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Short Note

Chemosensory response in stunted prairie rattlesnakes *Crotalus viridis viridis*

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Abstract Rattlesnakes use chemical stimuli in ambush site selection and for relocation of envenomated prey through strike-induced chemosensory searching. Shifts in responsiveness to prey chemicals have been documented in many snakes, and often correlate with prey commonly taken as snakes increase in age and size as well as geographical locations of the species. For instance, neonate rattlesnakes that prey primarily on ectotherms responded most strongly to chemical cues of commonly taken lizard prey, whereas adult rattlesnakes that prey primarily on small mammals responded significantly to chemical cues of commonly taken rodents. In the current study, 11 Prairie Rattlesnakes *Crotalus viridis viridis* which were classified as large neonates based on measures of snout-vent length (SVL) and body mass, yet chronologically were at or near adulthood, were tested for their responsiveness to chemical extracts of natural and non-natural prey items. Although the snakes had eaten only neonate lab mice (*Mus musculus*), they responded significantly more to chemical cues of natural prey items and particularly to chemical cues of prey normally taken by subadults (*Peromyscus* mice and *Sceloporus* lizard). These results suggest that ontogenetic shifts in responsiveness to natural prey chemical cues are innately programmed and are not based on body size or feeding experience in *C. v. viridis*. This does not imply, however, that growth and experience are without effects, especially with novel prey or rare prey that have experienced recent population expansion [*Current Zoology* 59 (2): 175–179, 2013].

Keywords Chemical cues, Chemoreception, *Crotalus viridis viridis,* Prey choice, Vomeronasal

It is well established that many squamate reptiles, especially snakes, are excellent at recognizing and discriminating between chemical cues of prey sources (Burghardt, 1970; Cooper et al., 1990, Chiszar et al., 1992; Clark, 2004). Behavioral plasticity has also been documented, as shifts in responsiveness to prey-derived cues often correlate with shifts in diet as snakes increase in age and size (Mushinsky and Lotz, 1980; Cooper, 2008; Saviola et al., 2012a). Saviola et al. (2012a) found that neonate prairie rattlesnakes *Crotalus viridis viridis* responded most strongly to chemical cues from prairie lizard prey *Sceloporus undulatus*, whereas adult *C. v. viridis* responded less strongly to these cues and more strongly to chemical cues from deer mice *Peromyscus maniculatus*. Subadults responded strongly to both lizard and rodent cues. These chemosensory response patterns also correlated with the natural prey most commonly taken by the three age groups (Hammerson, 1999; Mackessy, 1988).

Rattlesnakes are ambush predators that use chemical cues in ambush site selection and visual-thermal cues to deliver the envenomating strike (Chiszar et al., 1981; Kardong and Mackessy, 1991; Kardong, 1992; Clark, 2004). Following the strike and release of prey, rapid tongue flicking (strike-induced chemosensory searching) detects volatile and non-volatile chemical cues, allowing for relocation of the envenomated carcass (Chiszar et al., 1977, 1992). Prey-handling behaviors also vary as neonates typically hold on to lizard prey (Mackessy, 1988), whereas adults release rodent prey immediately after the strike (Mackessy, 1988; Hayes, 1991). Likewise, shifts in venom composition are often seen, as neonate rattlesnakes tend to have higher concentrations of toxic venom components, and less pre-digestive enzymes such as metalloproteinases, which are typically in much higher abundance in venoms of adult rattlesnakes

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(see Mackessy, 2008 for a review).

The present sample of *C. v. viridis* were collected as neonates in 2008 from the same population and location as the snakes studied by Saviola et al. (2012a), but the present animals had been underfed and, as a consequence, had not grown at a normal rate. It has been documented that prey preference correlates with skull shape and size in snakes (Vincent et al., 2004, 2009), and with maximum gape and head size being limiting factors in prey consumption, underfeeding and consumption of small prey may drastically inhibit snake growth. Based on measures of snout-vent length (SVL) and body mass, the present snakes were between the neonate and subadult categories defined by Saviola et al. (2012a), whereas chronologically the animals were at or near adulthood (Klauber, 1972). Hence, we were provided with a unique opportunity to assess whether the same shift in response to chemical cues occurred in these snakes as occurred in the snakes studied by Saviola et al. (2012a). This question is of additional interest for at least two reasons: (1) the present snakes had never encountered *P. maniculatus* or *S. undulatus*, as they had eaten only neonatal and adolescent lab mice (*Mus musculus*; 3–10 g, 2–3 cm in length) in captivity, and (2) because of the size of the stunted snakes, they were incapable of swallowing adult *P. maniculatus* (or adult *M. musculus*). Hence, if the stunted snakes nevertheless respond strongly to chemical cues derived from adult *P. maniculatus*, the implication would be that the transition described by Saviola et al. (2012a) was not dependent upon experience with natural prey but may be innately programmed.

1 Materials and Methods

Eleven *C. v. viridis* captured in Weld County, CO, October 8, 2008 (approx. one month after parturition) were maintained in captivity for approximately four years (at the time of this study). The snakes were underfed, but cage sanitation and drinking water were not neglected. During this period, snakes were offered *M. musculus* neonates and adolescents, as the snakes were and still are incapable of ingesting adults. Saviola et al. (2012a) classified wild caught neonates, subadults and adults as shown in Table 1. The present snakes had mean SVL of 349.1 mm and mean mass of 27.4 g, significantly higher than the means for the neonates of Saviola et al. (t_{34} = 9.52 and 9.75, respectively, *Ps* <0.01) but also significantly lower than the means for subadults (t_{29} = 18.73 and 11.09, respectively, *Ps* <0.01). Accordingly, the present sample would be classified as large neonates or small subadults, but not as adults, although chronologically these snakes would be nearing adulthood (Klauber, 1972). All snakes were maintained in individual plastic terraria (51 × 28 × 48 cm) and provided hide boxes, paper floor coverings and water *ad libitum*. The laboratory was maintained at 26–28 °C, and the photoperiod was automatically controlled on a 12:12 light-dark cycle.

Table 1 Mean snout-vent length and body mass (± *SEM*) for the 11 *C. v. viridis* tested

	Snout-vent length (mm)	**Mass (g)**
Current Subjects (*n*=11)	349.1 (7.1)	24.7 (2.0)
Neonate (*n*=25)	**280.9 (3.6)**	**13.4 (0.5)**
Subadult (*n*=20)	**552.0 (7.0)**	**103.0 (4.9)**
Adult (*n*=20)	**789.5 (10.9)**	334.5(15.1)

Bold lettering indicates subject classifications and data from Saviola et al. (2012a).

Chemical extracts of five adult potential prey organisms (*P. maniculatus, M. musculus, S. undulatus,* Side-blotched Lizard, *Uta stansburiana,* and Common House Gecko, *Hemidactylus frenatus*) were prepared by placing intact prey in one ml distilled water per gram of prey for 10 min (Clark, 2004). Extracts were always used within one hr of preparation, and distilled water was used as a control. Trials began by gently opening the lid of the terrarium. Using forceps, a cotton-tipped applicator containing one of the extracts or water was placed one cm from the snake's snout (Cooper and Burghardt, 1990). During the next 60 sec we counted the number of tongue flicks aimed at the applicator and the latency to strike if this occurred. Tongue flicking in squamates is activated by detection of chemical stimuli by the nasal olfactory system, or by visual, thermal or vibratory cues and is the main process for delivering volatile and non-volatile cues to the vomeronasal organs, which mediates definitive analysis of chemical information (Burghardt, 1970; Halpern, 1992; Schwenk 1995; Saviola et al., 2011, 2012b). Therefore, measuring tongue flicking in snakes is a useful assay for measuring a snake's response to any or all of these cues. Trials were separated by at least 24 hr, and the six chemical cues were presented in a different random order for each snake. Each cage was cleaned prior to and between trials with Quatricide-PV$^{®}$, a commercial disinfectant and deodorant. Snakes were always left undisturbed after a trial and allowed to come to rest after the cage lid was opened in preparation for the next trial.

Data were converted to tongue-flick-attack (TFA) scores in order to account for strikes (Cooper and Burghardt, 1990); four strikes occurred during all trials. So, TFA scores were essentially the same as the number of tongue flicks during the 60 sec tests. We used a log-10 transformation to normalize the data and to achieve homogeneity of variance in the six conditions.

Inferential analyses used Chi-square (χ^2) and repeated-measures analyses of variance (ANOVA) followed by non-orthogonal contrasts. Alpha was set at 0.01 for these contrasts to control type I errors. Use of both nonparametric and parametric tests was predicated on our desire to see different statistical tools converge on the same conclusions. Comparisons of characteristics of the present snakes with those of Saviola et al. (2012a) used *t*-tests.

2 Results

Nine of the eleven snakes had their highest TFA scores with *P. maniculatus* or *S. undulatus* cues as opposed to the other four cues ($\chi^2_1 = 4.44$, $P < 0.05$), including *M. musculus*, the only prey offered in captivity and likely the only prey ever eaten by these snakes. All snakes had higher TFA scores for the three potential prey extracts (*M. musculus, H. frenatus, U. stansburiana*) as opposed to the water control ($\chi^2_1 = 11.00$, $P < 0.01$).

The six means shown in Figure 1 differed significantly by repeated-measures ANOVA ($F_{5,50} = 3.92$, $P < 0.01$). Non-orthogonal contrasts revealed that the mean of the five extracts combined differed significantly from the mean for water ($F_{1,50} = 9.06$, $P < 0.01$). The means of *P. maniculatus* and *S undulatus* extracts were significantly higher than the means of all other extracts combined, including the mean for *M. musculus* extract ($F_{1,50} = 22.30$, $P < 0.01$). The latter means did not differ significantly among themselves ($F_{1,50} = 3.56$, $P > 0.05$).

3 Discussion

Rattlesnakes use chemical stimuli in both ambush site selection (Duvall et al., 1990; Clark, 2004; LaBonte, 2008) and relocation of prey during strike-induced chemosensory searching (Chiszar et al., 1977). Therefore, understanding how chemosensory responsiveness varies during the development of the snakes is important

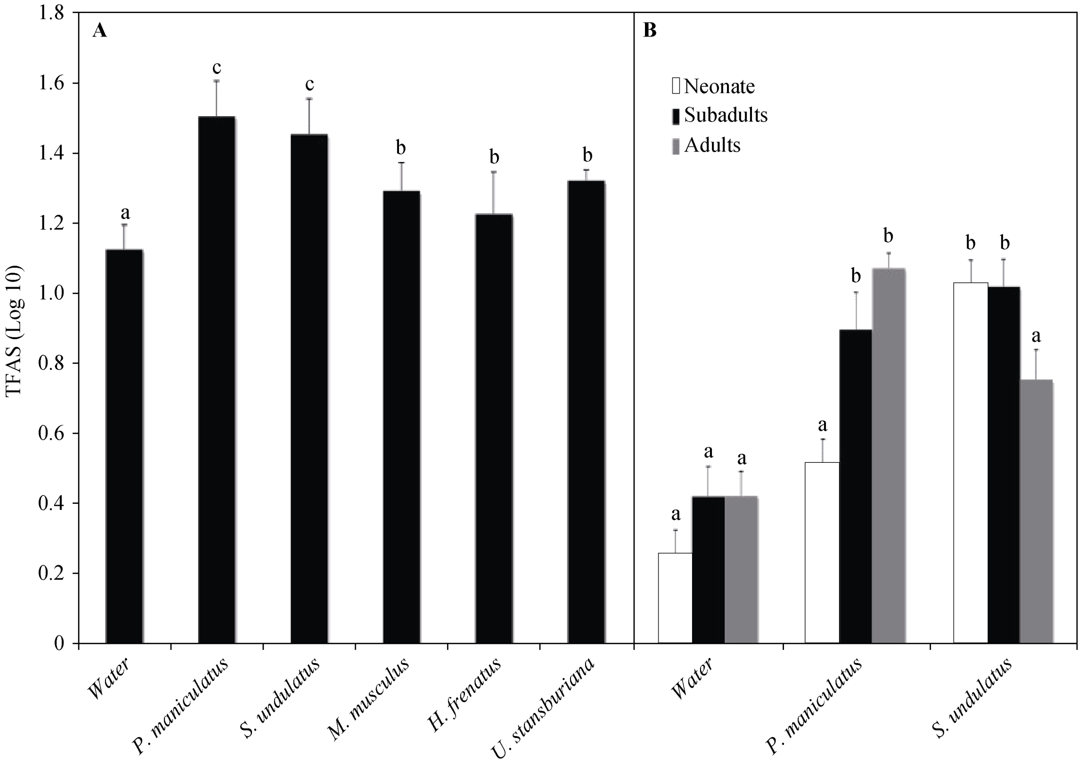

Fig. 1 Log10 transformation of tongue flicks attack score (TFAS) of the current *C. v. viridis* compared to wild-caught *C. v. viridis* from Saviola et al. (2012a)

A. Log10 transformation of mean TFAS ± standard error of the mean (*SEM*) for 11 stunted *C. v. viridis* toward 5 different prey types and water control. **B**. Log10 transformation of mean TFAS ± *SEM* towards chemical cues of natural prey items for wild-caught neonate, subadult, and adult *C. v. viridis* from Saviola et al. (2012a). Dissimilar letters above histogram bars indicate significant differences between responses; same letters indicate no significant differences.

for understanding the behavior and ecology of these species. Although the present snakes had eaten only *M. musculus*, the response to *P. maniculatus* had developed more-or-less on the same schedule as that of normally fed adult snakes, while the response to *S. undulatus* extract had not dropped significantly, as is seen in wild-caught snakes (see Saviola et al., 2012a). Further, the only strikes observed during trials were toward these native prey extracts (*P. maniculatus* and *S. undulatus*, two strikes towards each). Hence, these snakes behaved like normal subadults, even though they were closer to the size range of neonates and could not ingest adult mice.

Development of response to chemical cues of natural prey might be innately programmed, or experience with these or similar items might be required. In the present case, the responses to *P. maniculatus* and *S. undulatus* extracts were of normal intensity, even though the snakes probably had never seen or eaten these prey. This is suggestive of and might be taken as evidence for innate ontogenetic programming. It remains possible that feeding on *M. musculus* provided sufficient surrogate cues to promote development of response to *P. maniculatus* extracts. However, if this is true, then why did the snakes not respond strongly to *M. musculus* extracts? We cannot resolve this conundrum on the basis of present data, but we can provide two hypotheses: (1) response to *P. maniculatus* cues developed innately, perhaps even earlier than usual because of hunger and underfeeding, and (2) this sensitivity to rodent cues may have been the reason the snakes accepted *M. musculus* (rather than the other way around). Although captivity may influence chemosensory responses it has been documented that long-term captive rattlesnakes (*C. atrox, C. durissus, C. horridus, C. vegrandis, C. unicolor*) demonstrate no significant differences in strike-induced chemosensory searching when compared to wild-caught rattlesnakes (Chiszar et al., 1985). Similarly, long-term captivity did not appear to affect the ability of *C. o. oreganus* to strike, dispatch, or relocate prey normally (Alving and Kardong, 1994).

There is evidence that snakes can acquire responsiveness to new or unusual foods if the snakes are fed such foods but not their usual fare (Burghardt et al., 2000), and our data for *M. musculus* extracts agree with this finding. It is also known that naïve neonatal snakes respond to chemical extracts of natural foods (Cooper et al., 1990), and our data for *S. undulatus* extracts agree with those of Burghardt (working with garter snakes). The data for *P. maniculatus* extracts suggest that ontogenetic appearance of response to prey normally taken later in life does not require experience with that prey. Similarly, adult *C. horridus* born and raised in captivity showed significant responses to chemical cues of preferred natural prey, even though these snakes were fed entirely *M. musculus,* never encountering natural prey items (Clark, 2004).

Being gape-limited forces snakes to consume prey that are small enough for them to swallow, and chemosensory responses to prey cues have been shown to correlate with the most commonly consumed prey and with the foraging ecology of the snake (see Cooper, 1995, 2008; Saviola et al., 2012a, b). In Colorado, neonate *C. v. viridis* prey primarily on small ectotherms such as the lesser earless lizard *Holbrookia maculata*, prairie lizard *S. undulatus* and plains spadefoot toad *Spea bombifrons*; however, shifts to endotherm prey such as the western harvest mouse *Reithrodontomys megalotis* and deer mouse *P. maniculatus* occur in subadult and adult snakes (Hammerson, 1999). Although responsiveness to chemical cues changes over snake age, which typically correlates with increased size, our study demonstrates that *C. v. viridis* respond to chemical cues of prey most commonly taken in adulthood, regardless of the snakes' size, and we infer that changes in chemosensory responsiveness by *C. v. viridis* are innate.

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