



Risk assessment by grasshopper mice (*Onychomys* spp.) feeding on neurotoxic prey (*Centruroides* spp.)

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Predators should benefit from assessing the risks posed by prey that differ in their dangerousness. Assessment might come at any phase of the predatory sequence: search/recognition, pursuit/attack and handling/subjugation. Grasshopper mice are voracious carnivores in North American deserts, feeding almost exclusively on arthropods. In certain regions, grasshopper mice co-occur with extremely neurotoxic bark scorpions, whose sting can prove lethal to vertebrates. We presented three different prey items to wild-caught grasshopper mice at several field sites in the southwestern U.S. The three items, listed in increasing order of dangerousness, were: laboratory crickets, *Acheta domesticus*, having few or no defences; two sister species of *Vaejovis* spp., a non-neurotoxic genus of scorpion; and two sister species of toxic *Centruroides*. Grasshopper mice made no distinctions among the prey in either the recognition or pursuit phases of the encounter, attacking crickets and both genera of scorpions with little hesitation. There were, however, significant differences in how the mice handled the three different prey types, with *Centruroides* requiring significantly more effort to subdue. The difficulties that mice had in dispatching *Centruroides* were not related to the neurotoxic components of these scorpions' venoms, to which the mice are resistant. Instead, the difficulties appeared to result from other constituents of *Centruroides*' venom that cause intense, short-term pain.

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Adversaries engaged in intraspecific conflict should assess their opponent's strengths and weaknesses and modify their behaviour accordingly (e.g. Parker & Rubenstein 1981). This 'assessment strategy' also applies to predator-prey interactions, especially in the context of predator harassment (Swaisgood et al. 1999a). Maternal California ground squirrels, *Spermophilus beecheyi*, for example, use the rattling sounds that they provoke in rattlesnakes to assess both the snake's body size and body temperature; in defence of their pups, the females more aggressively harass smaller and cooler and hence less dangerous rattlesnakes (Swaisgood et al. 1999b, 2003).

Decision making by predators has received less attention (Lima 2002), although they, too, should benefit from assessment, especially when dealing with potentially

dangerous prey (Brodie & Brodie 1999; Williams et al. 2003). An appraisal of the costs and benefits of attacking might occur at any point during a predatory sequence, which historically has been divided into three phases: detection/recognition, pursuit/attack and handling/subjugation (Holling 1966; Vermeij 1982). Assessment of prey dangerousness might determine whether the predator attacks, how it attacks, and how it handles the prey following the initial attack. Southern toads, *Bufo terrestris*, for example, quickly learn to associate the sight and sound of honeybees, *Apis mellifera*, with the bee's painful sting, and ignore the bees after just a few encounters (Brower & Brower 1962, 1965). The spider-eating jumping spider, *Portia labiata*, modifies not whether but how it attacks *Scytodes pallidus*, a spider that spits a sticky gum it uses in turn to hunt jumping spiders (Jackson et al. 2002). *Portia* normally stalks *Scytodes* slowly and stealthily from behind; however, when a *Scytodes* female is carrying her egg sac in her mouth, and thus cannot spit effectively, *Portia* attacks directly and without hesitation (Jackson et al. 2002). For black-backed orioles, *Icterus galbula*, feeding on monarch butterflies, *Danaus plexippus*, assessment

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occurs during handling. The orioles do not appear to possess physiological adaptations that render the monarchs' cardenolide toxins ineffective; rather, the birds simply bite the wings and thorax of a captured butterfly, assess (taste) the level of toxins it contains, and reject those with high concentrations of cardenolides (Fink & Brower 1981; Brower & Calvert 1985).

Grasshopper mice provide an excellent system for studying assessment strategies in a predator that must, at least occasionally, deal with dangerous prey. The genus contains three species of carnivorous mice distributed throughout the deserts, shrub steppes and prairies of western North America (Riddle & Honeycutt 1990). Grasshopper mice are aggressive predators that feed primarily on arthropods, but their diet occasionally includes other small vertebrates (Egoscue 1960; Horner et al. 1965; Flake 1973; Hansen 1975). Some of the arthropods that grasshopper mice routinely consume have noxious defences, including the mists of irritating quinones sprayed by stink beetles, *Eleodes longicollis* (Eisner & Meinwald 1966) and the powerful spine-tipped legs used like saw blades by lubber grasshoppers, *Brachystola magna* (Whitman et al. 1986a). Scorpions lash out with their stinger-tipped tails, attempting to inject painful venom (Horner et al. 1965). Grasshopper mice have been reported to modify their attack behaviour to counteract each specific defence mechanism as, for example, by grasping and biting the head of a stink beetle while pushing the quinone-producing posterior end of the beetle into the ground (Eisner & Meinwald 1966; Cyr 1972; Langley 1981a). Similarly, reports indicate that these mice disable the dangerous legs of lubber grasshoppers by grasping and biting the appendages until they break (Whitman et al. 1986a), and they avoid the stings of scorpions by pinning and biting their tails (Eisner & Meinwald 1966; Cyr 1972; Langley 1981a). Collectively, these reports suggest that grasshopper mice assess prey prior to attack and match their attack strategy to prey-specific defence mechanisms.

Paradoxically, other studies suggest that grasshopper mice do not assess prey dangerousness before an assault, but instead inflexibly and unhesitatingly attack any small, moving object (Langley 1981b, 1983a, 1983b, 1989, 1991; Langley & Knapp 1982). Langley (1989), for example, showed that grasshopper mice attacked animated models of both crickets and scorpions indiscriminately, even when the mice had previous experience feeding on live crickets and scorpions. Likewise, grasshopper mice aversively conditioned to house crickets, *Acheta domesticus*, typically reject a cricket as prey only after it has been attacked and killed by the mouse (Langley 1981b; Langley & Knapp 1982). According to Langley (1991), such immalleable attack behaviour might be adaptive for a small carnivore inhabiting resource-poor environments such as deserts.

Contradictions in the literature regarding whether grasshopper mice adaptively adjust their predatory behaviour to match the defences of their prey may be a product of two factors. First, few studies have clearly distinguished among the different phases of a predatory sequence: search, attack and subdue. Second, few studies have examined the interactions of grasshopper mice with prey capable of inflicting 'severe and predictable' consequences

(sensu Brodie & Brodie 1999). All of the noxious prey used to date are species with biochemical or mechanical defences capable of causing only short-term discomfort (e.g. the quinone sprays of *Eleodes*). Even the scorpions used in previous investigations have been relatively benign, threatening pain but nonlethal injury to small mammals (e.g. *Hadrurus* spp.: Horner et al. 1965; Cyr 1972; Langley 1981a; *Vaejovis* spp.: Langley 1989). Perhaps such low-risk contexts do not demand precapture assessments of prey dangerousness. Bark scorpions, including the Arizona bark scorpion, *C. exilicauda*, and the striped bark scorpion, *C. vittatus*, may be good models for examining the adaptations used by grasshopper mice to mediate their interactions with dangerous prey. Of the various species of scorpion available to grasshopper mice throughout their distribution in the southwestern U.S., only bark scorpions produce vertebrate-specific neurotoxins lethal to a wide range of vertebrates, including humans, laboratory mice, chickens and fish (Russell & Madon 1984; Simard et al. 1992; LoVecchio & McBride 2003). Bark scorpions are extremely abundant, and at some sites constitute more than 90% of the scorpions active on the desert surface at night (Brown et al. 2002; A. H. Rowe & M. P. Rowe, unpublished data). Thus, encounters between the mice and bark scorpions are likely.

This study investigated the predator-prey relationship between grasshopper mice and bark scorpions. Feeding experiments were used to determine whether grasshopper mice prey on these highly toxic scorpions, and if so, what behavioural and/or physiological adaptations the mice use to mediate the interaction during each phase of the predatory sequence. Grasshopper mice may simply recognize and avoid *Centruroides*. Alternatively, grasshopper mice might modify their attack behaviour to minimize their risk of being stung. If, however, grasshopper mice cannot easily distinguish between toxic and nontoxic species of scorpions, and if being stung is unavoidable, we predict the mice to have evolved physiological mechanisms for detoxifying *Centruroides*' neurotoxins.

METHODS

Study Sites and Animals

Three genera of prey were selected to represent different degrees of dangerousness: two sister species of scorpions in the neurotoxic genus *Centruroides*, two sister species of scorpions in the nontoxic genus *Vaejovis* and laboratory crickets, *Acheta domesticus*, a prey lacking any formidable defences. Feeding trials were conducted using grasshopper mice from three different geographical locations sympatric with both *Centruroides* spp. and *Vaejovis* spp. Specimens of the Arizona bark scorpion, the Arizona stripetail scorpion, *V. spinigerus*, and southern grasshopper mouse, *O. torridus*, were collected from two locations in Arizona: the first study site was located in the Beaver Dam Mountains of Mohave County in northwestern Arizona, U.S.A. ($N = 7$ *O. torridus* trapped in July 1999); the second was at the Santa Rita Experimental Range, in the Santa Rita Mountains of Pima County, in south-central Arizona ($N = 22$ *O. torridus*

trapped in the summers of 1999, 2000 and 2002). Specimens of the striped bark scorpion, the lesser stripetail scorpion, *V. coahuilae*, and Mearns' grasshopper mouse, *O. arenicola*, were collected from a third site in the Organ Mountains of Doña Ana County, in southern New Mexico, U.S.A. ($N = 9$ *O. arenicola* trapped in August 1999).

Mice were captured using Sherman live traps baited with dry cat food. Traps were placed approximately 15–20 m apart, adjacent to either small mammal burrows or clumps of vegetation. Traps were set just before sunset and checked the following morning before sunrise. Captured grasshopper mice were sexed, weighed, measured and categorized as either juvenile or adult based on their dorsal pelage colour. Juvenile grasshopper mice have a grey coat until approximately 3 months of age, when their pelage turns cinnamon (Horner & Taylor 1968). Each mouse was housed in a standard-sized mouse cage with bedding. Cages were maintained in ice chests inside a temporary shelter, and a max/min thermometer was used to monitor the ambient temperature inside the chests. Water was provided ad libitum, but food was withheld before the feeding trials. Mean \pm SE weights for the 38 grasshopper mice used in this study were: 28.8 ± 1.49 g for adult ($N = 16$) and 22.6 ± 1.30 g for juvenile ($N = 13$) *O. torridus*; 23.7 ± 0.56 g for adult ($N = 6$) and 20.5 ± 0.93 g for juvenile ($N = 3$) *O. arenicola*.

Scorpions were collected the evening before feeding trials, from the same habitats where grasshopper mice were trapped. Collections were limited to adult specimens of either *Centruroides* spp. or *Vaejovis* spp. Scorpions were collected using UV illumination, usually between the hours of 2100 and 2400 hours. Scorpions received substrate and water before the feeding trials. Surplus specimens not consumed during the experiments were returned to the laboratory for morphometric and venom analyses. Based on these specimens, the mean \pm SE weights for the two genera of scorpions used in the feeding trials were 0.522 ± 0.011 g for *Centruroides* spp. ($N = 271$) and 0.506 ± 0.031 g for *Vaejovis* spp. ($N = 33$).

We obtained adult house crickets from local commercial sources for the feeding trials (mean \pm SE weight = 0.317 ± 0.29 g, $N = 40$).

Ethical note

Trapping was conducted under permits from the Arizona Game & Fish Department (permit numbers: 1999-SP853327, 2000-SP910791 and 2002-SP705800) and the New Mexico Department of Game & Fish (permit number: 1999-3034). Our protocol was approved by the Institutional Animal Care and Use Committee at North Carolina State University (number 99063-0). Pilot tests using *C. exilicauda* and *C. vittatus* showed that grasshopper mice suffered none of the systemic neurotoxic effects shown by diverse species of vertebrates (including laboratory mice) when envenomated by this genus of scorpions. With the exception of two voucher specimens collected from each of the three geographical locations in 1999, mice were maintained in captivity less than 18 h and were released unharmed at their site of capture during the early evening hours on the same day that they were trapped.

Apparatus and Procedure

Feeding trials were conducted in a Plexiglas terrarium ($8 \times 38 \times 26$ cm, $w \times l \times h$). The bottom was filled to a height of 4–5 cm with odourless, natural clay kitty litter as a substratum. Feeding trials were filmed using a digital video camcorder (Canon XL1 mini DV) equipped with a 3X wide-angle zoom lens (Canon XL 3.4–10.2 mm).

Feeding trials were conducted just after sunrise, using only ambient light, on the same day that mice were captured. A single mouse was placed in the terrarium before its feeding trial and given 5 min to habituate. Each mouse was tested once with each of the three prey types. Consecutive prey items were not introduced until the previous trial was completed. The order of introduction for prey items was systematically counterbalanced to avoid trial effects. A prey item was introduced while the mouse was either grooming or exploring, so that the mouse would not observe the addition of the test species. Mice discovered the prey item within seconds of its introduction, usually by attraction to movement of the prey. A feeding trial on a specific prey item was terminated when one of the following conditions was met: (1) the mouse incapacitated and consumed the prey, (2) the mouse incapacitated the prey but failed to consume it within 10 min or (3) the mouse attacked but failed to incapacitate the prey, then retreated from the prey and showed no interest in the prey for 10 min. The remains of each prey item were removed and the substratum was mixed at the end of each feeding trial for an individual mouse. The terrarium was cleaned and the kitty litter replaced at the completion of a given mouse's three feeding trials. All trials were videotaped. The tapes were later analysed using real-time and frame-by-frame playback modes on a Panasonic AG-DV2000 Pro-Line digital cassette recorder/player.

Behavioural Analyses and Measurement Variables

We evaluated the predatory behaviour of the grasshopper mice at each of the three phases of a predatory sequence: detection/recognition, pursuit/attack and handling/subjugation (Table 1). Trials were staged, so we could not measure behaviours that the grasshopper mice might use to search for and locate prey. We could, however, determine whether the mice recognized a prey item as 'acceptable' by scoring whether the mouse attacked, killed and ate the prey.

Three measures of duration lifted from the videotapes were used to determine whether grasshopper mice adjusted their behaviour to the dangerousness of a prey item during the pursuit/attack phase of a predatory sequence. These three measures were total inspection time, prey sniff time and total sniff time. Total inspection time was measured as the time that elapsed between the instant that a mouse first attended to a prey item and the instant that it attacked. Prey sniff time was measured as the time that a mouse spent sniffing within 5 mm of a prey item before attacking. Total sniff time was measured as the total

Table 1. Operational definitions applied to the predatory behaviour of grasshopper mice

Behaviour	Definition
Attend	Discernible change in the mouse's behaviour indicating that it has focused on the prey (e.g. a shift in the orientation of the mouse's head, eyes and ears towards the prey).
Sniff	Discernible movement of the mouse's nares and whiskers while nostrils are close to the prey or to the substratum traversed by the prey.
Attack	A mouse's attempt to capture its prey by lunging with forepaws outstretched and mouth open.
Sting	Scorpion's telson makes physical contact with the mouse and the mouse reacts (e.g. by flinching and/or by grooming the area contacted by the scorpion's stinger).
Drop	Mouse loosens its grasp or releases its bite on a prey item, permitting the prey to move away (and potentially escape) from the mouse.
Incapacitate	Mouse kills or immobilizes the prey, recorded as when the prey makes no further attempt to resist subjugation.
Retreat	Mouse breaks off an attack by turning and moving away from a prey item.

time, before its first attack, that a mouse spent sniffing the track deposited by a prey species on the substratum plus the time that it spent sniffing the prey. A fourth dependent measure was used descriptively; we determined which region (anterior, body or posterior/tail) of the prey item drew the initial attack by the mouse.

Differential handling of the various prey items during the subjugation phase of the predatory sequence was quantified using a single measure of duration along with several measures based on counts. The duration variable, handling time, was measured as the total elapsed time between a mouse's initial attack and the moment that the prey was incapacitated. Dependent variables based on counts included the number of times that a mouse attacked a prey, the number of times that a mouse retreated from the prey following initial attack, the number of times that prey were dropped, and (for scorpion prey only) the number of times that a mouse was stung.

Experimental Design

We tested 38 grasshopper mice (22 adults, 16 juveniles) from the three populations with each of the three prey genera. Data were analysed (SPSS, version 11.0, Chicago, Illinois, U.S.A.) using a three-factor ANOVA that included a single repeated-measures factor (prey type) and two between-subjects factors (mouse population, mouse age). None of the dependent variables met the standard parametric assumptions of normality and homogeneity of variance; duration variables were therefore logarithmically transformed using the equation $\log(X + 1)$, and count variables were square-root transformed using the equation $\sqrt{(X + 3/8)}$ (Zar 1996). Variables that failed to meet either assumption following transformation were subjected to the conservative Geisser–Greenhouse correction (Keppel 1991).

ANOVAs resulting in significant main effects for either prey type or population were further analysed using planned comparisons (JMP, version 3.0, SAS Institute, Cary, North Carolina, U.S.A.). For prey type, we made a priori predictions that either genus of scorpion would be more difficult than crickets for grasshopper mice to subdue; similarly, because of the potent neurotoxins possessed by bark scorpions, we predicted that grasshopper mice would have greater difficulty with *Centruroides* than with *Vaejovis*. Thus, we conducted two orthogonal planned contrasts for prey type: crickets versus *Centruroides* and *Vaejovis*, and *Vaejovis* versus *Centruroides*. For comparative purposes, we also ran a contrast on *Vaejovis* versus crickets.

We also made the a priori prediction that *O. torridus* from both the Santa Rita and Beaver Dam Mountains of Arizona would be more cautious than *O. arenicola* from the Organ Mountains of New Mexico, especially when feeding on *Centruroides*, because *C. exilicauda* sympatric with *O. torridus* are more neurotoxic than *C. vittatus* sympatric with *O. arenicola* (Rowe 2004; L. D. Possani, unpublished data). Thus, we conducted a planned contrast comparing *O. torridus* from the Beaver Dam and Santa Rita Mountains of Arizona versus *O. arenicola* from the Organ Mountains of New Mexico. We also ran the orthogonal contrast comparing *O. torridus* from the Beaver Dam versus Santa Rita populations.

We analysed two dichotomous variables, whether or not different types of prey were incapacitated and whether or not they were then eaten, using the Cochran Q test for repeated measures (Siegel 1956).

Descriptive results are presented as means \pm 1 SE. Unless indicated, all two- and three-way interactions were nonsignificant.

RESULTS

Recognition Phase

None of the prey items were rejected by the grasshopper mice; each cricket and scorpion, from both genera, was attacked within just a few seconds of its introduction into the test chamber. Typically, movement by the prey attracted the attention of the mouse, which then approached, briefly sniffed the prey item and immediately lunged. The latency from the moment that the prey item was introduced until the mouse's first attempt to grasp or bite the prey was 3.11 ± 0.57 s. Of this 3.11-s latency, 1.11 ± 0.36 s was spent sniffing the track traversed by the prey, with another 1.00 ± 0.13 s sniffing within 5 mm of the prey (mean total sniff time = 2.11 ± 0.39 s).

In the context of this experiment, prey defences appeared to have no effect on prey survivorship: all of the crickets, all of the *Vaejovis*, and all but two of the *Centruroides* were eventually incapacitated by the mice ($Q_2 = 2.00$, $P = 0.368$). Prey noxiousness, however, may have deterred some mice from feeding once the prey was killed, because grasshopper mice partially or entirely consumed every cricket, 35 of 38 scorpions in the genus *Vaejovis*, but only 28 of 38 scorpions in the genus *Centruroides* ($Q_2 = 14.36$, $P < 0.001$).

Pursuit/Attack Phase

None of the three duration variables associated with the pursuit phase of a predatory sequence varied significantly between prey types. There was no significant prey-type effect on total inspection time (ANOVA: $F_{2, 64} = 0.49$, $P = 0.615$), total sniff time ($F_{2, 64} = 1.38$, $P = 0.258$) or prey sniff time ($F_{2, 64} = 2.68$, $P = 0.077$). There were also no significant population effects on total inspection time ($F_{2, 32} = 0.38$, $P = 0.690$), total sniff time ($F_{2, 32} = 0.58$, $P = 0.564$), or prey sniff time ($F_{2, 32} = 1.94$, $P = 0.160$). The behaviour of juvenile grasshopper mice during the attack phase was not significantly different from that of adults. There was no significant effect of mouse age on total inspection time ($F_{1, 32} = 0.63$, $P = 0.434$), total sniff time ($F_{1, 32} = 0.22$, $P = 0.642$) or prey sniff time ($F_{1, 32} = 0.01$, $P = 0.909$).

We found limited qualitative evidence to suggest that grasshopper mice orient their initial predatory attacks to different body regions of the various prey. Mice directed their initial attacks to the tail region of scorpion species (66.7%), less often to the head (21.3%) and only rarely towards the scorpion's body (12%). In contrast, initial attacks on crickets were oriented most often towards its body (63.2%), frequently to the head (36.8%), but never to the cricket's posterior.

Subjugation/Handling Phase

Prey defences strongly influenced a grasshopper mouse's ability to handle a prey item once it had been attacked. In general, crickets and scorpions in the genus *Vaejovis* required little effort, but scorpions in the genus *Centruroides* were difficult to dispatch.

Effects of prey type

Grasshopper mice required 1.63 ± 0.31 s to subdue a cricket, 6.76 ± 0.70 s for *Vaejovis*, but 93.72 ± 31.34 s for *Centruroides* (ANOVA: $F_{1.3, 42.5} = 71.42$, $P < 0.0005$; Fig. 1). Handling times were significantly longer for scorpions than for crickets ($F_{1, 70} = 91.75$, $P < 0.0005$), longer for *Vaejovis* than for crickets ($F_{1, 70} = 25.32$, $P < 0.0005$) and longer for *Centruroides* than for *Vaejovis* ($F_{1, 70} = 42.60$, $P < 0.0005$).

During handling, grasshopper mice often responded to scorpion stings by breaking off their attack and briefly grooming the body part that had been stung. There appeared to be differences in the ability of the two scorpion genera to deliver effective stings (*Centruroides*: 4.42 ± 0.69 stings; *Vaejovis*: 1.16 ± 0.20 stings; $F_{1, 32} = 50.39$, $P < 0.0005$; Fig. 2).

The number of times that mice dropped a prey item during an encounter also depended on prey type (crickets: 0.26 ± 0.07 times; *Vaejovis*: 0.29 ± 0.10 times; *Centruroides*: 2.21 ± 0.36 times; $F_{1.6, 49.9} = 39.90$, $P < 0.0005$; Fig. 3). Scorpions were dropped significantly more often than were crickets ($F_{1, 70} = 17.94$, $P < 0.0005$) and *Centruroides* were dropped more often than were *Vaejovis* ($F_{1, 70} = 48.08$, $P < 0.0005$).

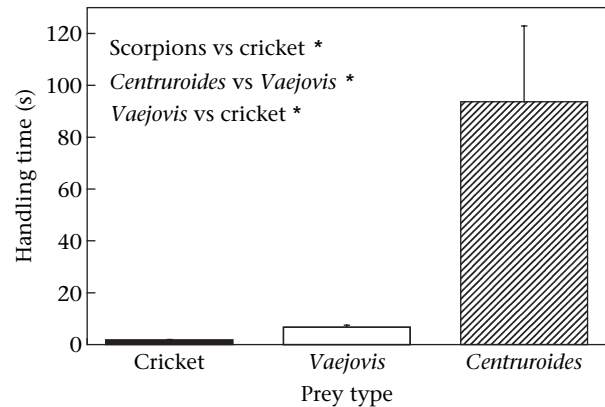


Figure 1. Mean + SE interval, for each prey type, between a mouse's first attack and when the prey item was incapacitated ($N = 38$ mice tested once with each of the three prey types). * $P < 0.0005$.

Grasshopper mice almost never retreated from a cricket after beginning an attack (0.03 ± 0.03 per encounter), rarely retreated from *Vaejovis* (0.05 ± 0.04), but frequently turned and retreated from *Centruroides* (1.68 ± 0.64) ($F_{1, 33.2} = 8.41$, $P = 0.006$). The number of retreats during trials with scorpions was significantly greater than that for crickets ($F_{1, 70} = 4.56$, $P = 0.036$), with more retreats from *Centruroides* than from *Vaejovis* ($F_{1, 70} = 12.28$, $P = 0.0008$).

In the confines of the test terrarium, retreats were temporary and reattacks were the norm. Crickets required only 1.34 ± 0.09 attacks before they were incapacitated and *Vaejovis* required 1.95 ± 0.20 attacks, whereas *Centruroides* took 4.61 ± 0.66 attacks ($F_{1.4, 46.1} = 37.72$, $P < 0.0005$). Scorpions required significantly more attacks than did crickets ($F_{1, 70} = 35.52$, $P < 0.0005$) and *Centruroides* required more attacks than did *Vaejovis* ($F_{1, 70} = 41.45$, $P < 0.0005$).

Age effects

The main effect of mouse age (juvenile versus adult) was not significant for four of the five dependent variables associated with the subjugation/handling phase of

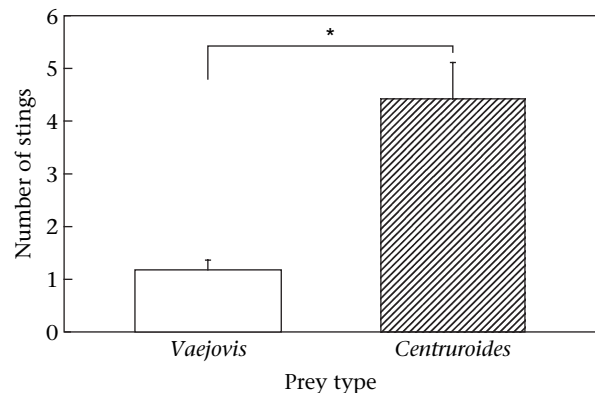


Figure 2. Mean + SE number of stings delivered by either *Vaejovis* spp. or *Centruroides* spp. during an encounter with *Onychomys* ($N = 38$ mice tested once with each genus of scorpions). * $P < 0.0005$.

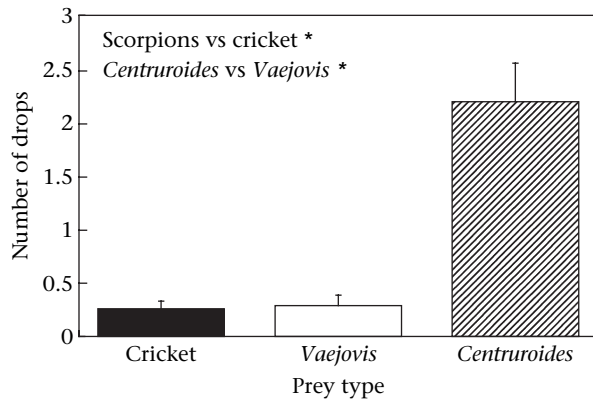


Figure 3. Mean + SE number of times that each prey type was dropped during the handling phase of an encounter with *Onychomys* ($N = 38$ mice tested once with each of the three prey types). * $P < 0.0005$.

a predatory sequence. There were no significant effects of mouse age on handling time ($F_{1, 32} = 2.13$, $P = 0.154$), number of times the mouse was stung ($F_{1, 32} = 0.07$, $P = 0.793$), number of times that the mouse retreated ($F_{1, 32} = 0.62$, $P = 0.438$) or the number of attacks by the mouse ($F_{1, 32} = 0.60$, $P = 0.446$). Adult mice did, however, appear to be more adept at hanging on to a prey item during an encounter; prey were dropped only 0.59 ± 0.13 times per trial by adults, but 1.38 ± 0.31 times per trial by juveniles ($F_{1, 32} = 6.00$, $P = 0.020$). Young mice had an especially difficult time hanging on to scorpions in the genus *Centruroides*, as evidenced by the significant interaction between mouse age and prey type ($F_{1.6, 49.9} = 3.82$, $P = 0.038$; Fig. 4).

Population effects

There were no significant differences between populations in handling time ($F_{2, 32} = 0.13$, $P = 0.879$), the number of times that prey were dropped during handling ($F_{2, 32} = 0.97$, $P = 0.392$) or the number of retreats by the mouse during the encounter ($F_{2, 32} = 0.62$, $P = 0.547$). The populations did, however, differ in the number of times that they were stung while handling a scorpion (Santa Rita population: 1.82 ± 0.28 times during a trial; Beaver Dam population: 3.71 ± 1.14 times; Organ Mountains population: 4.33 ± 1.21 times; $F_{2, 32} = 35.96$, $P = 0.042$; Fig. 5), but neither population contrast was significant. Similarly, Santa Rita mice required 2.11 ± 0.21 attacks per trial to subdue their prey, Beaver Dam mice, 2.62 ± 0.56 attacks and Organ Mountain mice, 3.93 ± 0.86 attacks ($F_{1, 32} = 3.67$, $P = 0.037$); only one of the population contrasts was significant, with *O. arenicola* requiring more attacks than *O. torridus* ($F_{1, 35} = 7.05$, $P = 0.012$).

The number of attacks was the only variable showing a significant two-way interaction between mouse age and population ($F_{2, 32} = 3.41$, $P = 0.045$). While juvenile mice from the Santa Rita and Beaver Dam populations required more attacks to incapacitate prey than did adults, mice from the Organ Mountains showed the opposite pattern, with adults requiring more attacks than did juveniles.

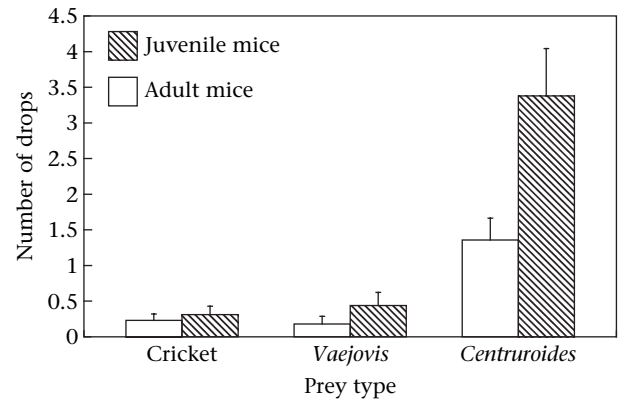


Figure 4. Mean + SE number of times that a prey item was dropped during handling as a function of prey type and age of the grasshopper mouse ($N = 22$ adult and 16 juvenile mice tested once with each of the three prey types).

Moreover, adult mice from the Organ Mountains directed an exceptionally high number of attacks at *Centruroides*, generating our only significant three-way interaction between age, population and prey type ($F_{2.9, 46.1} = 4.61$, $P = 0.007$; Fig. 6).

Trial Effects

There were no significant influences of trial number on any of our measurement variables, nor were there any significant trial by treatment interactions.

DISCUSSION

Our results show that grasshopper mice do not discriminate between the three prey types during the first phase of a predatory sequence. Mice recognized all three genera as potential prey, because they unhesitatingly and relentlessly attacked and killed both toxic and nontoxic genera of scorpions as well as defenceless crickets.

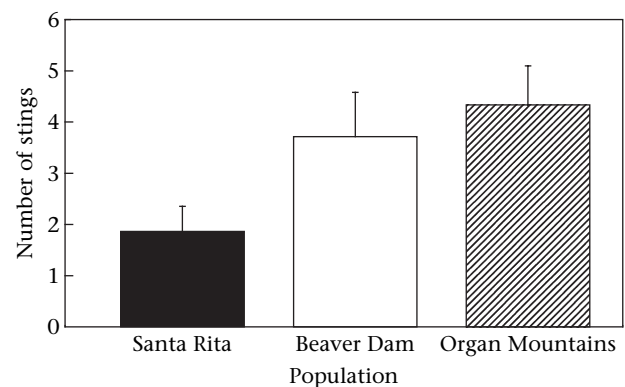


Figure 5. Mean + SE number of stings delivered by scorpions to *Onychomys* from the three different study populations (Santa Rita Mountains, $N = 22$ *O. torridus*; Beaver Dam Mountains, $N = 7$ *O. torridus*; Organ Mountains, $N = 9$ *O. arenicola*) tested once with each genus of scorpion.

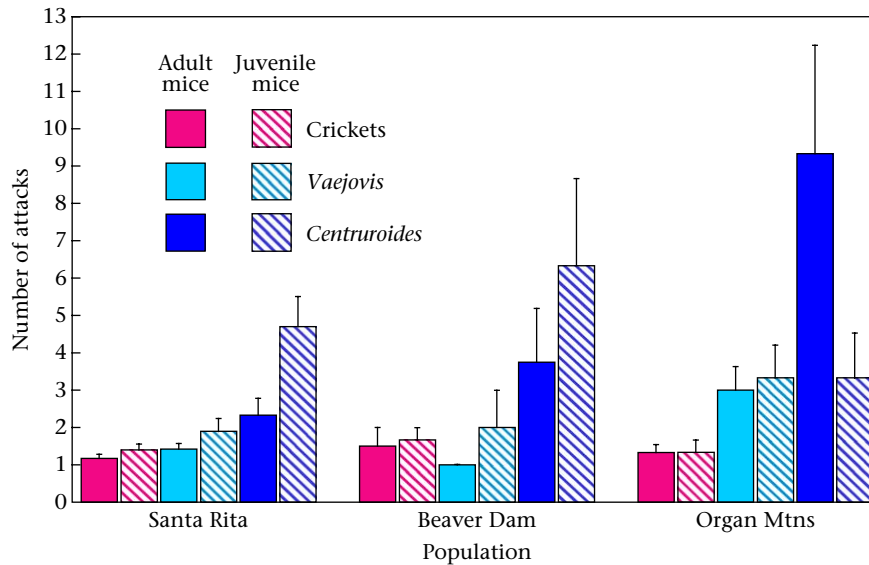


Figure 6. Mean + SE number of attacks directed by juvenile and adult mice from each population (Santa Rita Mountains, $N = 10$ juvenile and 12 adult *O. torridus*; Beaver Dam Mountains, $N = 3$ juvenile and 4 adult *O. torridus*; Organ Mountains, $N = 3$ juvenile and 6 adult *O. arenicola*) towards single presentations of each of the three prey types.

Do grasshopper mice differentiate between the three prey types during the second phase of a predatory sequence? Cues sufficient for such decisions might be extracted during the seconds that mice spent attending to a prey item before attacking. However, grasshopper mice did not appear to use these cues to discriminate between prey species that differ in their antipredator defences. We found no significant differences between scorpions and crickets, or between *Vaejovis* and *Centruroides*, in the latency between attending and attacking, in how long the mouse spent tracking the prey, or in the time spent sniffing the prey from within just a few millimetres. Our results confirm those of other studies that suggest a simple rule of thumb for grasshopper mice during this phase of a predatory sequence: if an appropriately sized object is moving and it smells like food, attack.

Labelling the grasshopper mouse's preattack strategy a 'rule of thumb' does not mean that these desert carnivores never modify their behaviour in the precontact phase of a predatory sequence. One result, which we could assess only qualitatively, suggests that grasshopper mice oriented their initial attacks differently to undefended crickets than to scorpions; crickets were grabbed mainly by the body (63%), but scorpions of both genera were seized mostly by the tail (67%). This might suggest that grasshopper mice recognize the sting of a scorpion as a potent defensive weapon and respond by directing their attacks to immobilize the scorpion's tail. Alternatively, grasshopper mice may simply be reacting to the body part that they initially encounter. For example, mice might first contact a scorpion's tail, either when approaching a scorpion from the rear or during a head-on confrontation while contending with the scorpion's defensively wielded stinger (Cyr 1972). Crickets do not have long, probing tails, seldom confront mice head-on, and were simply pounced on by the mice as the crickets attempted to escape. Thus, our qualitative observations provide only

equivocal support for precontact behavioural modification by grasshopper mice.

Whitman et al. (1986b) presented evidence that *O. torridus* makes cost-benefit decisions about the dangerousness of a prey before an attack. Southern grasshopper mice received a series of grasshopper prey in quick succession, including the chemically protected species *Taenio-poda eques*. The authors presented *T. eques*, then palatable *Brachystola magna*, followed by a *B. magna* that had been smeared with the defensive secretions from *T. eques*, and finally an untreated *B. magna*. Mice frequently rejected both the *T. eques* and the chemically coated *B. magna* in the 'sniff at close range' phase leading up to a pounce. Nevertheless, many mice appeared to use the standard 'attack first, assess later' rule of thumb, because half of the *T. eques* and 65% of the treated *B. magna* were attacked, often killed, and, at least for the treated *B. magna*, occasionally eaten (killed *T. eques*, however, were never ingested). Thus, Whitman et al. (1986b) showed that, for grasshopper mice presented with unpalatable *T. eques*, rejection can occur either during the attack or the handling phase of the predatory sequence.

Our results similarly suggest that grasshopper mice assess prey during the subjugation stage, because they handled the various prey species differently. The highly significant effect of prey type that we observed for every one of the subjugation-related dependent variables is the most robust result of this study. For example, grasshopper mice required more time, and thus exerted more effort, to subdue prey species having antipredator defences than those that did not (i.e. both scorpion genera were more difficult and took longer to incapacitate than crickets).

Interestingly, the defences of the two scorpion genera were not equally effective. Grasshopper mice, for instance, required significantly more time to incapacitate *Centruroides* than they did *Vaejovis*. *Centruroides* also appears capable of delivering more, or perhaps just more irritating,

stings than does *Vaejovis*, because the number of drops, retreats and attacks were each significantly higher for grasshopper mice grappling with the *Centruroides* than with the *Vaejovis*. Moreover, none of these three handling-related variables differed between *Vaejovis* and crickets, suggesting that the significant cricket versus scorpion contrasts were driven by the difficulty that mice had in subduing bark scorpions. Collectively, these results show that *Centruroides* is extremely troublesome to handle, but *Vaejovis* is not. Our findings parallel those of another study that used *Centruroides* as dangerous prey. O'Connell & Formanowicz (1998) showed that whiptail lizards, *Cnemidophorus gularis*, required more time and more attacks to subdue bark scorpions than house crickets.

Our results are similarly consistent with those of other studies showing that prey handling behaviour in grasshopper mice improves with experience (Cyr 1972; Langley 1986); adults dropped their prey less often than did juveniles and significantly so when feeding on *Centruroides*. Furthermore, our results tentatively suggest that prey handling behaviour may be influenced by population-level factors. *Onychomys arenicola* from the Organ Mountains of New Mexico required significantly more attacks to subdue their prey and sustained more stings from scorpions than did *O. torridus* from the Santa Rita and Beaver Dam Mountains of Arizona. These results counter our original prediction that highly toxic *C. exilicauda* would be more difficult for *O. torridus* to handle than would moderately toxic *C. vittatus* for *O. arenicola*. However, we cannot make strong inferences regarding population-level differences (including interactions involving 'population' as a factor) without larger samples of mice from the Beaver Dam and Organ Mountain populations.

Grasshopper mice neither avoid *Centruroides* nor attack them differently than *Vaejovis* because they are resistant to the bark scorpions' neurotoxins. California ground squirrels, *Spermophilus beecheyi*, show similar resistance to the venoms of their rattlesnake (*Crotalus* spp.) enemies (Poran et al. 1987; Biardi et al. 2000), as do predaceous garter snakes, *Thamnophis sirtalis*, to the neurotoxins of one of their prey, the rough-skinned newt, *Taricha granulosa* (Brodie & Brodie 1999; Geffeney et al. 2002). Comparisons of how grasshopper mice, ground squirrels and garter snakes deal with their toxic adversaries illustrate the interplay between venom resistance, risk assessment and adaptive modification of behaviour. Although highly resistant to rattlesnake venom, California ground squirrels nevertheless treat rattlesnakes more cautiously than they do nonvenomous gopher snakes, *Pituophis melanoleucus* (Hennessey & Owings 1978), and they take fewer risks when harassing larger and warmer, and hence more dangerous rattlesnakes (Swaigood et al. 1999b, 2003). For *S. beecheyi*, assessment occurs at the recognition and attack stages; envenomations are still sufficiently harmful that the squirrels modify their harassment behaviour to minimize their risk when dealing with more dangerous snakes. For garter snakes attacking tetrodotoxin-wielding newts, assessment and adaptive behavioural modification occur during the subjugation phase. Garter snakes evaluate the toxicity of a given newt while biting it, rejecting newts too toxic for the snake's own level of tetrodotoxin

resistance; snakes that misjudge can be killed (Williams et al. 2003). Our results show that assessment by grasshopper mice feeding on crickets and scorpions occurs primarily during the handling phase. We found little evidence, however, of postassessment adaptive modification in the mice's behaviour; *Centruroides* were simply attacked, dropped and reattacked until incapacitated, generating significantly longer handling times for bark scorpions than for crickets or for *Vaejovis*. Although stings are temporarily painful to the mice, envenomations by *Centruroides* apparently entail little risk, suggesting a high level of resistance by *Onychomys* to *Centruroides*' neurotoxins. Resistance levels of the mice will be confirmed in future experiments using *in vivo* toxicity tests.

Grasshopper mice may have developed resistance to vertebrate-specific neurotoxins as an evolutionary response to selection by *Centruroides*. In turn, the extreme potency of venom in certain groups of scorpions, including *Centruroides*, may be the result of selection by tenacious and resistant mammalian predators such as *Onychomys*. Comparative differences in the potency of scorpion venoms across species may shed light on strategies that scorpions use to evade their predators. Vermeij (1982) has argued convincingly that selection on any given prey species will be most intense during a single one of the three phases of detection/recognition, attack/pursuit and handling/subjugation. Our results strongly suggest, for example, that predator-induced selection has operated most intensely on *Centruroides* during the subjugation phase, because scorpions in this genus are extremely difficult to handle. By comparison, the antipredator adaptations of *Vaejovis* are probably associated with either avoiding detection or escaping pursuit, because these scorpions are barely more difficult to subdue than are crickets. Support for these predictions awaits more detailed ecological studies than are now available for either genus of scorpions. However, we suggest several proximate explanations for why *Centruroides* is more difficult than *Vaejovis* for *Onychomys* to handle. Perhaps the tails of *Centruroides* are more agile than the tails of *Vaejovis*, or their stingers more penetrating, or their venom more debilitating. In a separate study (A. H. Rowe & M. P. Rowe, unpublished data) we found that *Centruroides* does have more potent venom than *Vaejovis*, but not in the manner that we originally suspected. Of relevance here is evidence that scorpion venoms, although a complex cocktail of chemicals, can coarsely be divided into components causing immediate pain and components responsible for toxicity and death (Inceoglu et al. 2003). We originally predicted that grasshopper mice would be especially cautious when interacting with *Centruroides* given their potentially lethal neurotoxins. We were incorrect because *Onychomys* can afford to attack *Centruroides* with the same ferocity as they attack *Vaejovis* or crickets, because the mice have evolved resistance to *Centruroides*' neurotoxins. What appear to make both species of *Centruroides* so difficult for grasshopper mice to handle are not their neurotoxins, but rather the pain components of their venom. A highly painful sting causes the mouse to drop a *Centruroides* and, in a habitat more complex than our experimental

terrarium, might allow the scorpion to live to sting another day.

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