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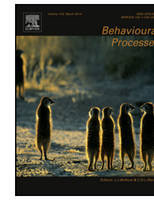

Venom-spraying behavior of the scorpion *Parabuthus transvaalicus* (Arachnida: Buthidae)

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ABSTRACT

Many animals use chemical squirting or spraying behavior as a defensive response. Some members of the scorpion genus *Parabuthus* (family Buthidae) can spray their venom. We examined the stimulus control and characteristics of venom spraying by *Parabuthus transvaalicus* to better understand the behavioral context for its use. Venom spraying occurred mostly, but not always, when the metasoma (tail) was contacted (usually grasped by forceps), and was absent during stinging-like thrusts of the metasoma apart from contact. Scorpions were significantly more likely to spray when contact was also accompanied by airborne stimuli. Sprays happened almost instantaneously following grasping by forceps (median = 0.23 s) as a brief (0.07–0.30 s, mean = 0.18 s), fine stream (<5° arc) that was not directed toward the stimulus source; however, rapid independent movements of the metasoma and/or telson (stinger) often created a more diffuse spray, increasing the possibility of venom contact with the sensitive eyes of potential scorpion predators. Successive venom sprays varied considerably in duration and velocity. Collectively, these results suggest that venom spraying might be useful as an antipredator function and can be modulated based on threat.

1. Introduction

Many animals are capable of predator risk (or threat) assessment, allowing them to choose an appropriate response once the nature of a specific threat is identified (reviewed by Bednekoff, 2007; Eisner et al., 2007; Ferrari et al., 2009; Lima and Steury, 2005). To reduce the risk of predation, many animals rely on both primary and secondary defensive tactics (reviewed by Ruxton et al., 2004). Primary tactics reduce initial detection, whereas secondary tactics render prey capture more difficult. As a secondary tactic, chemical defenses can be highly effective for eluding capture. When under attack, many animals deploy a remarkable diversity of irritants, toxins, and venoms, and some can spray these substances in the direction of their attackers (reviewed by Ruxton et al., 2004). These chemicals often repel, temporarily immobilize, or even kill the predator, allowing the targeted prey to escape.

A wide range of animals employ chemical spraying behavior as a defensive response. In some species, the chemicals are relatively non-toxic but serve an important defensive function. For example, the bombardier beetle (*Brachinus* spp.) ejects an extremely hot (100 °C) spray of aqueous benzoquinones as a defensive mechanism against would-be predators (Eisner, 1958; Eisner et al., 1977). This secretion, accurately aimed and delivered through a pair of spray nozzles, can effectively stun a predator, thus allowing the beetle to escape (Eisner, 1958; Eisner and Aneshansley, 1999). Skunks and a few other members of the mammalian families Mephitidae and Mustelidae similarly expel a malodorous spray from their anal sac when threatened, aiming it in the direction of the aggressor (Stankowich et al., 2011, 2014).

Some species spray highly toxic venom when threatened. Spitting cobras, the best-studied representatives among this group, accurately aim a stream of venom toward the face of an aggressor (Berthe et al., 2013; Westhoff et al., 2005, 2010). These snakes can generate 40 or more spits in rapid succession, traversing a distance up to 3 m, via behavioral (i.e., decision-making) control of the venom glands (Cascardi et al., 1999; Hayes et al., 2008; Rassmussen et al., 1995; Westhoff et al., 2005, 2010). Venom contact with an eye induces immediate, intense pain, and may cause subsequent blindness by destroying the cornea (Chu et al., 2010). Thus, these snakes clearly target a vulnerable part of a potential predator.

Among arachnids, at least two genera of spiders appear to spit venom. *Scytodes* spiders spit a glutinous mixture of silk, adhesive, and venom up to 2.5 cm or more to enmesh and immobilize both prey and predators (Jackson and Pollard, 2001; Suter and Stratton,

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2009, 2013), though recent study of *Suillia pallida* casts some doubt on whether toxins are a part of this mixture (Clement and Li, 2005). *Peucetia* spiders spray a narrow stream of venom up to 20 cm for defense only, and may do so several times with decreasing quantities of ejecta (Fink, 1984). These arachnids also deploy their venom by biting, and therefore, use their toxins via both injection as a venom and spraying as a toxungen (Nelsen et al., 2014b).

Within another arachnid group, at least seven species of the Afrotropical scorpion genus *Parabuthus* reportedly spray venom in a defensive context (Newlands, 1974). Venom spraying has not been documented in any other scorpion group. If the venom contacts sensitive tissues, such as those of an eye, this behavior could potentially deter a predator. The reported symptoms of eye envenomation are similar to those of spitting elapid snakes that result in immediate pain and temporary blindness (Chu et al., 2010; Newlands, 1974). Newlands (1969) hypothesized that venom spraying by *Parabuthus* was reflexive. He speculated that when these scorpions are startled, caudal (metasomal) muscles and muscles of the telson surrounding the venom gland contract, causing venom expulsion. Regardless of the mechanism of expulsion, a large body of evidence suggests that venom can be an expensive commodity that animals cannot afford to waste (Hayes et al., 2002; Hayes, 2008; Wigger et al., 2002), and therefore, we expect that the scorpion has some level of control of this behavior. Venoms are often biochemically complex, requiring sometimes high metabolic costs for their production, storage, and regeneration (McCue, 2006; Nisani et al., 2007, 2012; but see Smith et al., 2014). Accordingly, many animals meter the venom they expend (Hayes et al., 2002; Hayes, 2008; Herbert and Hayes, 2008; Nelsen et al., 2014a; Wigger et al., 2002). Scorpions, for example, often sting only large and difficult-to-handle prey; thus, conserving their venom for relevant situations (Bub and Bowerman, 1979; Casper, 1985; Rein, 1993). When stinging defensively, *Parabuthus* scorpions rely initially on pain-inducing, potassium-rich "prevenom" (Inceoglu et al., 2003), using their metabolically expensive, protein-rich venom only at the highest levels of threat, and deploying quantities that correspond to level of threat (Nisani and Hayes, 2011). If venom-spraying similarly serves an antipredator role, then we would expect this behavior also to be sensitive to threat perception, and exhibited more often at higher levels of threat.

To assess the behavioral context of venom spraying and its potential to function as an antipredator behavior subject to risk assessment, we experimentally examined the stimuli that elicit venom expulsion in *P. transvaalicus*. This scorpion is a large, nocturnal, semi-psammophilous, medically significant species occurring in the arid and semiarid regions of southeast Africa (Prendini, 2004). We predicted that venom spraying would occur more frequently with the higher levels of threat associated with a more complex stimulus configuration. We tested this by comparing responses toward a single predator-associated cue (a lower threat) with responses toward a combination of two cues (a higher threat). Multiple cues about predators are generally additive in the way they contribute to threat assessment and corresponding antipredatory behaviors (e.g., Kim et al., 2009; Lima and Steury, 2005; Smith and Belk, 2001). We also characterized the capacity of *P. transvaalicus* to expel its venom by measuring the duration, velocity, direction, and arc (stream width) of venom expulsion from video recordings of venom spraying. Collectively, this information may hint at whether the scorpion can control venom expulsion during spraying (c.f. Hayes, 2008). Knowledge of the various stimuli that elicit a certain behavior also offers insight on why that behavior might be exhibited in some situations but not in others.

Although the terms "spraying" and "squirting" appear frequently in the literature on antipredatory behaviors and are used interchangeably, the former is applied more often and we have chosen to use it here. Moreover, although the toxic secretion of this scorpion can function as both a venom (when injected) and a toxungen (when sprayed), we refer to it here only as a venom to avoid confusion.

2. Materials and methods

2.1. Experimental subjects

Adult *P. transvaalicus* scorpions (5.10–8.75 g; $n = 8$; all female) were purchased from Glades Herp, Inc. (Bushnell, Florida, USA), and Hatari Invertebrates (Portal, Arizona, USA). Juvenile scorpions (1.36–1.77 g; $n = 8$; all female) were purchased from Hatari Invertebrates. Adults were housed in clear plastic containers measuring 35 × 16 × 11 cm ($L \times W \times H$), and juveniles were housed in clear circular containers (diameter = 11 cm, height = 7 cm). Each cage included sand substrate and a wet sponge within a small plastic cup. The room in which the scorpions were housed was kept at 25 ± 1 °C on a 12:12 light–dark cycle. Scorpions were fed one cricket per week, but were fasted 10 days prior to testing and not fed for the duration of the study. None of the adult females were gravid.

2.2. Stimuli eliciting venom spraying

Each scorpion from both age classes was tested twice, once in each of two threat conditions incorporating different stimuli. Scorpions were transferred individually to a 30 × 16 × 7.5 cm ($L \times W \times H$) plastic box and allowed to acclimate 5 min. We transferred scorpions to the box by manipulating them into and then out of a 150-ml glass beaker while avoiding significant body contact. For the high-threat condition, including both direct contact and airborne stimulation (two predator-related cues), we grasped the scorpion by the metasoma (tail) with a pair of 29-cm forceps and blew a light puff of air (1 s duration) toward the front of the scorpion from a distance of 3–5 cm using a partial pull on the trigger of a Falcon Dust-Off® Disposable Compressed Gas Duster (Falcon Safety Products Inc., Branchburg, New Jersey, USA). Scorpions possess trichobothria, which are elongate setae or hairs that react to air flow and have directional, but not chemosensory, sensitivity (as cited by Ignatyev et al., 1976 and MeBlinger, 1987). Air disturbance might be expected from a predator's attack (e.g., limb thrusts or respiratory exhalation), though we cannot compare the light puff of air we employed to an actual predatory encounter. For the low-threat condition, the same procedure was repeated without any air being blown; thus, only one predator-related cue (contact) was provided. For each trial, we recorded whether the scorpion sprayed venom. We tested half the scorpions in the high-threat condition first, followed by the low-threat condition. The remaining scorpions received the opposite treatment order. Although Newlands (1974) reported that some *Parabuthus* specimens spray venom when blown upon, preliminary observations showed that our specimens refused to do so unless simultaneously grasped. The inter-trial interval was 6–7 days.

2.3. Characteristics of venom spraying

To videotape venom spraying, we tested scorpions individually in a 30 × 16 × 7.5 cm plastic box with a black poster board background and a metric ruler taped in place horizontally against the background. A 100-W incandescent light within a 22-cm-diameter metal reflector was situated 0.5 m horizontally from the box to provide illumination. A Panasonic digital camcorder (model PV-GS120, Panasonic, Secaucus, New Jersey, USA) was placed 1 m directly in front of and at a 20° angle above the plane of the plastic box. After transferring a scorpion to the box by glass beaker, we prodded the legs and body with forceps to manipulate it into a filming position with the body perpendicular to the front of the box and facing left. The forceps was always introduced from above and