), and a nocturnal nonthreatening species that feeds entirely on blind snakes (bandy-bandy, Vermicella annulata). Geckos showed similar antisnake behaviours (tail waving, tail vibration), and a similar intensity of responses (reducing activity, freezing), to chemical cues from all five snake species, even though the snakes differed in their degree of danger and foraging modes. Our results suggest that velvet geckos display generalized antipredator responses to chemicals from elapid snakes, rather than responding in a graded fashion depending upon the degree of threat posed by a particular snake species.
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Predation poses a major risk for most organisms, resulting in the evolution of a complex array of antipredator tactics that involves behavioural modifications (e.g. responses to predator approach), physiology (e.g. toxins), morphology (e.g. defensive spines) and performance capacities (e.g. locomotor ability; Greene 1988; Stanford 2002; Bollache et al. 2006; Boyer et al. 2006). Evolutionary theory predicts that such modifications entail costs as well as benefits, and the degree of elaboration of antipredator traits in a specific situation will depend upon the balance between these two (Lima & Dill 1990). Because responding to predators can involve costs (e.g. loss of foraging or reproductive opportunities), there should be strong selection on prey not only to identify predators, but also to modify antipredator responses according to the level of risk posed by the predator (Helfman 1989; Lima & Bednekoff 1999). Threat-sensitive responses to predation risk occur in a wide variety of taxa, including invertebrates, fishes, reptiles, amphibians, mammals and birds (e.g. Jędrzejewski et al. 1993; Mathis & Vincent 2000; Chivers et al. 2001; Persons & Rypstra 2001; Amo et al. 2004; Palleroni et al. 2005).

Many animals use chemical cues to identify predators and to assess the risk of predation (Kats & Dill 1998), and some species show threat-sensitive responses to chemical cues. For example, goldfish, Carassius auratus, show stronger antipredator responses as the concentration of predator odours increases (Zhao et al. 2006). Similarly, naïve Arctic charr, Salvelinus alpinus, only avoided odours of predatory brown trout, Salmo trutta, when the predators were fed on charr (Hirvonen et al. 2000). Despite numerous studies on aquatic organisms, few studies have investigated whether terrestrial vertebrates also show threat-sensitive responses to predator chemicals. Lizards are ideal model organisms for examining this question because they have a highly developed chemosensory system and can identify predators using chemical cues (Schwenk 1993a, b; Cooper 1994). Several lizard species also display stereotyped antipredator behaviours, including tail waving, foot shaking and freezing in the presence of snake chemicals (Thoen et al. 1986; Dial & Schwenk 1996; Downes & Shine 1998). However, it is less

* Correspondence: J. K. Webb, School of Biological Sciences A08, University of Sydney, NSW 2006, Australia.
E-mail address: jwebb@bio.usyd.edu.au (J.K. Webb).
[1] W. G. Du is at the Department of Environmental Sciences, School of Life Sciences, Hangzhou Normal College, 310036 Hangzhou, Zhejiang, People's Republic of China.

clear whether lizards generally show threat-sensitive responses to predator chemicals. Some species display antipredator behaviours in response to chemicals from dangerous snakes (i.e. those that eat lizards), but not in response to chemicals from nondangerous species (Thoen et al. 1986; Dial & Schwenk 1996; Downes & Shine 1998; Van Damme & Quick 2001; Bealor & Krekorian 2002). By contrast, Amo et al. (2004) reported that wall lizards, Podarcis muralis, did not respond differently to chemicals of saurophagous and nonsaurophagous snakes that posed different risks and that had different foraging modes. Similarly, Stapley (2003) found that mountain log skinks, Pseudemoia entrecasteauxii, offered the choice between an unscented and a snake-scented refuge avoided the snake-scented refuge, irrespective of the degree of threat posed by the snake.

This variation in the responses of lizards to predator chemical cues makes it difficult to determine the causal factors responsible for the maintenance of species-specific antipredator behaviours. In the absence of predators, costly antipredator behaviours can be lost, and prey may respond only to syntopic predators (e.g. Berger et al. 2001). However, when traits are not costly, prey may show strong antipredator responses to absent or rare predators (Byers 1997; Coss 1999). The 'multipredator hypothesis' (Blumstein et al. 2004) predicts that when antipredator behaviours are genetically linked, or function in multiple contexts, prey species that occur with multiple predators may evolve specific behaviours to reduce predation risk in response to each predator, but their expression is not predicted to vary independently. In other words, the presence of a single predator can maintain antipredator behaviours for other predators that are rarely encountered (Blumstein et al. 2006). For example, although we might expect prey to show differential responses to predators that hunt in different ways (e.g. ambush versus active foraging) or that use different habitats, prey may show generalized antipredator behaviours (e.g. reducing activity levels) that lower their risk of predation in the presence of multiple predators (Sih et al. 1998). Provided that costs associated with antipredator behaviours are low, species-specific responses to different predators may be difficult to evolve and maintain (Coss 1999; Blumstein 2006). Mathematical models for the evolution of phenotypic plasticity under different environmental conditions predict the same result: specific responses to different enemies are difficult to evolve when prey encounter multiple predators, but each predator is only encountered rarely (Via & Lande 1985; van Tienderen 1991).

The Australian velvet gecko, Oedura lesueurii, is an ideal model for investigating whether lizards show threat-sensitive antipredator behaviours to odours of snake predators that differ in their dangerousness. Previous studies have shown that velvet geckos display predator-specific behaviours to chemicals from a predatory snake species that consumes geckos (the broad-headed snake, Hoplocephalus bungaroides) but do not respond to odours from a snake that feeds mostly on skinks (the small-eyed snake, Cryptophis nigrescens, Downes & Shine 1998). When velvet geckos encounter broad-headed snake chemicals, they reverse or run away from the scent, and some individuals raise their tails into the air and slowly move them from left to right (Downes & Shine 1998). This tail-waving display occurs in several other species of geckos in response to live snakes or snake chemicals (Congdon et al. 1974; Dial 1978). Experiments involving both predators and prey have shown that the tail-waving display of geckos serves to direct the predator's attack to the tail, which is then autotomized, allowing the lizard to escape (Congdon et al. 1974; Daniels et al. 1986). Thus, tail-waving displays have probably evolved to promote prey survival during encounters with predators.

The species-specific response by velvet geckos to a single snake predator, the broad-headed snake, is unexpected, because velvet geckos are syntopic with several species of elapid snakes that prey on geckos (Cogger 2000). Some of these snakes (e.g. whip snake, Demansia psammophis, small-eyed snake) occur on the same rock outcrops, and use the same shelter sites (rocks) as velvet geckos, so that velvet geckos are likely to have encountered multiple snake predators over evolutionary time. Unless avoiding snake scent or tail waving involves significant energetic costs, a gecko that responds to a nondangerous snake is unlikely to be disadvantaged. More importantly, because most elapid snakes that velvet geckos encounter are potentially lethal, the optimal response by a gecko should be to treat all snakes as potentially dangerous (e.g. Amo et al. 2006), because the benefits of doing so far outweigh the costs involved with making a wrong decision (i.e. death of the gecko).

The multipredator hypothesis (Blumstein 2006; Blumstein et al. 2006) predicts that velvet geckos should display general antipredator behaviours to the scent of syntopic elapid snakes, irrespective of how frequently they are encountered, or the degree of threat they pose. In contrast, the threat-sensitive paradigm predicts that velvet geckos should ignore the scent of nonthreatening species, but should respond intensely (and perhaps even in different ways to different predator species) to snakes that pose a higher degree of threat (Helfman 1989; Downes & Shine 1998). To test between these alternative predictions, we videotaped the behaviour of adult velvet geckos in the presence of chemical cues from five species of elapid snakes that differ both in their attack strategy (ambush versus active foraging) and in their degree of danger to geckos.

METHODS

The Prey and the Predators

Velvet geckos occur in sandstone rock outcrops throughout coastal regions of New South Wales, Australia. These small (to ca. 65 mm snout–vent length) nocturnal lizards thermoregulate underneath sandstone rocks or inside crevices during the day (Schlesinger & Shine 1994; Webb 2006), and emerge at dusk to forage for invertebrate prey in leaf litter (Cogger 2000). Thus, geckos may encounter several species of ambush and actively foraging snakes that hunt in leaf litter or on rock outcrops, or that use rocks as shelter sites.

We used five species of elapid snakes that are all sympatric with velvet geckos. The bandy-bandy, Vermicella annulata, feeds entirely upon blind snakes (genus Ramphotyphlops) and hence poses no threat to geckos (Shine 1980a; Keogh & Smith 1996). Small-eyed snakes shelter under stones on rock outcrops (Webb et al. 2003), and feed mostly on sleeping skinks (Shine 1984). This species will consume velvet geckos in the laboratory, but rarely does so in the wild, apparently because both species forage at night, and alert geckos can easily escape these slow-moving predators (J.K. Webb & R. Shine, unpublished data). Whip snakes are diurnal active searchers that feed mostly on skinks, and occasionally consume velvet geckos (Shine 1980b). Whip snakes not only occur on the same rock outcrops as velvet geckos, but also shelter under rocks, therefore posing a risk to geckos. Death adders, Acanthophis antarcticus, ambush lizards (including geckos) and small mammals from camouflaged sites in leaf litter (Shine 1980c). Finally, broad-headed snakes feed on lizards, including velvet geckos, and shelter under rocks similar in size to those used by geckos; these ambush foragers clearly pose a major risk to the lizards (Webb & Shine 1998). With the exception of the broad-headed snake, each of these snake species has a large geographical range that overlaps with the velvet gecko's geographical range (Cogger 2000). Broad-headed snakes have a small geographical range, which overlaps entirely

with that of velvet geckos (Cogger 2000). In summary, the bandy-bandy poses no threat whatsoever to geckos, the small-eyed snake is a potential predator that rarely consumes geckos, the whip snake and death adder occasionally eat velvet geckos, and the broad-headed snake is a major predator of velvet geckos.

Origin and Maintenance of Study Animals

We collected 55 adult velvet geckos (30 males, 25 females), eight adult small-eyed snakes (five males, three females) and eight adult broad-headed snakes (four males, four females) by carefully turning over loose surface rocks on exposed rock outcrops adjacent to Morton National Park during late winter and spring (August–October) 2003. Death adders, whip snakes and bandy-bandy snakes also occur in Morton National Park, but for logistical reasons, we collected these snakes from Ku-ring-gai Chase National Park. Four death adders (three adults, one juvenile), four adult whip snakes (two males, two females) and eight adult bandy-bandy snakes (five males, three females) were captured from West Head, Ku-ring-gai Chase National Park, during spring and summer in 2003 and 2004. Upon capture, each animal was placed in a numbered cloth bag and its location was recorded using a hand-held GPS. We housed the geckos and snakes at the University of Sydney in separate temperature-controlled rooms maintained at 18 °C with lighting set to match the natural photoperiod. We housed lizards individually in ventilated plastic cages (22 × 13 cm and 7 cm high), with a paper substrate, a plastic shelter and a water dish. We also housed snakes individually, but in larger ventilated plastic cages (31 × 22 cm and 10 cm high) with paper substrates, a plastic shelter and a water dish. Snake and lizard cages were placed on automated heating racks (1000–1600 hours) that provided a thermal gradient within each cage (18–32 °C) so that the reptiles could thermoregulate. We fed geckos crickets dusted with a calcium supplement twice weekly, but we did not feed snakes during their brief stay in captivity. We kept geckos in captivity for several months and snakes for up to 2 weeks. At the end of the study, we released all animals to their exact site of capture in the field. All animals were collected under permits issued by the New South Wales Parks and Wildlife Service, and laboratory housing and experimental procedures were approved by the University of Sydney Animal Ethics Committee.

Antipredator Behaviour of Velvet Geckos

We conducted behavioural trials in a controlled temperature room (20 °C) illuminated by a single red 25 W globe. For each trial, we used forceps to place a black piece of cardboard (110 × 100 mm) on the bottom of a clean test arena (110 × 100 mm and 90 mm high, N = 9, with tight-fitting lids and small holes in the sides for ventilation). The test arena was large enough to permit geckos to reverse or run away from snake scent, but was small enough to allow us to quantify tongue-flicks on the videotape. We thoroughly washed test enclosures with bleach, rinsed them with fresh water, and dried them with paper towelling before each trial. Snake-scented cards were used for experimental trials, unscented cards were used for control trials, and cards sprayed with cologne (Autumn Leaf, Arion Perfume & Beauty Inc., Schertz, TX, U.S.A.) were used in pungency control trials. To obtain snake-scented cards, we placed a fresh card inside the plastic shelter of the donor snake predator for 2 days. For each trial, we used a new card to ensure that the scent from previous trials did not influence the behaviour of the new test gecko. To begin a trial, we gently placed a gecko on the card in the test arena, closed the lid, and turned on a video recorder. We then exited the room and left the lizards undisturbed for 10 min. We carried out trials after dusk, and the order of trials was randomized.

We used a between-subjects design, whereby each gecko (N = 55) was tested once in a single trial, with eight geckos randomly allocated to each scent treatment, except in the whip snake trials (N = 7). We used this design, rather than a repeated measures design, to minimize stress to geckos, and to avoid carryover effects that could occur if a gecko's behaviour in multiple trials was influenced by its previous exposure to snake scent. To avoid pseudoreplication, we used each broad-headed snake, small-eyed snake and bandy-bandy once as a scent donor. In the death adder and whip snake trials, only four snakes were available as scent donors, and each snake donated scent cues in two different trials.

We viewed videotapes on a large television screen, and scored the frequency (1–6) and duration (7–10) of the following antipredator behaviours after allowing the gecko to acclimate to the test chamber for 10 s. (1) Tongue-extrusion: the lizard extrudes and retracts its tongue, either onto the substrate or into the air. (2) Tail wave: the gecko raises its entire tail above the horizontal, and holds it stationary or slowly moves it from side to side. (3) Tail vibration: the tail or tail tip is wiggled from side to side, but is not raised above the horizontal plane. (4) Reverse: the lizard reverses either slowly or rapidly. (5) Lunge: the gecko throws its body forward and then stops. (6) Run: the gecko suddenly runs forward and attempts to escape from the test arena. (7) Freezing: the gecko remains motionless, either on the floor, walls or roof of the test chamber. (8) Slow motion: the lizard crawls slowly with stalking movements, sometimes accompanied by jerky or waving movements of the forelimbs, with the ventrum in contact with or close to the substrate. (9) Crawl: the gecko crawls slowly while pressing its ventral surface against the substrate. (10) Walk: the gecko displays continuous forward movement, with the ventrum raised, typically observed in unrestrained geckos.

The above behaviours and locomotor patterns are identical to those described by Downes & Shine (1998), except that we included the behaviour 'stand up' in our category 'walk'. Previously, Downes & Shine (1998) described the behaviour 'stand up' as follows: 'the lizard stands in an upright position against the wall of the test box and attempts to adhere to this vertical wall with its forelegs'. We also observed geckos standing upright and pushing their snouts against the clear arena wall, but the animals had no trouble adhering to the vertical wall, and they displayed 'stand up' behaviours while walking on the arena floor and vertical walls. Hence, we included the behaviour 'stand up' in our category 'walk'. Our general methodology followed Downes & Shine (1998), except that our test chambers were smaller, the snake scent was on cardboard rather than on sandstone, and we used up to eight individuals (rather than two) of each predator species as scent donors.

Statistical Analyses

Prior to statistical analyses, we checked that the data were normally distributed and that variances were homogeneous, and we transformed data where necessary. We square-root transformed the number of tongue-extrusions and arcsine transformed the proportion of time spent walking or freezing prior to statistical analysis. Because the behaviours freezing, crawling and slow motion have been interpreted as antibroad-headed snake locomotor behaviours, we summed these behaviours and analysed the single variable 'antisnake locomotor behaviour' after arcsine transformation (Downes & Adams 2001). We used contingency table analysis to compare the proportion of geckos in each treatment that performed tail displays (tail waving or tail vibration) or that reversed or ran during trials, and analysis of variance (ANOVA) to compare the number of tongue-flicks and the proportion of time spent walking or freezing among scent trials.

RESULTS

Velvet geckos displayed four striking antipredator behaviours (tail vibration, tail waving, reversing and running) in the presence of snake-scented cards, but not in trials involving unscented or cologne-scented cards. The proportion of geckos that displayed one or more of these behaviours was higher in snake scent treatments than in control treatments ($\chi^2_6 = 21.39$, $P = 0.002$), but was similar among snake treatments (Fig. 1a). In trials involving snake scent, the most commonly observed behaviours were tail vibration (44% of geckos) and tail waving (36%), whereas fewer geckos reversed (15%) or ran (15%) during trials (Fig. 1b). Few geckos lunged in our trials, and this behaviour was always followed by running and, occasionally, tail vibration. The proportion of geckos that vibrated or waved their tails did not differ between the five snake species ($\chi^2_4 = 5.09$, $P = 0.28$), and ranged from 25 to 75% for individuals exposed to death adder and small-eyed snake scent, respectively (Fig. 1b). By contrast, a higher proportion of geckos ran or reversed in trials involving small-eyed snake (50%) and whip snake scent (57%) than in other scent treatments ($\chi^2_4 = 18.27$, $P = 0.006$; Fig. 1b).

Geckos extruded their tongues most frequently in trials involving snake scent and least frequently in trials involving cologne (ANOVA: $F_{6,54} = 5.52$, $P = 0.0002$; Fig. 2). However, when we excluded control trials from the analysis, the number of tongue-extrusions performed by geckos did not differ between snake species (ANOVA: $F_{4,38} = 1.69$, $P = 0.17$; Fig. 2). The proportion of time that geckos spent walking differed between treatments ($F_{6,54} = 2.51$, $P = 0.03$), and was highest in trials involving unscented cards, and lowest in trials involving snake scent (Fig. 3a). However, when we excluded control trials from the analysis, the proportion of time that geckos spent walking did not differ between the five snake species ($F_{6,54} = 1.56$, $P = 0.21$).
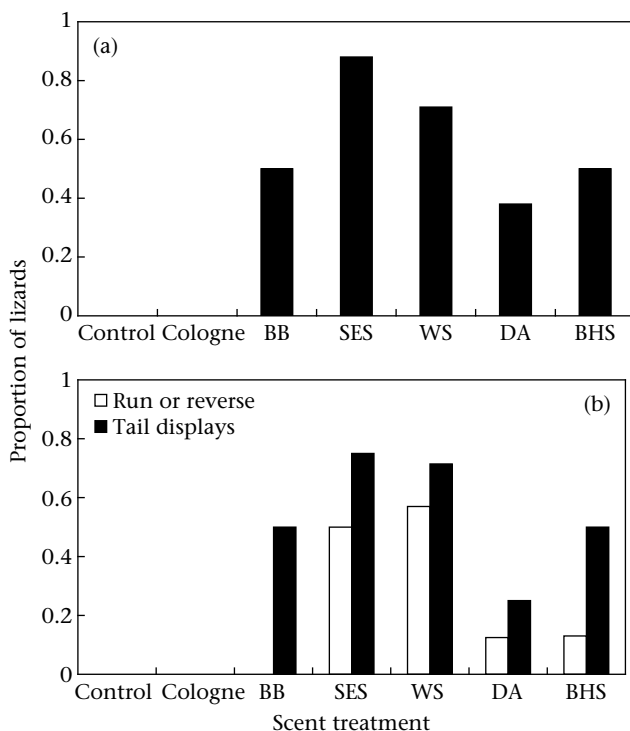

Figure 1. The proportion of velvet geckos that (a) performed one or more antipredator behaviours (tail displays, running, reversing) and (b) performed either tail displays (black bars) or ran or reversed (open bars) when exposed to unscented cards (controls), cologne-scented cards (pungency controls) or cards containing chemical cues from elapid snakes. The degree of danger posed by snakes varied from the harmless bandy-bandy (BB), moderately dangerous small-eyed snake (SES) and whip snake (WS), to the highly dangerous death adder (DA) and broad-headed snake (BHS).
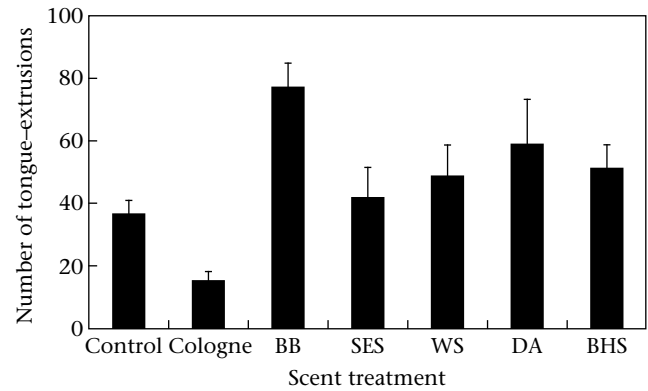

Figure 2. The mean number of tongue-extrusions made by velvet geckos in 10 min during exposure to unscented cards (controls), cologne-scented cards (pungency controls) and cards containing chemical cues from nondangerous and dangerous elapid snakes (as listed in Fig. 1). Error bars denote SEs.

Velvet geckos spent more time engaged in antisnake locomotion (freezing, slow motion and crawling) in trials involving snake scent than in control trials (ANOVA: $F_{6,54} = 2.51$, $P = 0.03$). However, when control trials were excluded from the analysis, this behaviour did not differ between snake species (ANOVA: $F_{4,38} = 1.51$, $P = 0.22$; Fig. 3b).

DISCUSSION

Velvet geckos showed similar behavioural responses, and similar intensities of response, to five snake predators that differed in their foraging modes and degree of dangerousness. In the presence of snake chemicals, velvet geckos reduced their activity, spent more time using antisnake locomotion (freezing, crawling or moving very slowly), and/or performed striking tail displays, or reversed or ran during trials (Figs 1a, 3). In contrast, in the presence of unscented or cologne-scented cards, velvet geckos did not reduce their activity (Fig. 3), nor did they perform tail displays, or reversals or running (Fig. 1a). The lack of antipredator behaviours in control or pungency control trials shows that geckos detected chemicals from elapid snakes, and displayed behaviours that are thought to be adaptive responses to predators (see below). However, we found no evidence that geckos distinguish between the harmless bandy-bandy and the more dangerous snake species. This intriguing result was unexpected, but there are several possible explanations, which we discuss below. In general, our results suggest that velvet geckos show generalized antipredator responses to elapid snakes, rather than a species-specific response to the most dangerous predator (the broad-headed snake).

In response to snake chemical cues, velvet geckos spent less time walking, and increased the time spent freezing or crawling slowly. Reduced activity is a common response to predator odours or alarm cues from injured conspecifics; presumably because it lowers the risk of detection, immobility is elicited by predator cues in a diverse array of taxa (Lima & Dill 1990; Kats & Dill 1998). The most striking antipredator behaviours performed by velvet geckos were tail waving and tail vibration displays (Fig. 1b). Similar behaviours have been observed in other lizard species in response to snake chemicals or live snakes (Thoen et al. 1986; Van Damme & Castilla 1996; Labra & Niemeyer 2004). In geckos, tail displays direct predatory attacks towards the lizard's tail, which is then autotomized, allowing the lizard to escape (Congdon et al. 1974; Daniels et al. 1986). Tail displays occurred when velvet geckos were motionless, focusing the predator's attention on the moving tail rather than the body (Daniels et al. 1986). Previously, Downes & Shine (1998) reported that velvet geckos performed tail vibration displays only in the presence of broad-headed snake chemicals, but not in the presence of
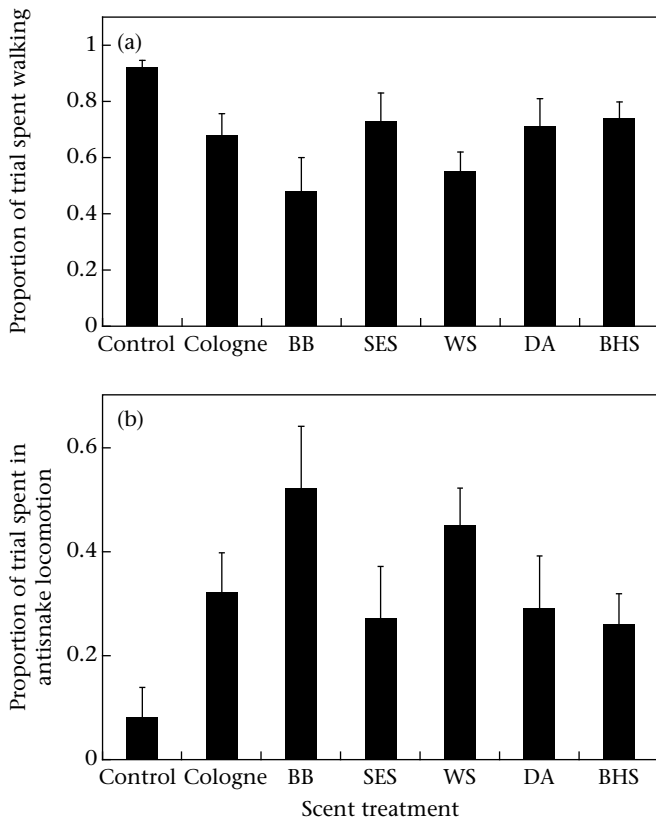
Figure 3. The proportion of time that velvet geckos spent (a) walking or (b) engaging in antisnake locomotion (freezing, crawling slowly, slow motion) during exposure to unscented cards (controls), cologne-scented cards (pungency controls) or cards containing chemical cues from nondangerous and dangerous elapid snakes (as listed in Fig. 1). Error bars denote SEs.

small-eyed snake chemicals. By contrast, we found that geckos performed tail displays in response to chemicals from all five species of elapid snakes we tested, including the small-eyed snake. We suggest that tail displays are a general antipredator behaviour, rather than a species-specific response to a single predator.

Previous studies on velvet geckos have interpreted high rates of tongue flicking as a species-specific response to the broad-headed snake (Downes & Shine 1998; Downes & Adams 2001). For example, Downes & Shine (1998) reported that velvet geckos performed twice as many tongue-extrusions to broad-headed snake chemicals as to small-eyed snake chemicals. In contrast, we found no difference in the mean number of tongue-extrusions performed by velvet geckos to any of the snake species, including the small-eyed snake (Fig. 1b). Our study is not unique in this respect; some lizard species perform more tongue-flicks in the presence of predatory snake chemicals (Thoen et al. 1986; Van Damme & Quick 2001), whereas other lizard species do not (Cooper 1990; Mori & Hasegawa 1999; Labra & Niemeyer 2004).

Unlike most snakes and lizards, geckos have a highly developed nasal olfactory system (Stebbins 1948; Rehorek et al. 2000) and can use nasal inhalation to deliver airborne chemicals to the nasal epithelium (Schwenk 1993a, b; Dial & Schwenk 1996). Some geckos can use nasal inhalation to detect and discriminate between chemicals of different snake predators, without relying on tongue flicking (Dial et al. 1989; Dial & Schwenk 1996; Cooper 1998). For example, Dial & Schwenk (1996) presented snake chemicals to the gecko Coleonyx brevis, and found that 79% of trials in which geckos performed tail displays did not involve prior tongue flicking. In our trials, several geckos responded strongly to snake scent but rarely

tongue-flicked. For example, an adult female gecko that vibrated and waved its tail and ran in the presence of small-eyed snake scent only extruded its tongue 17 times during the 10 min trial. Hence, in the absence of other behavioural data, tongue-flick rates may provide little indication of a gecko's ability to discriminate between chemicals of different snake predators (Dial et al. 1989; Dial & Schwenk 1996; Cooper 1998).

Tongue-extrusion rates of velvet geckos were lower in trials involving cologne than in control trials or trials involving snake chemicals (Fig. 2). Dial & Schwenk (1996) reported that the gecko *C. brevis* labial-licked but did not tongue-flick in trials involving cologne, which they interpreted as evidence that cologne was an irritant that suppressed tongue flicking. We did not discriminate between labial-licks and tongue-extrusions, but we agree that undiluted cologne may not be a suitable pungency control (Cooper et al. 2003). Future studies could use diluted cologne (Cooper et al. 2003) or a more biologically relevant odour (e.g. herbivorous marsupial) as a pungency control.

Overall, we found no evidence that geckos distinguished between the bandy-bandy and the more dangerous snake species. Similarly, geckos did not show graded responses according to the degree of risk posed by the snake species. These results do not support the threat sensitivity hypothesis (Helfman 1989), but there are two caveats. First, the small size of our test enclosure may have precluded some antipredator behaviours (e.g. running away, avoiding the scent). Future studies using larger, more realistic enclosures, in which geckos are given the choice between unscented versus snake-scented retreat sites (e.g. Downes & Shine 1998), would provide a stronger test of the threat sensitivity hypothesis. Encouragingly, our preliminary studies using larger test arenas (60×40 cm and 40 cm high) support the results presented here. For example, when given the choice between an unscented crevice and a crevice scented by a small-eyed snake, velvet geckos avoided the small-eyed snake-scented retreat site. Thus, the responses of geckos to snake scent in our test chambers may be indicative of their responses in natural settings. A second factor that we could not control was that snakes could have deposited varying amounts of chemicals on the cardboard. Thus, some geckos could have been tested with scent-rich cards, and others with cards containing little or no scent. Such variation in cue intensity might explain why not all geckos responded to snake-scented cards (but see below). However, despite methodological differences, our results are very similar to those of previously published studies. For example, in this study, 50% of geckos performed antipredator behaviours in the presence of broad-headed snake chemicals, similar to the figure (33–53%) reported by Downes & Adams (2001). None the less, snakes of different sizes, or with different diets or foraging modes, could deposit different amounts of chemicals in the environment. If velvet geckos use chemical concentration to assess predation risk (as do some fish: Zhao et al. 2006), their antipredator behaviours might depend on the concentration of snake chemicals. Future studies involving lizards could improve our design by developing a better technique to obtain snake chemicals (e.g. using hexane to remove skin lipids from the snake's skin), and by examining the responses of geckos to different odour concentrations, or to chemicals from snakes fed different diets.

Despite these minor ambiguities, our study provided no evidence that geckos discriminated between dangerous and non-dangerous snakes. The simplest explanation is that velvet geckos respond to a chemical cue (probably a skin lipid) that is common to all elapid snakes. This is particularly likely in Australia, where most lizard-eating snakes belong to a single phylogenetic lineage (the Elapidae) that presumably shares many attributes of chemical cues through evolutionary conservatism (Keogh 1998). Provided that the appropriate responses to different snake predators are similar, then

from the prey's perspective, different predators may function as a single enemy (Van Buskirk 2001). Why, then, did some geckos not perform tail displays in response to snake chemicals? First, if geckos respond to odour concentration (of the same cue), then geckos may have received different odour concentrations (as discussed above). Second, some individuals may use alternative escape tactics. For example, during encounters with a large harmless snake, 44% of individuals of the gecko *Coleonyx variegatus* ran away without raising their tails (Johnson & Brodie 1974). Third, velvet geckos may use multiple sensory modalities to assess current predation risk (like many other prey species: Bouwma & Hazlett 2001; Smith & Belk 2001). The lipid-based chemical cues that snakes deposit on the substrate are detectable for several days after the snake has left the area (Ford 1986) and, hence, are unreliable indicators of a snake's current location. Visual cues, or volatile airborne chemicals emanating from nearby snakes, may be more reliable indicators of a predator's current location and, thus, predation risk (Dial et al. 1989; Dial & Schwenk 1996). Studies on fish show that many species do not show innate recognition of predator chemicals, but require learning to identify predators (Chivers & Smith 1994). It is possible that geckos learn to discriminate between snakes via conditioning with chemical cues paired with visual cues or after unsuccessful predatory attacks (Chivers & Smith 1994; Darwish et al. 2005). Finally, an alternative interpretation for the velvet gecko's lack of predator species discrimination is that gene flow has prevented adaptive evolution (e.g. Storfer & Sih 1998). This last hypothesis is difficult to test, but geographical comparisons of gecko behaviour could shed light on this issue.

Mathematical models for the evolution of phenotypic plasticity suggest that specific responses to different enemies are difficult to evolve when prey encounter multiple predators, but each predator is only encountered rarely (Via & Lande 1985; van Tienderen 1991). In support of these models, empirical studies on rock squirrels suggest that species-specific responses evolve only when prey co-occur with few predators (Coss 1999; Owings et al. 2001). For example, the California ground squirrel, *Spermophilus beecheyi*, is syntopic with just two species of snake, the venomous rattlesnake, *Crotalus viridis*, and the nonvenomous gopher snake, *Pituophis melanoleucus*, and can distinguish between them. By contrast, the rock squirrel, *S. variegates*, co-occurs with at least five rattlesnake species and the gopher snake, and does not discriminate between them (Owings et al. 2001). Rates of encounter between prey and predators are also important. For example, California ground squirrels from a population that frequently encounters snakes discriminate between rattlesnakes and gopher snakes, whereas squirrels from a population that rarely encounter snakes do not (Owings et al. 2001).

The multipredator hypothesis posits that when antipredator traits function in multiple contexts, the presence of a single predator can maintain antipredator behaviours for other predators that are rarely encountered (Blumstein 2006; Blumstein et al. 2006). The gecko–snake predator–prey system fulfils these requirements. In many lizards, including velvet geckos, tail displays function in both social and antipredator contexts (Bustard 1965; Thoen et al. 1986; Cooper 2001; Kelehear & Webb 2006). Tail displays are also effective against a wide range of visually oriented predators (Dial 1978; Daniels et al. 1986; Mori 1990). Three of the snake species we tested use visual cues to orient feeding strikes (personal observation), so it is not surprising that geckos perform tail displays to scents of different snake species. At our study sites in Morton National Park, velvet geckos are syntopic with nine species of elapid snakes (Cogger 2000). However, based on our long-term (1992–2008) mark–recapture study of snakes in this system, geckos are most likely to encounter small-eyed snakes and broad-headed snakes (Webb & Shine 2008). Encounters between velvet geckos

and snakes probably occur infrequently, even on rock outcrops where snakes are common. Juvenile velvet geckos have high survival during their first 6 months of life, and move about relatively little during this time (Webb 2006). Overall, our results are consistent with the idea that the number of snake predators that a lizard encounters will influence whether or not a particular prey species displays species-specific responses to snake predators.

Why, then, have previous studies concluded that lizards respond in a graded and species-specific manner to scent cues from different species of predatory snakes (Thoen et al. 1986; Cooper 1990; Downes & Shine 1998; Van Damme & Quick 2001; Stapley 2003)? Part of the answer may lie in experimental design; all of these previous studies used only one or two individual snakes to provide scent stimuli, so that results can be influenced by problems associated with pseudoreplication (Hurlbert 1984). This problem is widespread in behavioural studies involving chemical cues (Ramirez et al. 2000), and hence may be of general significance. For example, prey may respond differently to chemical cues from the same predator species depending on the predator's size, sex, dietary history, hunger levels or the methods used to obtain chemical cues (e.g. Mirza & Chivers 2001; Bell et al. 2006). A study that lacks replication of scent donors could therefore compare prey responses to a scent-free individual of predator species A versus a scent-rich individual of predator species B, or any combination thereof. Any resulting differences in response caused by stimulus intensity would thus be misinterpreted as a species effect rather than a consequence of variation among individual predators within each species. We do not doubt that some lizard species can use chemical cues to discriminate between predator species, but we predict that such cases are likely to be restricted to systems involving prey that are syntopic with two or three snake predators.

Ultimately, whether or not a species shows species-specific responses will depend on the costs and benefits involved with antipredator tactics (Lima & Dill 1990; Lima 1998). In geckos, a lizard that briefly wiggles its tail or freezes in response to a non-dangerous snake is unlikely to incur substantial energetic costs (Johnson & Brodie 1974; Van Damme & Castilla 1996). From the lizard's perspective, the potential 'cost' of failing to respond to a potential predator's presence (high risk of death) will exceed the energetic 'cost' of a brief behavioural response (running away from a nondangerous predator). Clearly, some situations may reverse this inequality. For example, if a nondangerous predator species is encountered frequently, the 'costs' of avoiding it are higher. The same might be true if sudden flight away from an individual's usual home range incurs substantial fitness decrements through intraspecific or interspecific interactions. In such a situation, discriminating dangerous from nondangerous predators may enhance lizard fitness. Similarly, if alternative predator species are best responded to in very different ways (e.g. crypsis versus flight) then species-specific, rather than generic, responses may evolve. Whether these conditions are satisfied will presumably influence the relative fitness accruing to generalized versus species-specific antipredator responses in lizards.

Acknowledgments

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