

Ontogenetic shift in response to prey-derived chemical cues in prairie rattlesnakes *Crotalus viridis viridis*

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Abstract Snakes often have specialized diets that undergo a shift from one prey type to another depending on the life stage of age group for 25 neonate, 20 subadult, and 20 adult (average SVL = 280.9, 552, 789.5 mm, respectively) wild-caught *C. v. viridis* to chemical stimuli presented on a cotton-tipped applicator; water-soluble cues from two ectotherms (prairie lizard, *Sceloporus undulatus*, and house gecko, *Hemidactylus frenatus*), two endotherms (deer mouse, *Peromyscus maniculatus* and lab mouse, *Mus musculus*), and water controls were used. Neonates tongue flicked significantly more to chemical cues of their common prey, *S. undulatus*, than to all other chemical cues; however, the response to this lizard's chemical cues decreased in adult rattlesnakes. Subadults tongue flicked with a higher rate of tongue flicking to both *S. undulatus* and *P. maniculatus* than to all other treatments, and adults tongue flicked significantly more to *P. maniculatus* than to all other chemical cues. In addition, all three sub-classes demonstrated a greater response for natural prey chemical cues over chemical stimuli of prey not encountered in the wild (*M. musculus* and *H. frenatus*). This shift in chemosensory response correlated with the previously described ontogenetic shifts in *C. v. viridis* diet. Because many vipers show a similar ontogenetic shift in diet and venom composition, we suggest that this shift in prey cue discrimination is likely a general phenomenon among viperid snakes [*Current Zoology* 58 (4): 549–555, 2012].

Keywords Gape-limited predator, Prey choice, Trophic adaptation, Vomeronasal chemoreception

Squamate reptile response to prey is often associated with chemical cues of prey integument, visual cues, or visual-thermal cues associated with prey movement (Burghardt, 1970; Ford and Burghardt, 1993; Cooper, 1995). Reliance on chemical cues by lizards and snakes is also critical in mate selection, exploratory behavior, predator identification, prey choice and location, and kin selection (Kubie et al., 1978; Chiszar and Scudder, 1980; Weldon and Burghardt, 1979; Chiszar et al., 2008; Clark, 2004; Pernetta et al., 2009), and many studies have examined chemical cue discrimination and chemosensory responses to multiple chemical cue sources. Chemical cue discrimination between extracts of multiple prey types is often correlated with evolutionary changes in diet, such that snake response to prey cues is greatest to that of most commonly taken prey (Cooper and Burghardt, 1990; Cooper, 1994, 1997, 2008; Clark, 2004). Since snakes are gape-limited predators, swallowing prey whole, head size is a limi-

ting factor in what can be consumed. Therefore, shifts in prey taken as well as response to specific prey cues may also change as snakes increase in age and size (Mushinsky and Lotz, 1980).

Neonate rattlesnakes primarily take smaller ectothermic prey such as lizards and anurans, and adults often specialize on larger endotherms such as rodents and birds (Klauber, 1972; Mackessy, 1988; Mackessy et al., 2003). Rattlesnakes are ambush predators, and the use of chemical cues in ambush site selection has been examined, and likewise selection of ambush sites is correlated with the presence of chemical cues of the most commonly consumed prey (Clark, 2004; LaBonte, 2008). During predatory events, adult rattlesnakes use visual-thermal cues to strike, envenomate, and release endotherm prey (Hayes and Duvall, 1991; Kardong, 1992), inducing strike-induced chemosensory searching (SICS) and further use of chemical stimuli to relocate the envenomated carcass (Chiszar et al., 1977; 1992).

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However, neonate rattlesnakes demonstrate different prey handling behaviors. Mackessy (1988) noted that neonate pacific rattlesnakes (*Crotalus oreganus helleri* and *C. o. oreganus*) often hold onto small ectotherm prey and therefore do not have the task of relocating prey after venom has taken its course. Hayes (1991) showed that juvenile *C. v. viridis* released small endotherm prey after the strike; however, the duration of holding onto prey was much longer than that seen in medium or large *C. v. viridis*. Therefore, although extended contact with prey may increase risk of retaliation from struggling prey, the prolonged holding behavior may be advantageous for neonate feeding success, perhaps by allowing more venom to enter prey or by limiting the distance released prey can retreat before succumbing.

The prairie rattlesnake *Crotalus v. viridis* is one of three species of rattlesnak

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tongue flicks than the two control treatments; no other pairwise comparisons were significant. The six means, ordered from low (open-and-close control) to high (*S. undulatus* extract), were 1.4, 2.3 (water control), 4.2 (*P. maniculatus* extract), 4.3 (*M. musculus* extract), 7.9 (*H. frenatus* extract), and 13.7, respectively. Fifteen pairwise comparisons are possible. The differences (observed ranges, ORs) between the highest mean and all others were significant, since the least significant range (LSR) was 5.08, and all ORs exceeded this value. The two control means were significantly lower than the mean for *H. frenatus* extract, because the ORs were greater than the LSR of 4.86 (all $P_s < 0.05$).

For subadult *C. viridis*, response to *S. undulatus* and *P. maniculatus* extracts were significantly higher than some but not all other treatments. Response to *S. undulatus* extract was as strong as was seen in the neonates, but response to *P. maniculatus* extract was equally strong in the subadults. A significant effect of treatments (extracts) was revealed by ANOVA ($F_{5, 95} = 8.64$, $P < 0.01$) and NKRT revealed that the means for the *S. undulatus* extract and for the *P. maniculatus* extract were significantly higher than the means for the two control treatments. No other differences between treatments were significant. The mean numbers of tongue flicks, ordered from low (open-and-close control) to high (*S. undulatus* extract), were 2.8, 3.6 (water control), 7.7 (*M. musculus* extract), 8.8 (*H. frenatus* extract), 12.7 (*P. maniculatus* extract), and 14.4, respectively. The LSRs for comparisons with *P. maniculatus* and *S. undulatus* extracts were 6.19 and 6.47, respectively, and the ORs for both control means were greater than these values; hence, these differences were significant (all $P_s < 0.05$). All treatments except for *P. maniculatus* and *H. frenatus* extracts had ORs with *S. undulatus* extract that were greater than $LSR = 6.47$. Therefore, these three treatment means (the two controls and *M. musculus* extract) differed significantly from the mean for *S. undulatus* extract (all $P_s < 0.05$). Mean response to *M. musculus* extract did not differ significantly from *P. maniculatus* extract (OR=5.0, LSR=6.19, $P > 0.05$). Mean response to *H. frenatus*

means had significantly higher ORs with all means except for *P. maniculatus* extracts presented to adult and

showed that ambush site selection shifted from ectotherm to endotherm cues with increased snake age in southern pacific rattlesnakes *C. o. helleri*. All of these results are consistent with our data reported here which indicate a shift in responsiveness to age-appropriate prey.

In addition to shifts in diet and chemosensory responses to prey, ontogenetic shifts in venom composition have also been documented, as neonate venoms often contain more toxic components, while adult venoms, which are less toxic, contain higher levels of pre-digestive and digestive enzyme toxins (Fiero et al., 1972; Mackessy, 1988, 2008). This shift in venom composition is correlated with prey surface-to-volume ratios. With smaller body sizes and longer limbs, ectotherms are structurally easier to digest than endotherm prey, and many of the higher mass lytic components of rattlesnake venoms (specifically metalloproteinases) are more abundant in venoms of adult snakes (Mackessy, 1988, 2008). It therefore appears that concomitant changes in several aspects of behavior and physiology occur as rattlesnakes age.

Our results support the hypothesis that chemosensory responses stages of *C. v. viridis* are highly correlated with chemical cues of prey commonly taken at different life. Similarly, Mushinsky and Lotz (1980) found that the plain-bellied water snake *Nerodia erythrogaster* shifted response preference from fish to frog extract at approximately 8 months of age. Anurans make up nearly 85% of the diet of large *N. erythrogaster*, whereas fish are primarily taken by smaller snakes, indicating that the shift in chemosensory response as snakes mature closely follows actual dietary shifts (Mushinsky and Lotz, 1980). Additionally, feeding experience has been shown to lead to shifts in chemosensory responses. Burghardt et al. (2000) showed that an isolated population of *Thamnophis sirtalis* with a natural diet primarily of earthworms, but fed exclusively fish in captivity, exhibited a stronger response to fish cues at the end of the experimental period. It should be stressed that the present study was not designed to assess the extent of flexibility within each of the life stages. These studies are needed before we can conclude that neonates and adults exhibit different specialized strategies, each relatively resistant to modification. However, prairie rattlesnakes are clearly differentially responsive to chemical cues derived from prey typical of a given life stage, and this ontogenetic change may be typical of other rattlesnakes which show age-related changes in diet.

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