

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

Anthony J. SAVIOLA^{1*}, David CHISZAR², Stephen P. MACKESSY¹

¹ School of Biological Sciences, University of Northern Colorado, 501 20th Street, CB 92, Greeley, CO 80639 USA

² Department of Psychology, CB 345, University of Colorado, Boulder, Colorado 80309 USA

Abstract Snakes often have specialized diets that undergo a shift from one prey type to another depending on the life stage of the snake. *Crotalus viridis viridis* (prairie rattlesnake) takes different prey at different life stages, and neonates typically prey on ectotherms, while adults feed almost entirely on small endotherms. We hypothesized that elevated rates of tongue flicking to chemical stimuli should correlate with particular prey consumed, and that this response shifts from one prey type to another as individuals age. To examine if an ontogenetic shift in response to chemical cues occurred, we recorded the rate of tongue flicking 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).

Received Sep. 8, 2011; accepted Dec. 1, 2011.

* Corresponding author. E-mail: Anthony.Saviola@unco.edu

© 2012 Current Zoology

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 rattlesnakes found in Colorado, with a broad distribution throughout most of the state at elevations under 2,890 m (Hammerson, 1999). The diet of *C. v. viridis* in Colorado consists primarily of the lesser earless lizard *Holbrookia maculata*, prairie lizard *Sceloporus undulatus* and plains spadefoot toad *Spea bombifrons*, as well as endotherms such as western harvest mouse *Reithrodontomys megalotis* and deer mouse *Peromyscus maniculatus*; other prey are also taken (Hammerson, 1999). As with many other species of rattlesnakes, ontogenetic shifts are seen in prey type taken, and neonates primarily consume ectotherms, while adults primarily specialize on small mammals (Mackessy, 1988; Hammerson, 1999). Prairie rattlesnakes therefore represent a species for which many aspects of behavior, sensory processes, ecology and venom toxicology have been well studied, and it can serve as a model for behavior of rattlesnakes generally.

Tongue flicking in snakes is a stimulus-seeking behavior and is the main process for delivering volatile and non-volatile cues to the vomeronasal organs (Halpern, 1992), which mediate definitive analysis of chemical information (Cowles and Phelan, 1958; Schwenk, 1995). Tongue flicking is activated by detection of volatile chemical cues by the nasal olfactory system and by visual, thermal, or vibratory stimulation (Burghardt, 1970; Chiszar et al., 1981; Ford and Burghardt, 1993; Saviola et al., 2011); therefore, the rate of tongue flicking can be used as a convenient measure of a snake's response to any or all of these stimuli. In this paper we present data from laboratory experiments examining the responses of neonate, subadult, and adult wild-caught prairie rattlesnakes to aqueous extracts

from two ectotherms (*S. undulatus* and *H. frenatus*) and two endotherms (*P. maniculatus* and *M. musculus*) to determine if an ontogenetic shift in response to chemical cues occurs within this species. In addition, we examine if *C. v. viridis* can discriminate between prey extracts of natural (*S. undulatus* and *P. maniculatus*) and non-natural (*H. frenatus* and *M. musculus*) prey items. This latter point is of interest because both of these non-natural prey are taken by *C. v. viridis* in captivity; indeed, *M. musculus* of various sizes are probably the most common food items in captivity.

1 Materials and Methods

1.1 Study animals

The snakes used in this study consisted of 65 wild-caught *C. v. viridis* from Weld Co., Colorado, USA. Snakes were classified as neonates ($n=25$), subadults ($n=20$), and adults ($n=20$) based on snout-vent length (SVL) and body mass (Table 1), which were measured immediately after testing. Snakes were randomly collected using snake hooks from two den sites that were frequently visited by our lab group. All snakes were housed in groups based on age (size) classification from visual inspection, as actual measurements and associated handling were not conducted until after testing. Snakes were tested approximately 48 hrs and within 96 hrs after being brought into captivity. Snakes were released at the exact location of capture and were in captivity for no more than 10 days. Snakes were maintained with water *ad libitum*, and none of the snakes were fed during their time in captivity. 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 (\pm SEM) for the 65 rattlesnakes tested

	Snout-vent length (mm)	Mass (g)
Neonate	280.9 (3.6)	13.4 (0.5)
Subadult	552.0 (7.0)	103.0 (4.9)
Adult	789.5 (10.9)	334.5 (15.1)

1.2 Experimental design

The test cages for neonate and subadult rattlesnakes consisted of glass terraria (51×28×48 cm); adults were tested in larger glass terraria (122×33×35 cm). Testing cages contained paper flooring, and paper was wrapped around the transparent sides. Cages were cleaned prior to and between tests with Quatricide-PV[®], a commercial disinfectant and deodorant. Before testing, a snake was placed into the test cage, the top was affixed, and the

subject was allowed to acclimate undisturbed for 10 min.

Trials began by gently opening the lid of the testing cage, and using forceps we placed a cotton-tipped applicator containing one of five chemical cue extracts 1 cm from the snake's snout (Cooper and Burghardt, 1990). Trials were of 60 sec duration, and we counted tongue flicks directed at the cotton-tip applicator simultaneously using a hand counter. To minimize stress to the animals and to keep them in captivity for as little time as possible, trials were separated by 10 min, during which the lid was affixed to the cage and the snake was left undisturbed. However, this short duration between trials did not influence subsequent tongue flicking, as indicated by an absence of trials effects in statistical tests.

Chemical extracts of deer mouse *P. maniculatus*, lab mouse *M. musculus*, prairie lizard *S. undulatus*, and house gecko *H. frenatus*, were prepared by placing intact prey (entire animal) in 1ml of distilled water per gram of prey for 10 minutes (Clark, 2004). Extracts were always used within 1 hr of preparation. Distilled water was used as a control, and we added an additional control treatment of opening and closing the lid of the testing cage. This additional control consisted of an observer opening the lid of the testing cage for 60 secs, counting tongue flicks, and closing the lid of the cage. This treatment was included to take into consideration

any effect of opening the test cage as well as the observer being present in front of the snake that may have led to elevated levels of tongue flicking.

1.3 Statistical analysis

Data were analyzed by Chi-square (χ^2) and repeated-measures, and mixed analyses of variance (ANOVA) followed by Newman-Keuls Range Test (NKRT, 5%). Repeated-measures ANOVA and mixed ANOVA (treating conditions as a repeated measures factor and age as a between-subjects factor) were completed since we have multiple observations on each individual and three age-groups of individuals. The concept of analyzing data by both non-parametric and parametric statistics is based on the desire to see different methodologies converge on a common outcome, thus strengthening our conclusions (Siegel, 1956).

2 Results

The mean number of tongue flicks toward chemical extracts during the 60 sec trials varied markedly among treatments and age groups of snakes (Fig. 1). For neonates, 19 of 25 snakes directed more tongue flicks at *S. undulatus* extract than toward any other prey extract ($\chi^2_1 = 6.76$, $P < 0.01$). Repeated-measures ANOVA revealed a significant effect of prey cue type ($F_{5, 120} = 16.74$, $P < 0.01$) and NKRT showed that *S. undulatus* extract received significantly more tongue flicks than all other treatments. Also, *H. frenatus* extract received more

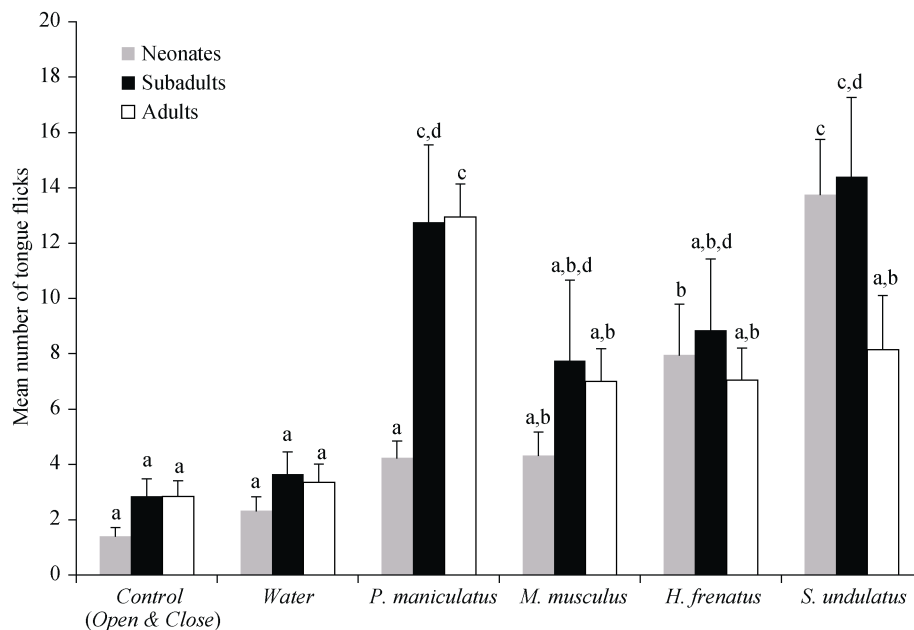


Fig. 1 Mean number of tongue flicks per minute \pm standard error of the mean (SEM) from neonate, subadult, and adult prairie rattlesnakes toward extracts of four different prey types and two control treatments

Dissimilar letters above histogram bars indicate significant differences between responses; same letters indicate no significant differences.

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* extract differed significantly from the open-and-close control (OR=6.0, LSR=5.83, $P < 0.05$). No other pairwise comparisons were significant.

For adult *C. v. viridis*, ANOVA indicated a significant effect of treatments ($F_{5, 95} = 12.24$, $P < 0.01$). NKRT showed that the two controls did not differ from each other, but both of these means differed significantly from all others. Most conspicuously, the mean for re-

sponses to *P. maniculatus* extract was significantly higher than all other means. This was confirmed by Chi-square ($\chi^2_1 = 5.00$, $P < 0.05$) and NKRT. In addition, 17 of 20 adults had higher scores for *P. maniculatus* than for *M. musculus* extract ($\chi^2_1 = 9.8$, $P < 0.01$), indicating that adults discriminated between natural and non-natural prey cues. The six means, arranged from low (open-and-close control) to high (*P. maniculatus* extract), were 2.8, 3.3 (water control), 7.0 (*M. musculus* extract), 7.1 (*H. frenatus* extract), 8.1 (*S. undulatus* extract), and 12.9. The LSR for comparisons involving the latter mean was 4.26, and all ORs were higher than this value, including that for *M. musculus* extract. Hence, *P. maniculatus* extract generated significantly more tongue flicks than all other conditions. The LSR for comparisons involving *S. undulatus* extract was 4.07, and both controls had ORs that were larger (all $P_s < 0.05$). The mean for the open-and-close control generated ORs that were higher than the LSRs for *M. musculus* and *H. frenatus* extracts (3.49, 3.83, respectively, $P_s < 0.05$). The mean for the water control had an OR with the mean for *M. musculus* extract that exceeded the corresponding LSR (3.49; $P < 0.05$). No other pairwise comparisons were significant.

Integrating all three sub-analyses into a single 3 (age) \times 6 (treatments) mixed ANOVA, treating age as a between-subjects factor and treatments as a repeated-measures factor, demonstrated that the age effect was not significant ($F_{2, 62} = 1.47$, $P > 0.05$). The overall means across extracts for the age groups were similar and did not differ significantly. The significant effect of extracts ($F_{5, 310} = 54.89$, $P < 0.01$) was primarily due to the fact that the controls were lower than the other treatments. The interaction of age \times treatments was significant ($F_{10, 310} = 38.21$, $P < 0.01$). When comparing the 18 means with each other using NKRT, the *S. undulatus* extract had the highest means for neonates and subadults (significantly higher than for the adults); conversely, *P. maniculatus* extract had the highest means for adults (significantly higher than for neonates). Applying NKRT to the 18 means (see the lists of means from the three previous sub-analyses) from this 3 \times 6 mixed ANOVA produced 153 pairwise comparisons. First, all control means (from both controls and all three age groups) had ORs that did not exceed the corresponding LSRs. The highest OR in this subset of 15 contrasts was 2.2, whereas the corresponding LSR was 4.76. So, none of these treatments differed from each other. The highest means were for the *S. undulatus* extracts presented to subadults and to neonates, and these

means had significantly higher ORs with all means except for *P. maniculatus* extracts presented to adult and subadult snakes. The highest of these latter ORs was 4.8, while the corresponding LSR was 5.10. Hence, the means for *S. undulatus* extracts presented to subadults and to neonates were significantly higher than all but these last two means. Additionally, the means for *S. undulatus* presented to subadults and neonates did not differ from each other (OR = 0.3, LSR = 5.18). The mean for *P. maniculatus* extract presented to adult snakes was higher than all other means except for *P. maniculatus* extract presented to subadults, and *S. undulatus* extracts presented to neonates and subadults (ORs = 0.2, 0.8 and 1.5, respectively; LSRs = 5.49, 5.45, and 5.57, respectively). In short, this set of comparisons agrees with those reported above in showing that the youngest snakes tongue flicked most strongly towards *S. undulatus* extract, whereas the adult snakes tongue flicked the most to *P. maniculatus*. Subadult snakes tongue flicked approximately equally to both *S. undulatus* and *P. maniculatus* extracts.

3 Discussion

Natural selection can be expected to influence chemosensory responses of snakes to stimuli that are most likely to lead to capture of prey (Tinbergen, 1951; Cooper, 2008). Evolutionary shifts in snake diet have been well documented and are further correlated with shifts in response to specific cues associated with such prey (see Cooper, 2008). Whether this response is a learned or heritable trait has been examined in several species, and studies have indicated that prey generalists demonstrate learning behavior such that increased response is directed toward cues of the diet being fed to the snake (Burghardt, 1993). Prey specialists, on the other hand, may exhibit chemosensory responses only to specific prey, with such behavior exhibiting little flexibility, as is characteristic of heritable responses (Arnold, 1981; see also Cooper, 2008). The majority of rattlesnakes demonstrate different feeding strategies at different life stages; for example, gut content analyses indicated that juvenile pacific rattlesnakes had a diet consisting of more than 50% ectotherms, whereas adult snakes had exclusively mammals in their diet (Mackessy, 1988).

Neonate *C. v. viridis* showed a significantly higher rate of tongue flicking for chemical extract of *S. undulatus* when compared to all other treatments, including a non-prey lizard species *H. frenatus* extract. These results indicate that neonates discriminated between natu-

ral *S. undulatus* and non-natural *H. frenatus* lizard prey chemical cues. Interestingly, Chiszar and Radcliffe (1977) found that neonate *C. v. viridis* born in captivity exhibited no significantly different responses to chemical extracts of lizard and lab mouse *M. musculus* when compared to the control. However in this study, snakes were naïve, never having been fed prior to testing. Further, these snakes had never seen nor smelled prey, and snakes were tested immediately following the shed of natal skins (about 10 days following parturition). In comparison, in the current study, snakes certainly had feeding experience before they were caught, and that the observed difference between the results of these two studies is likely due to prior predatory experience among snakes in our study.

Subadult *C. v. viridis* responded with a higher rate of tongue flicking to both *S. undulatus* and *P. maniculatus* extracts and adult *C. v. viridis* tongue flicked significantly to the extract of *P. maniculatus* when compared to all other treatments. As with the neonates, rate of tongue flicking to chemical extract of native prey *P. maniculatus* was higher than that seen for non-native prey (*M. musculus*), indicating that adults also discriminated between natural and non-natural endothermic prey cues. Collectively, these results reveal a shift in chemosensory responses, presumably correlated with prey commonly taken between neonatal and adult life stages of *C. v. viridis* (Hammerson, 1999). Subadult snakes showed elevated levels of tongue flicking to both *S. undulatus* and *P. maniculatus*, indicating that the emergence of the adult response is not associated with an immediate decline of earlier dietary predilections. Indeed, although adult *C. v. viridis* mostly take endothermic prey, they are known to take *S. undulatus* and other ectotherms opportunistically throughout life (Hammerson, 1999).

Responses of rattlesnakes to chemical stimuli during ambush site selection demonstrate that rattlesnakes rely on prey chemical cues for ambush foraging strategy. Prairie rattlesnakes assumed ambush postures, both in the field and in the laboratory, in response to potential prey extracts (Duvall et al., 1990; Theodoratus and Chiszar, 2000), and dusky pygmy rattlesnakes *Sistrurus miliarius barbouri* in the field selected ambush sites based on presence of extracts of their most taken prey, leopard frogs *Rana pipiens* (Roth et al., 1999). In addition, yearling timber rattlesnakes *C. horridus* born in captivity responded significantly to chemical extracts of natural prey items, even though snakes were fed exclusively lab mice (Clark, 2004). LaBonte (2008) further

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.

Acknowledgements We thank the landowners (Loose family) for permission to work with snakes on their land.

References

- Arnold SJ, 1981. Behavioral variation in natural populations. I. Phenotypic, genetic and environmental correlations between chemoreceptive responses to prey in the garter snake *Thamnophis elegans*. *Evolution* 35: 489–509.
- Burghardt GM, 1970. Chemical perception in reptiles. In: Johnson Jr JW, Moulton DG, Turk A eds. *Communication by Chemical Signals*. New York: Appleton-Century-Crofts, 241–308.
- Burghardt GM, 1993. The comparative imperative: Genetics and ontogeny of chemoreceptive prey responses in natricine snakes. *Brain Behav. Evol.* 41: 138–146.
- Burghardt GM, Layne DG, Konigsberg L, 2000. The genetics of dietary experience in a restricted natural population. *Psychological Science* 11: 69–72.
- Chiszar D, Radcliffe CW, 1977. Absence of prey-chemical preferences in newborn rattlesnake (*Crotalus cerastes*, *C. enyo* and *C. viridis*). *Behav. Biol.* 21: 146–150.
- Chiszar D, Radcliffe CW, Scudder K, 1977. Analysis of the behavioral sequence emitted by rattlesnakes during feeding episodes. I. Striking and chemosensory searching. *Behav. Biol.* 21: 418–425.
- Chiszar D, Scudder KM, 1980. Chemosensory searching by rattlesnakes during predatory episodes. In: Müller-Schwarze D, Silverstein RM eds. *Chemical Signals: Vertebrates and Aquatic Invertebrates*, New York: Plenum Press, 125–139.
- Chiszar D, Taylor SV, Radcliffe CW, Smith HM, O'Connell B, 1981. Effects of chemical and visual stimuli upon chemosensory searching by garter snake and rattlesnakes. *J. Herp.* 15: 415–424.
- Chiszar D, Lee RKK, Radcliffe CW, Smith HM, 1992. Searching behaviors by rattlesnakes following predatory strikes. In: Campbell JA, Brodie ED Jr eds. *Biology of the Pitvipers*. Tyler, Texas: Selva, 369–382.
- Chiszar D, Walters A, Smith HM, 2008. Rattlesnake preference for envenomated prey: Species specificity. *J. Herp.* 42: 764–767.
- Clark RW, 2004. Timber rattlesnakes *Crotalus horridus* use chemical cues to select ambush sites. *J. Chem. Ecol.* 30: 607–617.
- Cooper WE Jr, 1994. Chemical discrimination by tongue-flicking in lizards: A review with hypotheses on its origin and its ecological and phylogenetic relationships. *J. Chem. Ecol.* 20: 439–487.
- Cooper WE Jr, 1995. Foraging mode, prey chemical discrimination, and phylogeny in lizards. *Anim. Behav.* 50: 973–985.
- Cooper WE Jr, 1997. Correlated evolution of prey chemical discrimination with foraging, lingual morphology, and vomeronasal chemoreceptor abundance in lizards. *Behav. Ecol. Sociobiol.* 41: 257–265.
- Cooper WE Jr, 2008. Tandem evolution of diet and chemosensory responses in snakes. *Amphibia-Reptilia* 29: 393–398.
- Cooper WE Jr, Burghardt GM, 1990. A comparative analysis of scoring methods for chemical discrimination of prey by squamate reptiles. *J. Chem. Ecol.* 16: 45–65.

- Cowles RB, Phelan RL, 1958. Olfaction in rattlesnakes. *Copeia* 1958: 77–83.
- Duvall D, Chiszar D, Hayes WK, Leonhardt JK, Goode MJ, 1990. Chemical and behavioral ecology of foraging in prairie rattlesnake *Crotalus viridis viridis*. *J. Chem. Ecol.* 16: 87–101.
- Fiero MK, Seifert MW, Weaver TJ, Bonilla CA, 1972. Comparative study of juvenile and adult prairie rattlesnake *Crotalus viridis viridis* venoms. *Toxicon* 10: 81–82.
- Ford NB, Burghardt GM, 1993. Perceptual mechanisms and the behavioral ecology of snakes. In: Seigel RA, Collins JT eds. *Snakes: Ecology and Behavior*. New York: McGraw-Hill, 117–164.
- Halpern M, 1992. Nasal chemical senses in reptiles: Structure and function. In: Gans C, Crews D eds. *Biology of the Reptilia. Physiology E, Hormones, Brain, and Behavior*, Vol. 18. Chicago, Illinois: University of Chicago Press, 423–523.
- Hammerson G, 1999. *Amphibians and Reptiles in Colorado*. Niwot, CO: University Press of Colorado and Colorado Division of Wildlife.
- Hayes WK, 1991. Ontogeny of striking, prey-handling and envenomation behavior of prairie rattlesnakes *Crotalus v. viridis*. *Toxicon* 29: 867–875.
- Hayes WK, Duvall D, 1991. A field study of prairie rattlesnake predatory strikes. *Herpetologica* 47: 78–81.
- Kardong KV, 1992. Proximate factors affecting guidance of the rattlesnake strike. *Zool. J. Anat.* 122: 233–244.
- Klauber LM, 1972. *Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind*. 2 Vols. 2nd edn. Berkeley: University of California Press.
- Kubie JL, Vagvolgyi A, Halpern M, 1978. The roles of the vomeronasal and olfactory systems in the courtship behavior of male garter snakes. *J. Comp. Physiol. Psych.* 92: 627–641.
- LaBonte JP, 2008. Ontogeny of prey preference in the southern pacific rattlesnake *Crotalus oreganus helleri*. In: Hayes WK, Beaman KR, Cardwell MD, Bush SP eds. *The Biology of Rattlesnakes*. Loma Linda, U.S.A.: Loma Linda University Press, 169–174.
- Mackessy SP, 1988. Venom ontogeny in the pacific rattlesnakes *Crotalus viridis helleri* and *C. v. oreganus*. *Copeia* 1988: 92–101.
- Mackessy SP, 2008. Venom composition in rattlesnakes: Trends and biological significance. In: Hayes WK, Beaman KR, Cardwell MD, Bush SP eds. *The Biology of Rattlesnakes*. Loma Linda, U.S.A.: Loma Linda University Press, 495–510.
- Mackessy SP, Williams K, Ashton K, 2003. Characterization of the venom of the midget faded rattlesnake *Crotalus viridis concolor*: A case of venom paedomorphosis? *Copeia* 2003: 769–782.
- Mushinsky HR, Lotz KH, 1980. Chemoreceptive responses of two sympatric water snakes to extracts of commonly ingested prey species: Ontogenetic and ecological considerations. *J. Chem. Ecol.* 6: 523–535.
- Pernetta AP, Reading CJ, Allen, JA, 2009. Chemoreception and kin discrimination by neonate smooth snakes *Coronella austriaca*. *Animal Behaviour* 77: 363–368.
- Roth ED, May PG, Farrell TM, 1999. Pigmy rattlesnakes use frog-derived chemical cues to select foraging sites. *Copeia* 1999: 772–774.
- Saviola AJ, Lamoreaux WE, Opferman R, Chiszar D, 2011. Chemosensory response of the threatened eastern indigo snake *Drymarchon couperi* to chemical and visual stimuli of *Mus musculus*. *Herpetological Conservation and Biology* 6: 449–454.
- Schwenk K, 1995. Of tongues and noses: Chemoreception in lizards and snakes. *Trends in Ecology and Evolution* 10: 7–12.
- Siegel S, 1956. *Non-parametric Statistics*. New York: McGraw-Hill.
- Theodoratus DH, Chiszar D, 2000. Habitat selection and prey odor in the foraging behavior of western rattlesnakes *Crotalus viridis*. *Behaviour* 137:119–135.
- Tinbergen N, 1951. *The Study of Instinct*. London: Oxford University Press.
- Weldon PJ, Burghardt GM, 1979. The ophiophage defensive response in crotaline snakes: Extension to new taxa. *J. Chem. Ecol.* 5: 141–151.