Ontogenetic Variation in Venom Composition and Diet of *Crotalus oreganus concolor*: 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*, and at similar body sizes, *C. o. concolor* show an ontogenetic shift in diet. Juvenile snakes primarily feed on small lizards, whereas adults typically consume small rodents. However, *C. o. concolor* do not show the same pattern of venom ontogeny as do *C. o. helleri* and *C. o. oreganus*.

Because of the presence of a phospholipase A_2-based β-neurotoxin (concolor toxin) and several myotoxins, *C. o. concolor* venom is particularly toxic, but mouse LD_{50} assays demonstrated no significant difference in toxicity between adult (0.38 μg/g) and juvenile (0.45 μg/g) venoms. Metalloprotease activity (correlated with extensive tissue damage and prey predigestion) was extremely low in both juvenile and adult venoms. Levels of peptide myotoxins and several serine proteases that interfere with hemostasis (specifically thrombin-like and plasmin-like activities) showed a positive correlation with size. Human envenomations recorded during this study showed symptoms consistent with biochemical analyses, with numbness associated with the bite, coagulation abnormalities and essentially no tissue damage. Results suggest that the occurrence of potent neurotoxic component(s) in a venom minimizes predigestive components (metalloproteases). Further, concurrence of these functional components in the venom of an individual may be selected against, and highly toxic venom in both juvenile and adult *C. o. concolor* may represent a form of venom paedomorphosis.

CHARACTERISTICS of organisms often change over an individual's lifetime. In snakes, ontogenetic shifts in diet are common (Mushinsky, 1987). Probably the most frequent type is a shift from smaller snakes feeding on primarily ectothermic prey to larger snakes feeding on endothermic prey (e.g., Mackessy, 1988; Rodríguez-Robles, 2002; Valdujo et al., 2002). Most rattlesnakes show this type of ontogenetic shift in diet (Klauber, 1956), which is particularly interesting because venom likely initially evolved for securing and digesting prey (Thomas and Pough, 1979). If the diet of an individual rattlesnake changes during its lifetime, then the properties of its venom may also be expected to change. Mackessy (1988, 1993a, 1996) addressed this possibility in a study of *Crotalus oreganus helleri* and *Crotalus oreganus oreganus* (Pacific Rattlesnakes). These subspecies show a distinct ontogenetic shift in diet, from feeding primarily on ectotherms to feeding on endotherms. They also show an ontogenetic shift in venom characteristics; venoms from smaller snakes exhibit higher toxicity, whereas venoms of larger snakes have greater predigestive properties. This shift in venom characteristics occurs at body sizes slightly larger than that for the ontogenetic shift in diet. In other words, venom properties change soon after *C. o. helleri* and *C. o. oreganus* shift from feeding mainly on ectotherms to endotherms.

Crotalus oreganus concolor (Midget Faded Rattlesnake) is a diminutive subspecies found in arid high grasslands of Colorado, Utah, and Wyoming. Typical adult body size seldom exceeds 700 mm, much smaller than other subspecies of *Crotalus oreganus*. Litter sizes are also generally smaller than other subspecies (Klauber, 1956; Ashton, 2001). Venom of *C. o. concolor* is more toxic than most other rattlesnakes (Glenn and Straight, 1977) because of the presence of a presynaptic phospholipase A_2-based β 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 Nedelhov, 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).

Crotalus oreganus concolor, a derived lineage, is nested within the *Crotalus viridis* complex, par-

ticularly with respect to *C. o. helleri* and *Crotalus oreganus oreganus* (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. viridis* 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 recognized here as subspecies are considered full species by other authors (Aird, 1984; Douglas et al., 2002).

The goal of this study is to test whether *C. o. concolor* have ontogenetic shifts in diet and venom characteristics. We compare the occurrence and timing of ontogenetic shifts in *C. o. concolor* to those observed for *C. o. helleri* and *C. o. oreganus* (Mackessy, 1988, 1993a, 1996) to evaluate evolutionary changes in ontogenetic shifts. Populations of *C. o. concolor* have limited contact with other populations of *C. oreganus* and, particularly in southern Wyoming, occur in a harsh environment with a short active season and large daily and seasonal changes in temperature (Ashton and Patton, 2001). Because of the extreme environment encountered by this population, we also address the effect of environment on venom composition. In particular, we predict that venom metalloproteases, responsible for predigestive effects (Mackessy, 1993a, b), are prominent among those components showing ontogenetic changes in activity. We also include information from two cases of human envenomation because they provide further evidence of venom properties.

MATERIALS AND METHODS

To obtain diet information, we examined 192 museum specimens from throughout the range of *C. o. concolor* (Appendix 1). Each specimen was measured (SVL; ± 1 cm), weighed (± 0.1 g), and classified by sex. A midventral incision was made on each specimen to examine stomach contents, and each prey item was removed and identified. Prey mass was gathered for intact prey items and estimated as possible for partially consumed prey by comparisons with museum specimens. Museum specimens presumed to have been held in captivity, in poor condition or of uncertain taxonomic affiliation, were excluded.

Additional prey records were gathered from field captures of *C. o. concolor* near Flaming Gorge National Recreation Area, Sweetwater County, Wyoming. If a snake contained a prey item, it was either forced to regurgitate or was housed until defecation occurred. Feces were then checked for hair or scales, and, when possible, prey was identified to species.

Snakes captured in Sweetwater County, Wyoming, were the source of all venom samples; one adult venom sample (not included here), collected from a snake from central Utah, showed biochemical characteristics identical to other adult venoms. Venom was extracted once from each snake using standard techniques (Mackessy, 1988). Most snakes were returned to capture sites within a week. Several gravid females were retained until parturition, and these newborns were the source of neonate venoms.

Venoms from 36 snakes of all size classes (neonate: < 240 mm, $n = 10$; juvenile: 250–450 mm, $n = 8$; adult: > 475 mm, $n = 18$) were assayed for protein content (Bradford, 1976) and for metalloprotease, thrombin-like, kallikrein-like, plasmin-like, phospholipase A_2, phosphodiesterase (exonuclease) and L-amino acid oxidase activities (see Munekiyo and Mackessy, 1998). All enzyme activity values were corrected for protein content and are expressed as specific activities (amount product formed/min/mg venom protein). An additional 26 venom samples from snakes in the same population collected more recently, and samples taken from two captive animals (all from the Sweetwater population) were used only in the analyses of venom yields and of venom mass/volume relations. Data analysis and presentation were completed using SigmaPlot 2001 for Windows 7.0 (SPSS Inc., Chicago, IL).

Venoms were subjected to reducing SDS-PAGE (Novex 14% acrylamide tris-glycine; Invitrogen, Inc.) to provide a molecular fingerprint of venoms from different age classes of snakes; approximately 35 μg protein was used per lane. Venoms (6 μg protein per lane) were also run on SDS-PAGE zymogram gels (Invitrogen) containing gelatin (a metalloprotease substrate) incorporated into the gel matrix (Heussen and Dowdle, 1980; Munekiyo and Mackessy, 1998). Gels were processed according to manufacturer's instructions, and metalloproteases visualized as a clear band of digested substrate on a dark field. This method provides an estimate of number and sizes of metalloproteases in individual samples.

Samples of adult and neonate snake venoms were fractionated on a Waters HPLC (Milford, MA) operating under Empower Pro software and using a Tosohaas G2000 SWXL size exclu-

TABLE 1. PREY OF *Crotalus oreganus concolor*. Frequency is number of times a prey taxon was recorded with number of snakes that had eaten a particular prey taxon in parentheses. Data are from museum specimens (listed by museum abbreviation and specimen number) and field observations.

Prey taxon	Frequency	% of total prey	Source
MAMMALIA			
Rodentia			
Muridae			
Peromyscus maniculatus	19 (17)	54.3	CM 12368; UCM 19882, 51485, 60074 UU 2357, 3378, 3549; field data
Reithrodontomys megalotis	1 (1)	2.9	CM 42853
Sciuridae			
Tamias minimus	2 (2)	5.7	BYU 2760, 16536
Unidentified mammal	4 (4)	11.4	CAS 38098; MVZ 30312; field data
REPTILIA			
Squamata			
Sceloporus graciosus	2 (2)	5.7	LACM 105202; field data
Sceloporus undulates	2 (2)	5.7	BYU 20751; UCM 7618
Sceloporus sp.	1 (1)	2.9	Field data
Uta stansburiana	1 (1)	2.9	UCM 18110
Teiidae			
Cnemidophorus tigris	1 (1)	2.9	BYU 16535
Cnemidophorus velox	2 (2)	5.7	UCM 18113, 58978
Total	35 (33)		

sion column. Venoms (adult male and combined yields of three neonates) were solubilized in 25 mM HEPES buffer pH 6.8 containing 100 mM NaCl and 5 mM $CaCl_2$ (fractionation buffer). Samples were then centrifuged at 10,000 × g and filtered through a 0.45 μm syringe tip filter to remove particulates. Two hundred μl (approximately 2 mg adult venom, 2.5 mg neonate venom) was injected on the column at a fractionation buffer flow rate of 0.3 ml/min and monitored at 280 and 220 nm, and one minute fractions (0.3 ml) were collected. This method provides highly repeatable fractionation patterns. Fractions were evaluated electrophoretically (14% Novex gels) to determine composition of specific protein peaks.

A small subset of juvenile and adult venom samples were evaluated for lethal toxicity (24-h intravenous LD_{50}) using 25–30 g female NSA mice. All injections were adjusted to body mass and administered in 100 μl 0.9% saline via the caudal vein.

We received two accounts of human envenomation during the course of this study. A 15-year-old male (5 ft. 10 in., 170 lbs.) was bitten on top of the foot while walking in the vicinity of Squaw Hollow near Flaming Gorge Reservoir, Sweetwater County, Wyoming, in summer of 1998; this appears to be a legitimate bite (sensu Hardy, 1986), since the snake was neither seen nor handled. The foot was put on ice and the patient was taken to the nearest hospital (30 min away) for observation and treatment. The second patient (14-year-old male) was bitten in the left index finger at approximately 7:00 P.M. while camping near Moab, Utah, in fall of 2000; this bite also appears to be legitimate. He received first aid and a tetanus vaccination in Moab shortly after the bite and then was transported to Grand Junction, Colorado, by ambulance, arriving four hours after the bite.

RESULTS

Thirty-three *C. o. concolor* contained 35 total prey items (Table 1; 19 museum, 14 field records). All individuals except one contained a single prey item; the exception was one adult that had consumed three *Peromyscus maniculatus*. Each prey item was swallowed headfirst. Mammals comprised 75% of all prey records, whereas phrynosomatid and teiid lizards accounted for the remaining 25% (Table 1). The most common prey (53% of all records) was *P. maniculatus*. Prey mass was positively related to snake mass ($n = 14$, $r^2 = 0.48$, $P < 0.01$). Prey shape (body length/mass) was negatively related to snake mass ($n = 13$, $r^2 = 0.40$, $P < 0.05$), indicating that larger snakes ate relatively bulkier prey. Lizards were the primary prey of juve-
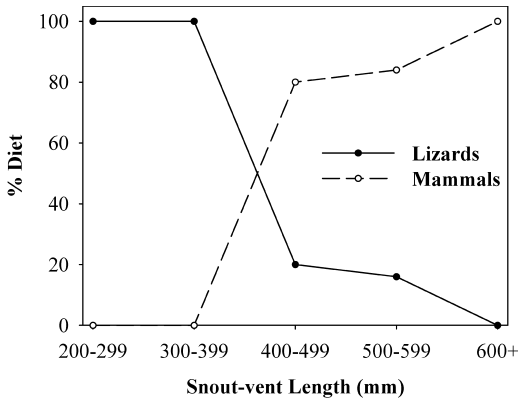
Fig. 1. Diet as a function of length in *Crotalus oreganus concolor*. There is a strong dependency of neonate and young snakes on lizards, followed by a switch to mammalian prey as snakes mature.

nile *C. o. concolor*, whereas larger *C. o. concolor* fed mostly on small mammals (Fig. 1). Typical prey of adult *C. o. concolor* were 15–40 g rodents (*Peromyscus* and *Tamias*), and the only observation of larger prey (*Neotoma*) taken occurred in mid-July.

Snake mass showed a close exponential relationship with snake total length ($n = 67$, $r^2 = 0.92$; Fig. 2A), indicating that length measures provide a relevant and consistent measure of overall snake size. Venom volume yields increased exponentially with snake length ($n = 69$, $r^2 = 0.69$; Fig. 2B). The relationship between venom volume and venom mass ($n = 25$, $r^2 = 0.79$; Fig. 2C) appeared to be linear.

Venom enzyme activities varied with size (Table 2). Plasmin-like protease (Fig. 3A), thrombin-like protease (Fig. 3B) and phosphodiesterase (Fig. 3D) activities showed a significant positive relationship with body size, whereas kallikrein-like protease (Fig. 3C), L-amino acid oxidase (Fig. 3G) and phospholipase A_2 (Fig. 3H) activities showed no apparent relationship with size. Metalloprotease activity (azocasein, Fig. 3E, and hide powder azure, Fig. 3F) showed a significant negative relationship with size; however, overall activity levels were quite low.

Reduced venoms showed 16–20 protein bands ranging in molecular mass from ~100 kD to < 6 kD (Fig. 4A—B). In general, patterns from adult and neonate snakes were similar; however, several high molecular weight bands were missing from neonate venoms, and the low molecular weight band (myotoxins; Engle et al., 1983) was faint in all neonate and juvenile venoms but prominent in all adult venom samples (Figs. 4A—B). These myotoxins have an actual molecular mass of approximately 4.8 kD, but
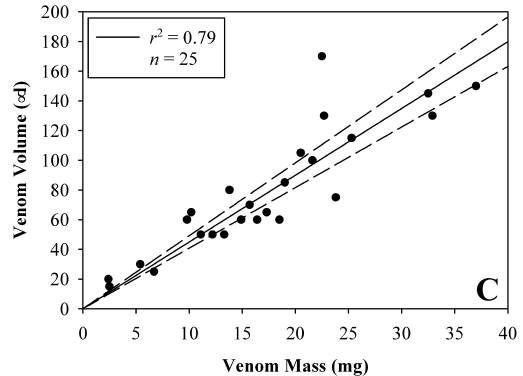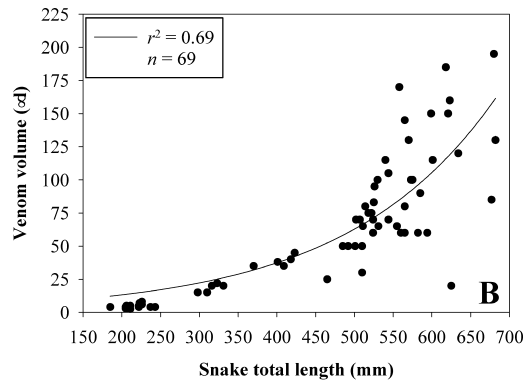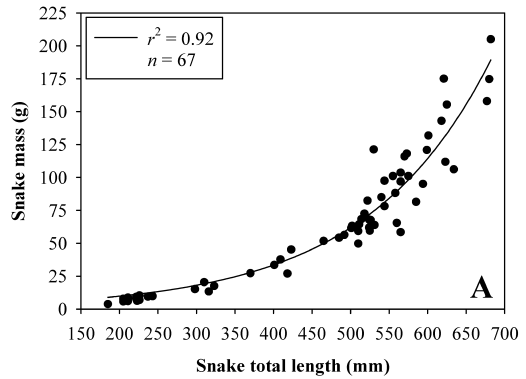

Fig. 2. (A) Relationship of snake mass to snake total length. (B) Relationship of venom volume to snake total length. (C) Relationship of venom volume to venom mass. Solid lines are regression lines, and dashed lines in C indicate 95% confidence intervals.

like many basic proteins, they migrate somewhat anomalously on SDS-PAGE, giving an apparent mass of ~6 kD.

Zymogram analysis revealed at least four gelatin-degrading proteases in *C. o. concolor* venom (Fig. 5A—B); the presence/absence of these proteins in individual venoms showed individual variation not associated with ontogeny. Unlike most other rattlesnake species, which pro-

TABLE 2.   ENZYME ACTIVITIES FROM NEONATE, JUVENILE AND ADULT *Crotalus oreganus concolor* 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$_2$	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. o. concolor* 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$_2$-based β-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$_{50}$ was 0.38 μg/g. Lethal toxicity of juvenile venom in mice was 0.45 μg/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, Wyeth polyvalent antivenin was administered. Symptoms in general resolved within one hour after antivenin administration, but the leg remained swollen for three days after the bite. No hemorrhage or necrosis at the bite site was noted, and no permanent damage appeared to result.

The second case of envenomation (Moab, Utah) occurred in the tip of the left index finger. When observed one hour after the bite, the patient complained of numbness around the mouth and at the back of the throat, and the finger was slightly swollen; three hours later, he experienced throbbing pain with minor edema in the left hand, numbness from the shoulders down on both sides, mild pain just below the ribs on the right side and very minor ecchymosis (small subcutaneous hemorrhage) at the bite site. A coagulation profile taken approximately five hours after the bite was markedly abnormal, with fibrinogen levels < 60, fibrin degradation products (FDP) > 640 μg/ml, a prothrombin time (PT) of > 120 sec, an international normalization ratio (INR) > 9.4, activated partial thromboplastin time (APPT) of > 120 sec and D-dimer levels at 1.6–3.2 μg/ml. All of these clinical tests indicated severe disruption of the capacity to form blood clots normally, and the patient appeared at risk of developing disseminated intravascular coagulation (DIC) syndrome, a potentially fatal condition. The patient received 10 vials of Wyeth antivenin approximately 8 h after the bite (complicated by an allergic reaction), and over the next 24 h, 30 more vials were administered. Fibrinogen levels, PT, APPT, and FDP remained at these abnormal levels for ~30 h, in spite of the administration of antivenin, but these signs resolved rather suddenly approximately 38 h after the bite.

DISCUSSION

Similar to many other rattlesnakes (Klauber, 1956), including *C. o. helleri* and *C. o. oreganus* (Mackessy, 1988), ontogenetic changes in diet occur in *C. o. concolor*. Small *C. o. concolor* feed mainly on lizards and then switch to mostly
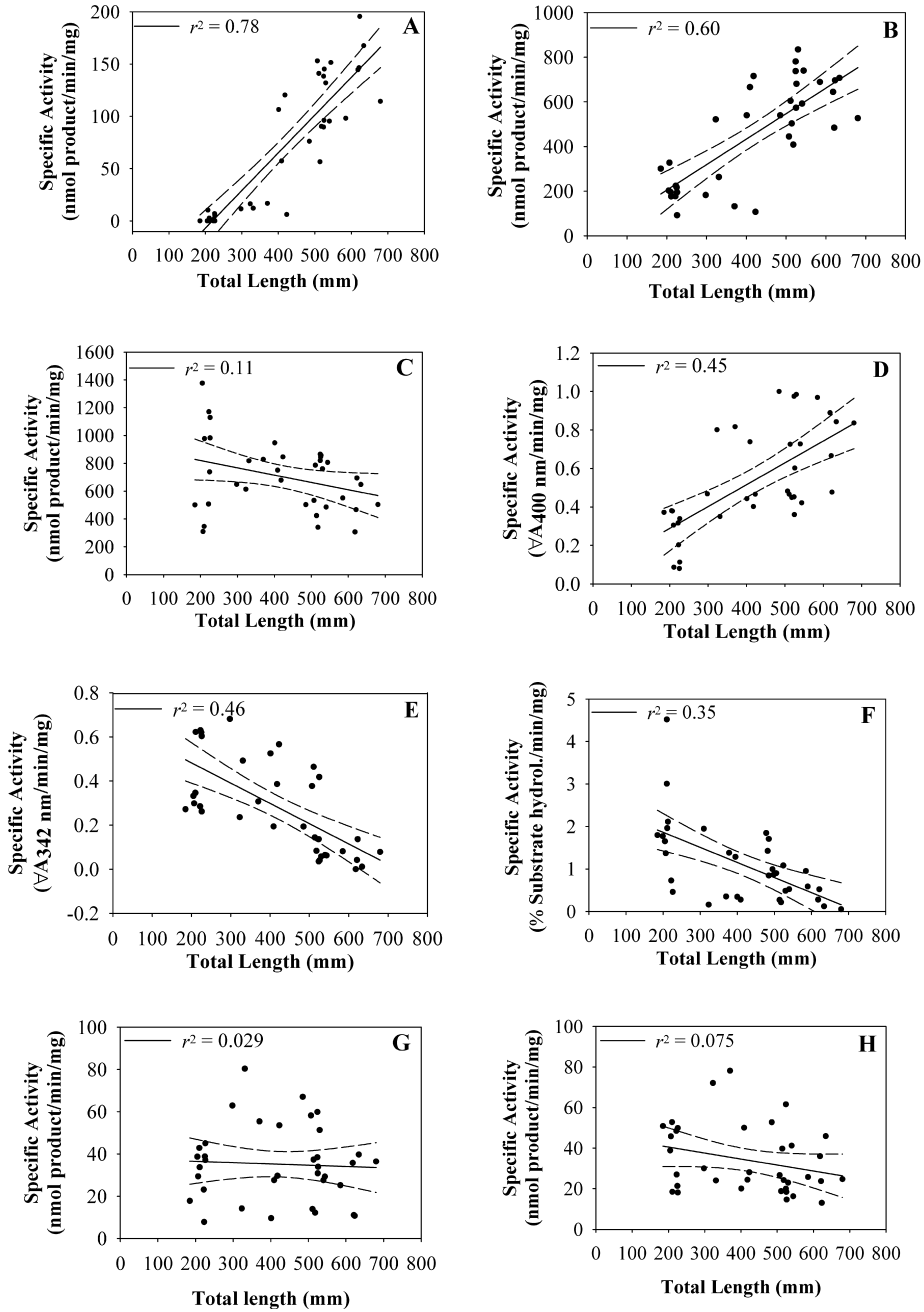
Fig. 3. Enzyme activities of crude venoms of *Crotalus oreganus concolor* as a function of snake total length. All activities are normalized to total protein content of venoms and are expressed as specific activities. (A) Plasmin-like activity; (B) thrombin-like activity; (C) kallikrein-like activity; (D) phosphodiesterase activity; (E) azocasein metalloprotease activity; (F) hide powder azure metalloprotease activity; (G) L-amino acid oxidase activity; (H) phospholipase A$_2$ activity. Solid lines are regression lines, and dashed lines indicate 95% confidence intervals.
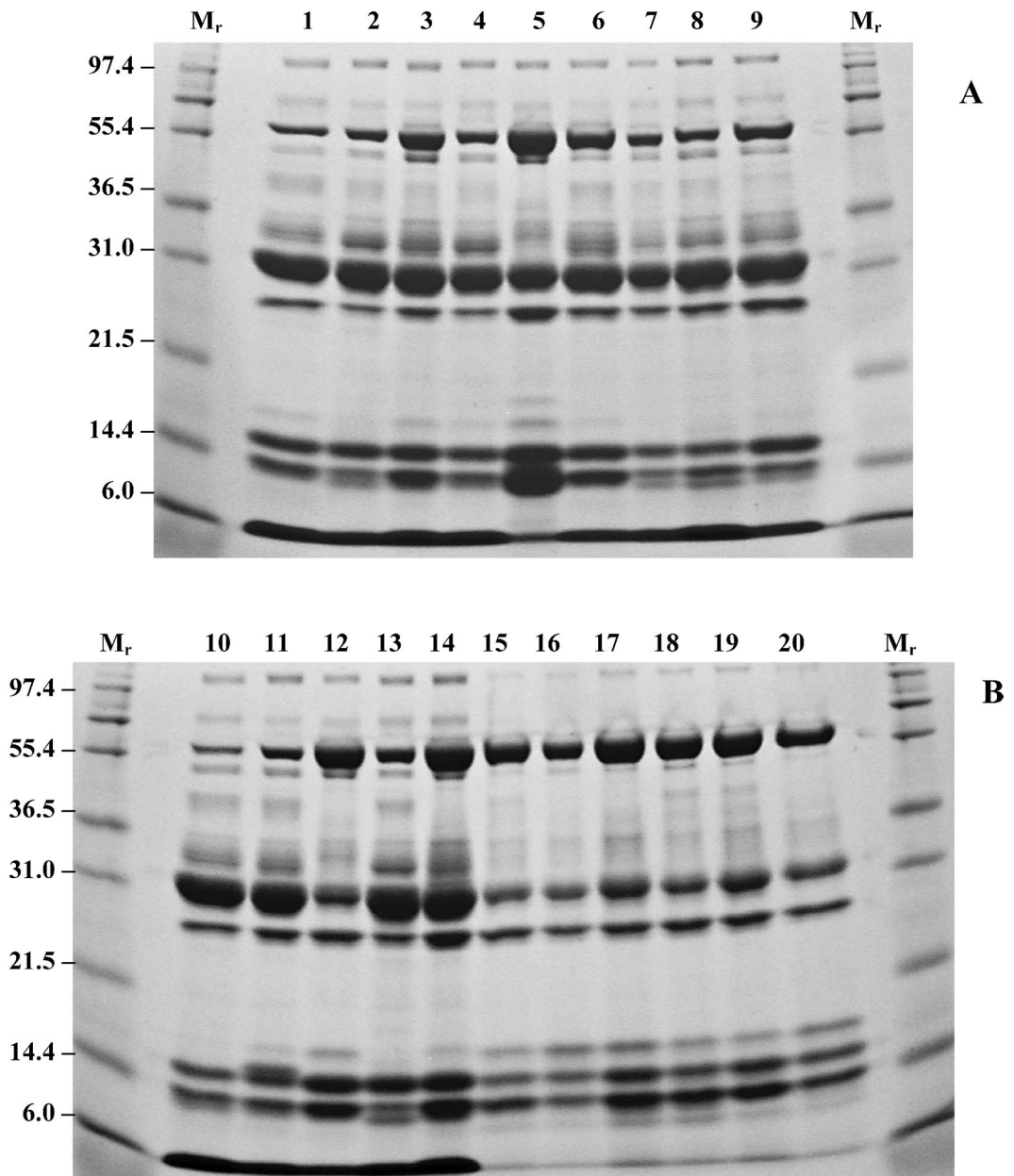
Fig. 4. SDS-PAGE analysis of *Crotalus oreganus concolor* crude venoms under reducing conditions. (A) Lanes 1–4, 6–9: adult venoms; lane 5: juvenile venom. (B) Lanes 10–14: adult venoms; lanes 15–20: neonate venoms. Note that the prominent myotoxin band (apparent mass = 6.0 kD) at the bottom of each 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. o. concolor* and for *C. o. helleri* and *C. o. oreganus*, despite the much smaller maximum adult size of *C. o. concolor*.

Ontogenetic shifts in diet also occur in other populations of *C. oreganus* (e.g., Fitch and Twining, 1946; Diller and Wallace, 1996), but they do not always involve a major shift in dominant prey type. For instance, juvenile *C. o. oreganus* in Idaho and British Columbia, Canada, feed primarily on shrews (*Sorex cinereus and Sorex vagrans*) and juvenile mammals (*Peromyscus* and *Microtus*), whereas adults feed mainly on larger mammals (Macartney, 1989; Wallace and Diller, 1990). Although dominant prey type does not
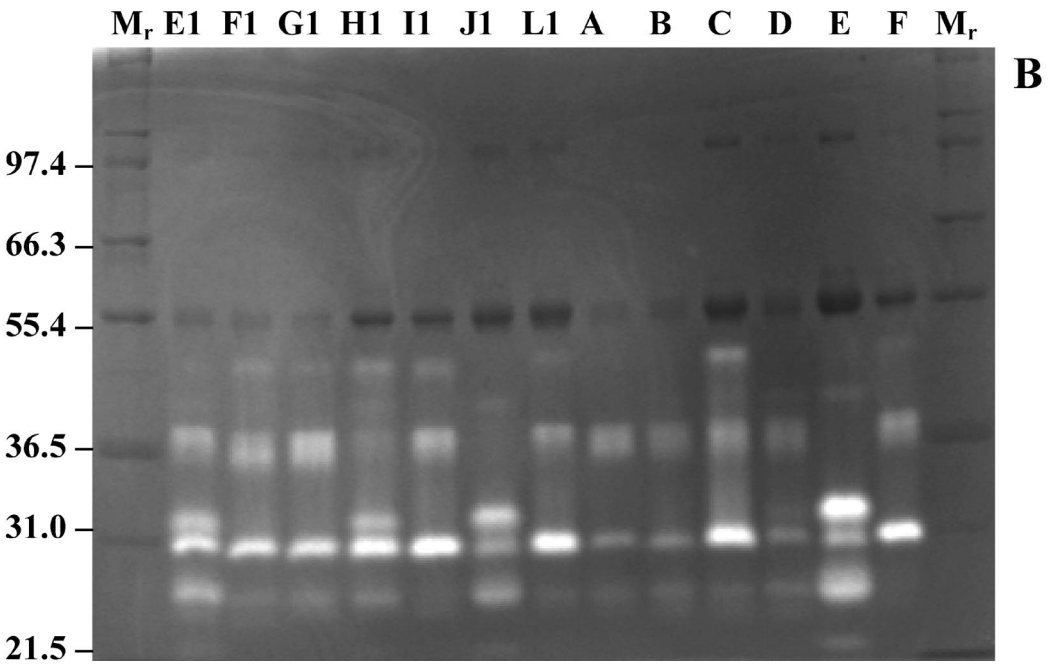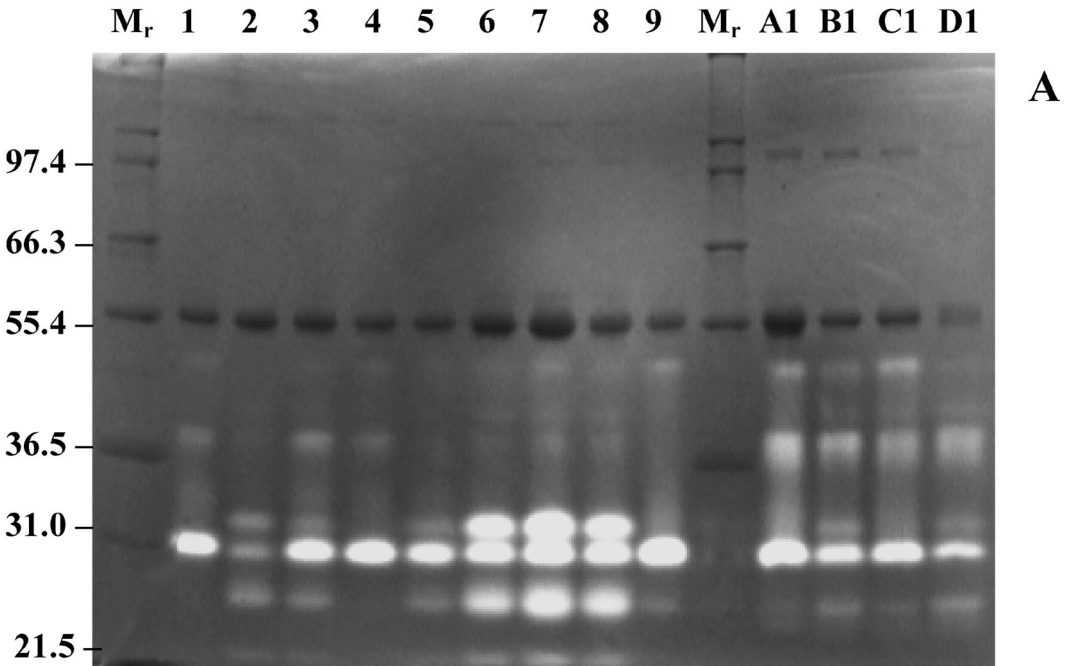
Fig. 5. Zymogram analysis of *Crotalus oreganus concolor* crude venom metalloprotease activity. (A) Lanes 1–9: neonate venoms; lanes A1–D1: adult venoms. (B) All samples are adult venoms. Bands containing activity are seen as a clear band on the dark background; no clear differences between neonate and adult venoms are apparent. M_r, Invitrogen Mark 12 protein standards; mass in kilodaltons.
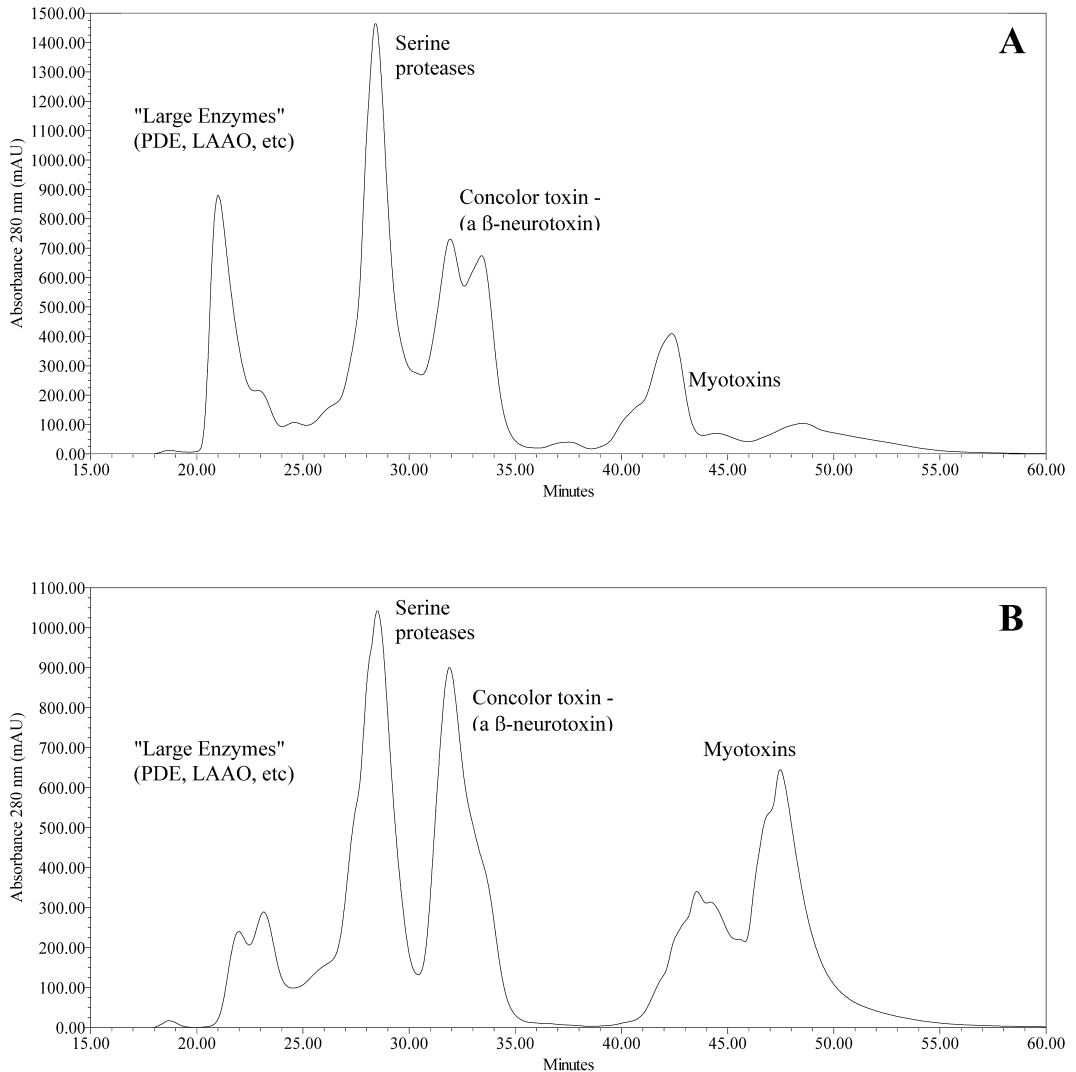
Fig. 6. Size exclusion HPLC fractionation of crude venom from (A) neonate and (B) adult *Crotalus oreganus concolor*. SDS-PAGE analysis of peak fractions was used to identify proteins present in each sample. The concolor toxin peak in A fractionated as two peaks with identical SDS-PAGE migration patterns; these may represent isoforms. Note that the myotoxin peak is greatly diminished in neonate venom (A), despite the greater total protein load. PDE, venom phosphodiesterase; LAAO, L-amino acid oxidase.

change with body size in these populations, larger snakes eat larger, more bulky prey as was observed for *C. o. concolor* (this study; Macartney, 1989; Wallace and Diller, 1990).

Venom yields were comparable to those obtained for other subspecies of *C. oreganus* of similar size (e.g., Glenn and Straight, 1982; Mackessy, 1988). As observed with *C. o. helleri* and *C. o. oreganus*, venom yields increased exponentially with size; the somewhat weaker association (r^2 = 0.69) was largely because of loss of venom during the extraction process, particularly for adult snakes. A study by W. K. Hayes (unpubl.

data, mentioned in Hayes et al., 2002) indicated that adult *C. o. concolor* injected similar quantities of venom into mouse (5.7 mg) and lizard (6.2 mg) prey, representing approximately 15–30% of the total venom yield for adult snakes.

Ontogenetic changes in venom composition for *C. o. concolor* from the Sweetwater County, Wyoming, area are different from those observed in *C. o. helleri* and *C. o. oreganus*. Metalloproteases, important to the predigestive roles of venoms (Thomas and Pough, 1979; Mackessy, 1988, 1993a), showed a significant *decrease* with size in *C. o. concolor*, whereas this activity showed
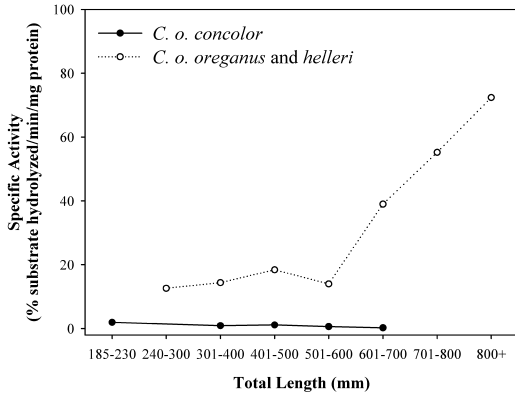
Fig. 7. Comparison of metalloprotease activity (toward hide powder azure) of crude venoms from *Crotalus oreganus concolor* (this study) and *Crotalus oreganus helleri* and *Crotalus oreganus oreganus* (data from Mackessy, 1988); spacing for the two smallest size classes is not linear with respect to the other size classes. Note that activities for samples of *C. o. concolor* venom for all size classes are below the lowest levels seen in Pacific Rattlesnake venoms.

a fivefold *increase* with size in venoms from *C. o. helleri* and *C. o. oreganus* (Mackessy, 1988). Other North American rattlesnakes (*Crotalus atrox, Crotalus mitchelli pyrrhus, Crotalus molossus molossus, Crotalus ruber*) also show a large ontogenetic increase in activity of these proteases (Mackessy, 1985, unpubl. data). Phospholipase A_2 activity, which decreased approximately fourfold with size in *C. o. helleri* and *C. o. oreganus*, showed no significant size-related variation in activity for *C. o. concolor*. These results were contrary to initial predictions and suggest that ontogeny of venom composition for *C. o. concolor* is different than that observed for many conspecifics and congenerics.

Comparative analysis of hide powder azure proteolytic data (Mackessy, 1988; this study) showed that although metalloprotease activity in *C. o. concolor* venom does decrease with size, the highest values (neonates) are below the lowest values (neonates and juveniles) for *C. o. helleri* and *C. o. oreganus* (Fig. 7). The prominent increase in activity with body size seen in venoms of *C. o. helleri* and *C. o. oreganus*, and those of other large species of rattlesnakes (e.g., Mackessy, 1985, 1988), does not occur in *C. o. concolor*. Therefore, although values appear to decrease with age, metalloprotease activity is already at low values and then declines slightly. Additionally, venom toxicity for both juvenile and adult *C. o. concolor* is extremely high (approximately 5–10 times greater than *C. o. helleri* and *C. o. oreganus*), and prey death occurs rapidly. Although several components do show age-related changes in activity, toxicity does not vary ontogenetically as was observed with *C. o. helleri* and *C. o. oreganus* venoms (Mackessy, 1988) and *C. v. viridis* venoms (Fiero et al., 1972).

Venoms from subspecies of *C. oreganus* show ontogenetic patterns of venom composition similar to those seen in other viperids. For example, venoms from *C. o. helleri* and *C. o. oreganus* (Mackessy, 1988) undergo an ontogenetic shift in composition similar to that seen in *Crotalus durissus durissus* (Gutiérrez et al., 1991). Juvenile *C. d. durissus* venom was also similar in composition (high toxicity, low protease activity) to venom from adult *Crotalus durissus terrificus*, which produces life-threatening neurotoxic symptoms in human envenomations. Similar to *C. o. concolor*, a lack of ontogenetic toxicity change was observed for venoms from *Bothrops alternatus*, which feed primarily on endotherms; however, there were toxicity differences between venoms of adult and juvenile *Bothrops jararaca* (Andrade and Abe, 1999). Some species of *Bothrops* showed ontogenetic shifts in venom, whereas others did not, and aspects of *Bothrops* venom toxicity appeared to be related to diet.

Information on ontogenetic changes in diet and venom of *C. o. concolor* (this study) and *C. o. helleri* and *C. o. oreganus* (Mackessy, 1988) facilitates inferences about the evolution of ontogenies, because *C. o. helleri* and *C. o. oreganus* are basal to *C. o. concolor* within the *C. viridis* group (Ashton and de Queiroz, 2001; Douglas et al., 2002). *Crotalus oreganus concolor, C. o. helleri*, and *C. o. oreganus* undergo ontogenetic changes in diet and do so at similar body sizes (Fig. 1; fig. 4 of Mackessy, 1988), indicating that sizes at which ontogenetic shifts in diet occur may be relatively fixed. Unfortunately, information on sizes at which ontogenetic shifts take place is not available for other populations of *C. oreganus*. In contrast to diet, ontogenies of venom characteristics are much different. Venoms of *C. o. concolor* of all sizes have low metalloprotease activity, no change in phospholipase A_2 activity, and high toxicity. These characteristics, specifically low protease activity and high toxicity, resemble juvenile, but not adult, venom characteristics of *C. o. helleri* and *C. o. oreganus*. This constitutes a case of venom paedomorphosis (= paedogenesis of Reilly et al., 1997).

In snake venoms generally, high concentrations of potent neurotoxins and high metalloproteolytic activity appear to be incompatible. Among elapid snakes, which generally produce venoms containing numerous neurotoxins (e.g., Mackessy and Tu, 1993; Tu, 1998), metalloproteolytic activity is typically very low (Tan and Ponnudurai, 1990, 1991, 1992). Among rattle-

snakes, species with highly toxic venoms (*C. d. terrificus*, *Crotalus scutulatus*, and *Crotalus tigris*) 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. o. helleri* and *C. o. oreganus*, individuals benefit from both strategies; in *C. o. concolor*, the necessity to stop prey quickly and with certainty has selected for a high titer of the presynaptic PLA$_2$-based β-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. o. concolor* may be constrained by climate and venom properties to feeding primarily on smaller mammalian prey. *Crotalus viridis viridis*, which are nearly sympatric with *C. o. concolor* and occur much farther northward on the Great Plains (Klauber, 1956), produce a venom with many of the same components as *C. o. concolor*. However, venom of *C. v. viridis* lacks the presynaptic neurotoxin and contains much higher metalloprotease content and activity than venom of *C. o. concolor* (Gleason et al., 1983; Ownby and Colberg, 1987; SPM, unpubl. data).

The cases of human envenomation are also consistent with low metalloprotease activity, as tissue damage (other than swelling) did not occur, and several symptoms (numbness, lack of coordination) suggest involvement of a neurotoxin. Rattlesnake envenomations of humans often result in extensive tissue necrosis and permanent damage to muscle (e.g., Russell, 1980; Gutiérrez and Rucavado, 2000), but these symptoms are generally lacking in bites by species with a high neurotoxin content in the venom. Serine proteases, which include fibrinogen-depleting activity (thrombin-like protease) and clot-degrading activity (plasmin-like protease), are common among rattlesnake venoms (Markland, 1998; Pirkle, 1998; Braud et al., 2000) and are very prominent in *C. o. concolor* venom, and these activities were responsible for the potentially life-threatening coagulation disorders observed in one envenomation victim. Injected into prey (and unwary humans), these serine proteases likely promote systemic effects of other toxins by eliminating clot formation, allowing the circulatory system to distribute components rapidly.

In evolutionary arms races between predators and prey (cf. Heatwole et al., 2000), single component venoms could lead to selection for resistance in prey, and smaller specific neurotoxins (such as concolor toxin) may be easily detoxified by resistant prey. The very potent α-neurotoxins prevalent in elapid venoms are ineffective against conspecifics and many other snakes because of relatively minor mutations in the acetylcholine receptor α subunits, and irreversible binding (resulting in muscular paralysis) does not occur (Ohana et al., 1991; Servent et al., 1998). Prey resistance responses are an important driving force maintaining venom compositional complexity and requiring doses for native prey that are orders of magnitude above LD$_{50}$ values (see also Chiszar et al., 1999). For inbred mice, the experimental LD$_{50}$ of *C. o. concolor* venom was < 0.5μg/g, but based on an unpublished study (Hayes et al., 2002), adult *C. o. concolor* when feeding inject mice with 570 times and lizards with 2480 times this dose of venom. Assuming that *C. o. concolor* are metering venom delivery (e.g., Hayes et al., 1995), these data indicate that native prey, particularly lizards, are much more resistant than inbred mice to *C. o. concolor* venom. Further, antihemostatic venom proteases (thrombin-like, plasmin-like, kallikrein-like), which affect regulation of blood clotting and pressure, and venom phosphodiesterase, which hydrolyzes second messengers such as cAMP and depletes ATP/ADP levels, are likely more effective against the highly regulated physiology of mammalian prey. The ontogenetic increase of these activities seen in *C. o. concolor* venom may increase the potency of the venom and disrupt selection for resistance to venom in prey. The ontogenetic increase in myotoxin content is also likely directed toward mammalian prey, as suggested by its near absence from neonate venoms. Retention of other typical rattlesnake venom components, including L-amino acid oxidase (apoptosis induction), metalloproteases (structural protein degradation, hemorrhage, predigestion) and phospholipase A$_2$ (membrane disruption, disruption of platelet aggregation, production of second messenger compounds, etc.), ensures that prey do not typically survive successful envenomation. This "shotgun effect" overwhelms homeostatic mechanisms of prey, and rapid immobilization and death result.

Venom composition in front-fanged snakes follows several well-defined patterns, and among rattlesnakes, specifically *C. oreganus* and *C. viridis*, the two dominant strategies occur

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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APPENDIX 1

Material examined (museum abbreviations follow Leviton et al., 1985): AMNH 58252, 58253, 64841, 68500, 112939, 115633; BYU 120, 361, 364, 575, 1636, 1670, 1672, 1675, 2760, 12962–12964, 14699, 14923, 16534–16537, 20751, 21392, 21489, 23802, 31187, 34661, 34662, 36928, 37100, 37677, 38375, 39687, 41653, 41746; CAS 38098, 103050, 148648, 170409–170411, 170416, 170417, 170426, 170434, 170435; CM 1429, 6162, 11307, 12342, 12355, 12368, 12426, 12427, 12451, 42850–42854; FMNH 2791, 4900, 25272, 25731 (2), 25732 (2), 25741, 28495, 62898; KU 23596; LACM 76506, 105186, 105201, 105202; MSB 44592, 44618, 44647; MVZ 17891, 17894, 21804, 28153, 30310–30314; SDSU 3895; UCM 452, 5790, 7618, 10136, 11586, 16937, 18110–18115, 19757, 19758, 19760, 19761, 19882, 21725, 47805, 51485, 51867, 51936, 55629, 56198, 56206, 57059, 58977, 58978; UMMZ 62143, 68612, 121412, 181699, 205012, 205038, 205053–205056; USNM 40195, 48680; UTA 2008, 5536; UU 876, 962, 1133, 1134, 1137–1139, 1141–1143, 1146–1152, 1157, 1159, 1160, 162, 1197–1201, 1304, 1311, 1359, 2357, 2359, 2833, 2852, 2858, 2859, 2868, 2875–2877, 2878–2884, 3321, 3323, 3378, 3548–3553, uncataloged (4).