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Molecular basis for prey relocation in viperid snakes

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leading to adaptations in behavior, life history, physiology and morphology of species [7]. Further, competition, predation and utilization of dangerous prey have been proposed as the most significant factors of selection on organisms [8]. The ability of predators to adapt to dangerous prey, such as garter snake (*Thamnophis sirtalis*) resistance to tetrodotoxin (TTX) of *Taricha* newts [2], provides strong evidence for a coevolutionary arms race between predators and prey. However, adaptations in predatory behaviors to avoid complete retaliation from dangerous prey may be rare. Nevertheless, natural selection can be expected to lead to adaptations influencing behaviors that are most advantageous to prey capture [1], and further examination of the molecular mechanisms allowing for these large scale behavioral adaptations is critical for understanding coevolution between predator-prey interactions. Many studies examining phenotypic plasticity in species address various forms of plasticity separately, yet this variety may have significantly different ecological consequences [9]. Among venomous snakes, venom characteristics are under positive directional selection [10], and the presence of specific venom components may have played a critical role in diversification of predatory behaviors of several snake taxa.

Rattlesnakes and other vipers demonstrate one of the most advanced modes of predation among vertebrates, utilizing a strike-and-release mode of envenomation. This behavior provides the benefit of minimal contact or retaliation from potentially dangerous prey, but adds the additional task of locating the trail left behind by the envenomated prey that may wander several meters or more from the attack site. By using rapid tongue flicking (strike-induced chemosensory searching) to detect, and the vomeronasal organs to analyze volatile and non-volatile chemical cues [11], snakes must then differentiate between the trail deposited by the prey before and after envenomation has occurred, as well as the trails left inadvertently by other potential prey and non-prey sources. Several hypotheses have addressed the source of chemical cues used to discriminate between trails of struck and unstruck prey. Cues emanating from the mouse when it is punctured during the envenomating strike, as well as other potential chemical cues, such as urine or volatiles from venom left on the prey's integument, have been examined, yet are not utilized by snakes [12-15]. These previous results indicate that

with either Peaks I, IIa, IIb or the peptide peaks, there was no significant difference between the mean number of tongue flicks or the percentages of tongue flicks directed towards either the E or NE carcasses (Table 2; see also Additional file 1, Table S1). However, for Peak III, there were significantly more tongue flicks directed towards the E mouse ($t = 4.24$, df = 10, $P < 0.01$; Table 2), and the mean percentage of tongue flicks toward the envenomated carcass (68%) was also significantly higher than the null ($t = 5.78$, df = 10, $P < 0.01$; Table 2). Analysis of variance (ANOVA) indicated a significant main effect of conditions ($F = 4.63$, df = 4, 54, $P < 0.01$). The Newman-Kewls range test also revealed that the mean for Peak III was significantly higher than the means for Peaks I, IIa, IIb and the peptide peaks ($P < 0.05$), which did not differ significantly among themselves (*P*

mass spectrometry of Peak III (Figure 1B) revealed only peptides with masses of approximately 7.5 kD. Further analysis of Peak III through reverse-phase high pressure liquid chromatography (HPLC) yielded two peaks (Figure 2A) that were subjected to Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometer analysis. These results yielded masses of 7,440.35 Da (Figure 2B) and 7,383.29 Da (Figure 2C), respectively, indicating that the proteins isolated were the disintegrins crotatroxin 1 and crotatroxin 2. N-terminal sequencing of Peak III proteins confirmed the identity of these disintegrins (Figure 3).

Discussion

Determining the molecular mechanisms leading to large-scale adaptations of predatory behaviors, including, in this case, relocation of prey2-2894.72o5503())2itiocnartetendinggrnte

mbyrerg(n)7thesca01l(n)-6odo(ar)7806(oe)]TJ0.0163TcT*pre(n0(ely)-)-5si
d

differing only by the presence of an additional N-terminal alanine in crotatroxin 1 ([22]; see also Figure 3). Disintegrins are non-enzymatic and are produced by the proteolytic posttranslational processing of the C-terminal domain of P-II snake venom metalloproteinases [23]. The presence of dimeric disintegrins in other viperid venoms has also been documented; however, only medium-sized monomeric disintegrins appear to be present in

[17,18], was placed into the snake's cage. The 100 μL volume of reconstituted venom is comparable to the volume of venom injected during a predatory strike [14]. Two injections (each containing 50 μL) were made in the thoracic region, dorsal and ventral to the shoulder blade, in areas most commonly struck during predatory episodes [45]. The control (non-envenomated) mouse was injected in the same regions with 100 μL of deionized water. Trials (10-minute trial duration) started as soon as the test apparatus was placed in the cage, with observers counting tongue flicks directed within 1 cm of either the envenomated or the non-envenomated mouse. All tongue flicking was recorded double blind to the condition; therefore, the observer was unaware of which mouse carcass was injected with the control or venom sample, as well as which condition was being tested. Tongue flicking in snakes represents a stimulus-seeking behavior that is the main process for delivering volatile and non-volatile cues to the vomeronasal organs [11]. Since tongue flicking is activated by the detection of volatile cues by the nasal olfactory system, or visual, thermal or vibratory stimuli, measuring the rate of tongue flicking is an accurate and convenient assay of nasal as well as vomeronasal chemoreception in snakes [11,46]. Cages and test apparatus were cleaned between trials.

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Lyophilized venom (250 mg, from the same venom pool used in Experiment 1) was dissolved in 1.0 mL HEPES buffer solution (10 mM, pH 6.8, with 60 mM NaCl and 5 mM $CaCl_2$) and briefly centrifuged at 9,000 rpm to pellet and remove insoluble material. This solution was then fractionated by size exclusion chromatography using a 90 × 2.8 cm column of BioGel P-100 equilibrated with the same HEPES buffer. Fractionation occurred at a flow rate of 6.3 mL/hr at 4°C, and 30-min-