

## Ontogenetic Variation in Venom Composition and Diet of *Crotalus oreganus helleri*: A Case of Venom Paedomorphosis?

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Ontogenetic shifts in diet are common for snakes, and such shifts in diet for venomous snakes may be associated with changes in venom composition. The present study investigated whether an ontogenetic shift in diet and venom composition, as observed for *Crotalus oreganus helleri* and *Crotalus oreganus oreganus*, occurs in *Crotalus oreganus concolor*. Like *C. o. helleri* and *C. o. oreganus*,

*C. o. concolor*-based  $\beta$  neurotoxin (concolor toxin; Pool and Bieber, 1981; Aird and Kaiser, 1985; Bieber et al., 1990) and potent nonenzymatic peptide myotoxins (Engle et al., 1983; Bieber et al., 1987; Bieber and Nedelkov, 1997). Because of these potent activities, some aspects of *C. o. concolor* venom biochemistry are well known, but few analyses of venom composition have been conducted (but see Aird, 1984).

*C. o. concolor*, a derived lineage, is nested within the *C. o.* complex, par-

ticularly with respect to *C. j. e. e* and *C. j. e. e* (Ashton and de Queiroz, 2001). As such, it provides an opportunity to study the evolution of ontogenetic shifts in diet and venom characteristics. Phylogenetic relationships within the *C. j. e. e* complex have been the focus of several studies, some using morphological, allozyme, venom, and DNA characters (Aird, 1984; Quinn, 1987) and others using DNA sequence data (Pook et al., 2000; Ashton and de Queiroz, 2001; Douglas et al., 2002). Throughout we use the taxonomy and relationships presented by Ashton and de Queiroz (2001), acknowledging that some taxa rec-



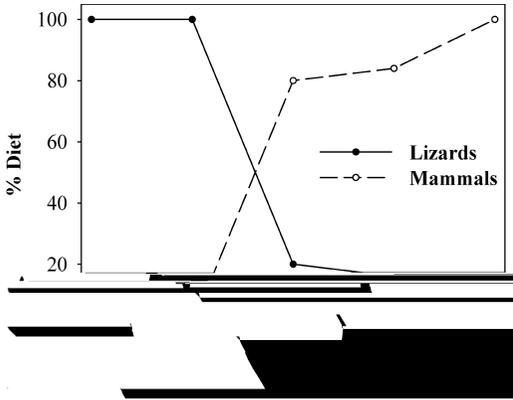


Fig. 1. Diet as a function of length in *C. ...*

TABLE 2. ENZYME ACTIVITIES FROM NEONATE, JUVENILE AND ADULT *C. . . .* VENOM.

Enzyme assayed	Neonate venom (n = 10)	Juvenile venom (n = 8)	Adult venom (n = 18)
Plasmin-like protease	2.72	43.44*	124.03*
Thrombin-like protease	210.74	390.92	621.58*
Kallikrein-like protease	803.65	766.09	621.79
Phosphodiesterase	0.257	0.561*	0.685*
Azocaseinase	0.427	0.423	0.135*
HPA metalloprotease	1.94	0.82*	0.76*
L-amino acid oxidase	31.43	41.65	34.37
Phospholipase A <sub>2</sub>	37.18	40.87	29.27

All values represent averages of specific activities (see Materials and Methods and Fig. 3). Averaged total length (mm) for size class: neonate = 214; juvenile = 332; adult = 556. \* = significant difference from neonate venom ( $P < 0.05$ ).

duce venoms containing both low and high molecular weight proteases, *C. . . .* venom proteases were smaller proteins, ranging in size from ~19–48 kD.

Size exclusion HPLC resulted in the separation of six major protein size class peaks from both neonate and adult venoms (Fig. 6A—B), but these differed qualitatively and quantitatively. In particular, the low molecular weight components (far right peaks; myotoxins) were much more prominent in the adult venom, even though the total protein load of the neonate venom was somewhat higher (based on total peak areas of the chromatograms). The peak containing the phospholipase A<sub>2</sub>-based  $\beta$ -neurotoxin accounted for a similar percentage of total venom protein in both samples.

Lethal toxicity in inbred mice was determined for venom from one adult snake, and the LD<sub>50</sub> was 0.38  $\mu$ g/g. Lethal toxicity of juvenile venom in mice was 0.45  $\mu$ g/g.

The first case of human envenomation (Sweetwater County, Wyoming) occurred on the top of the left foot, approximately two inches behind the toes. Within 10 min of envenomation, the subject complained of numbness in the face, and the mouth was difficult to open. Within 30 min, this numbness was more widespread on the left side of the face and appeared to travel down the left arm. Shortly thereafter, fasciculations occurred in both lips, and a parent commented that it looked "like worms crawling under the skin." Swelling of the leg to the calf occurred approximately 30–120 min after the bite. Approximately 45 min after the bite, the patient could not maintain balance, and approximately 120 min after the bite, Wy-



Fig. 3. Enzyme activities of crude venoms of *C. [illegible]* as a function of snake total length. All activities are normalized to total protein content of venoms and are expressed as specific activities. (A) Plasmin-like activity; (B) thrombin-like activity; (C) kallikrein-like activity; (D) phosphodiesterase activity; (E) azocasein metalloprotease activity; (F) hide powder azure metalloprotease activity; (G) L-amino acid oxidase activity; (H) phospholipase A<sub>2</sub> activity. Solid lines are regression lines, and dashed lines indicate 95% confidence intervals.

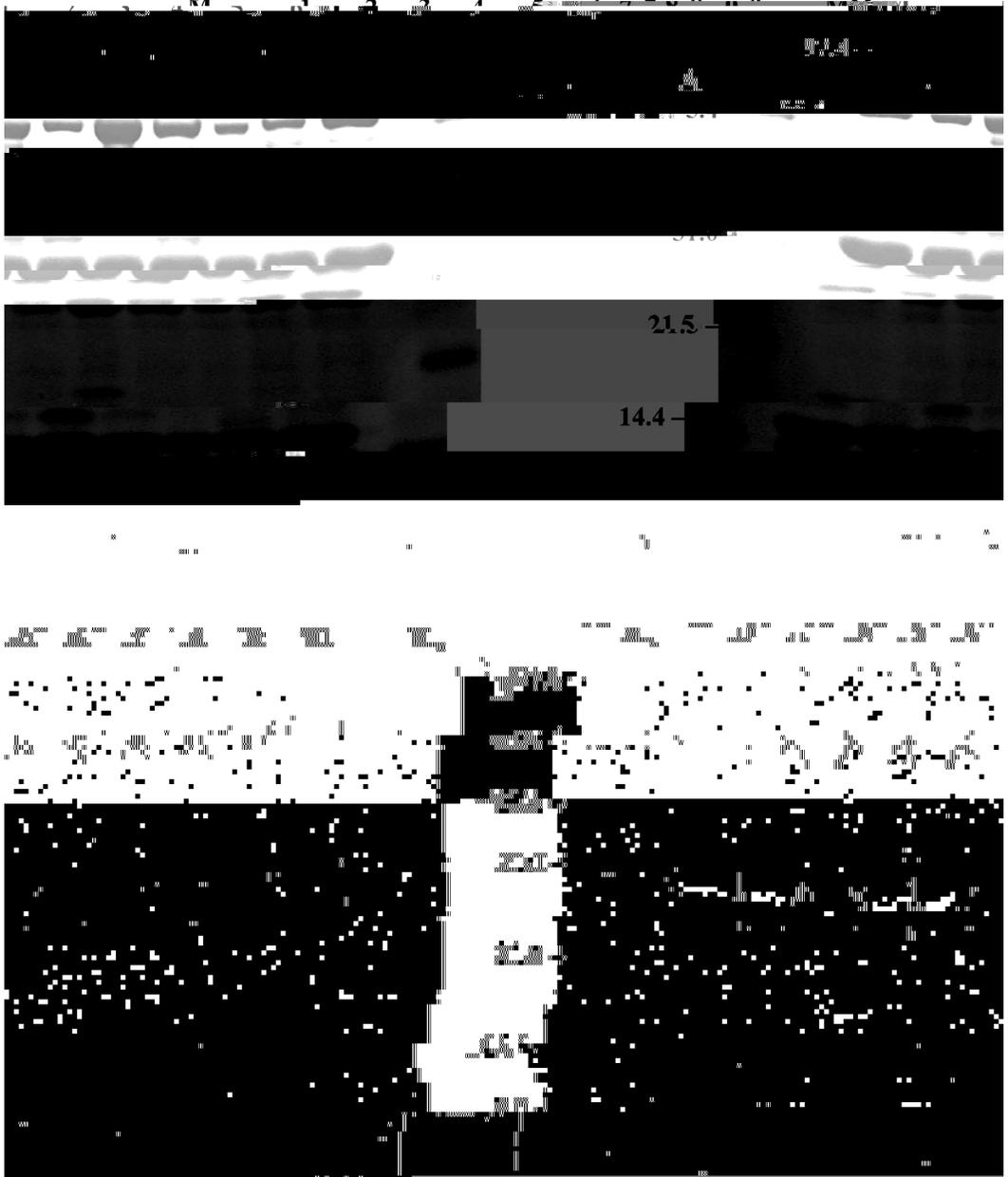


Fig. 4. SDS-PAGE analysis of *C. concolor* crude venoms under reducing conditions. (A) Lanes 1–4, 6–9: adult venoms; lane 5: juvenile venom. (B) Lanes 10–14: adult venoms; lanes 15–20: neonate venoms. Note that the prominent myotoxin band (apparent mass = 6.0 kD) at the bottom of each adult venom lane is very faint in neonate and juvenile venoms.  $M_r$ , Invitrogen Mark 12 protein standards; mass in kilodaltons.

mammals as they increase in size (Fig. 1). This shift in diet occurs at similar body sizes for *C. concolor* and for *C. oreganus* and *C. scottii*, despite the much smaller maximum adult size of *C. concolor*.

Ontogenetic shifts in diet also occur in other populations of *C. concolor* (e.g., Fitch and Twinning, 1946; Diller and Wallace, 1996), but they

do not always involve a major shift in dominant prey type. For instance, juvenile *C. concolor* in Idaho and British Columbia, Canada, feed primarily on shrews (*Peromyscus eremicus* and *M. pennsylvanicus*), whereas adults feed mainly on larger mammals (Macartney, 1989; Wallace and Diller, 1990). Although dominant prey type does not

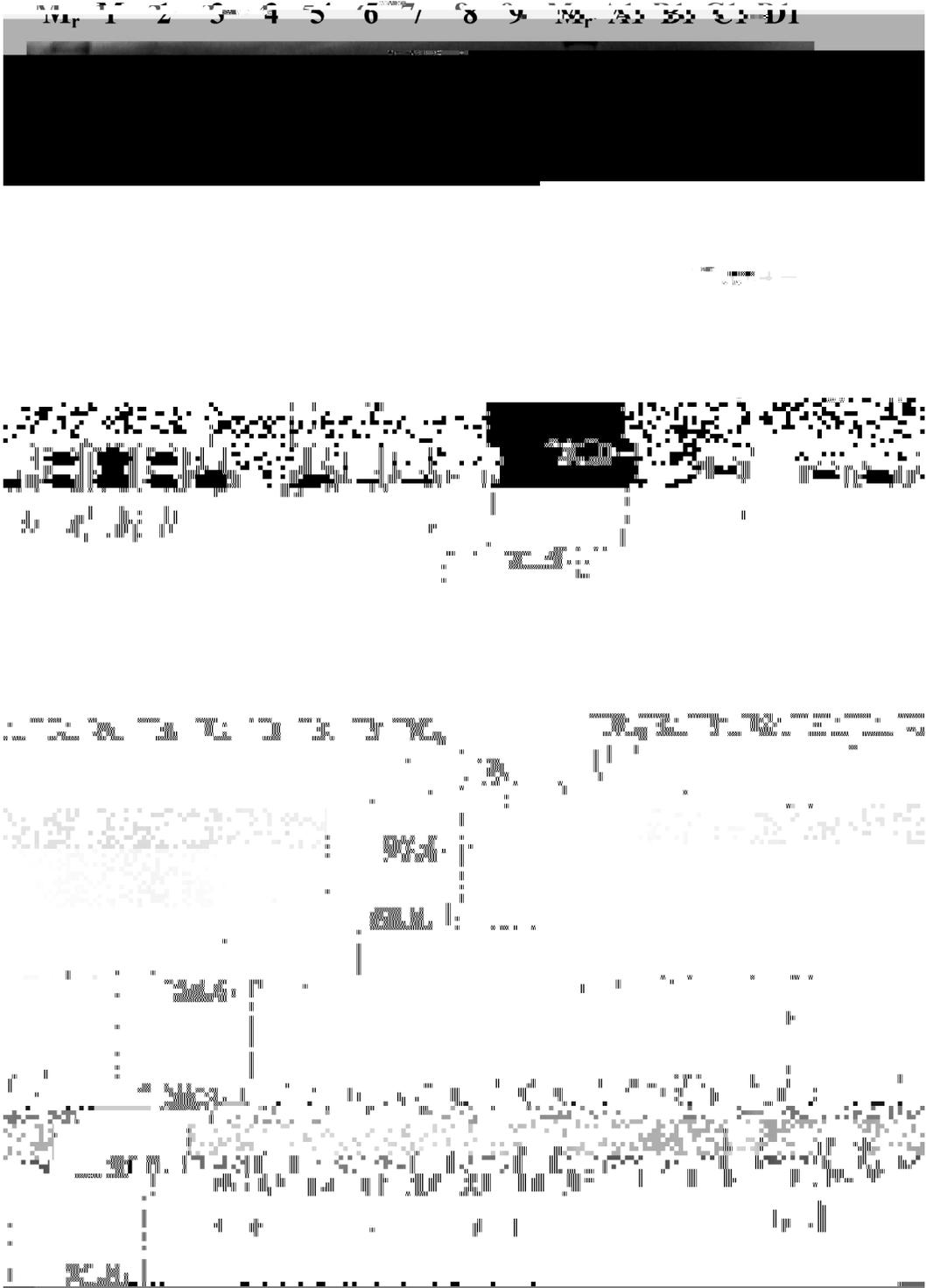


Fig. 5. Zymogram analysis of *C. ...* crude venom metalloprotease activity. (A) Lanes 1-9: neonate venoms; lanes A1-D1: adult venoms. (B) All samples are adult venoms. Bands containing activity are seen as a clear band on the dark background; no clear differences between neonate and adult venoms are apparent. M, Invitrogen Mark 12 protein standards; mass in kilodaltons.





snakes, species with highly toxic venoms (*C. . . . .*, *C. . . . .*, and *C. . . . .*) also show low metalloprotease activity (Glenn and Straight, 1978; Glenn et al., 1983; Gutiérrez et al., 1991). This incompatibility is consistent with the biological roles of metalloproteases including prey predigestion, because much of the venom bolus must remain localized if prey death is very rapid, minimizing distribution of venom in prey tissues. Evolutionarily, venomous snakes have “opted” for either a venom rich in lytic enzymes, which promotes predigestion, or a highly toxic venom which quickly kills prey with high efficacy (but see “intergrade venoms”; Glenn and Straight, 1989). With venom ontogeny in *C. . . . .* and *C. . . . .*, individuals benefit from both strategies; in *C. . . . .*, the necessity to stop prey quickly and with certainty has selected for a high titer of the presynaptic PLA<sub>2</sub>-based  $\beta$ -neurotoxin in this venom, and protease activity is very low. High toxicity venom in this population is not without a trade-off, however, and adult *C. . . . .*

within different populations of the same and closely related species. As trophic adaptations that facilitate numerous aspects of prey handling, venoms have been shaped by many factors impacting snake populations, but high toxicity and high metalloprotease activity appear to be mutually incompatible in most venoms. Venomous snakes are, therefore, constrained to adopt either one or the other strategy, and among some viperids, each strategy occurs at different stages of life history.

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- , AND ———. 1978. Mojave Rattlesnake *C. concolor* venom: variation in toxicity with geographical origin. *Toxicon* 16:81-84.
- , AND ———. 1982. The rattlesnakes and their venom yield and lethal toxicity, p. 3-119. *I* : Rattlesnake venoms: their actions and treatment. A. T. Tu

