The Biology of Rattlesnakes II

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Dust jacket illustration
An adult Tiger Rattlesnake (*Crotalus tigris*) set beautifully in its Sonoran desert habitat. Tiger Rattlesnakes occur from south-Central Arizona into southern Sonora, Mexico. The image titled, "Tiger Rattlesnake (*in situ*)," was painted by Tell Hicks and commissioned for the cover of Biology of the Rattlesnakes II. Limited edition prints of this painting are available at http://tellhicksprints.weebly.com/index.html.

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The Desert Massasauga (*Sistrurus tergeminus edwardsii*) in Colorado: From Biome to Proteome

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ABSTRACT.—The Massasauga (*Sistrurus catenatus*) is a small rattlesnake that occurs in grasslands of North America from northern Mexico to southeastern Canada. Although threatened in many parts of its range, the diminutive Desert Massasauga (*S. t. edwardsii*) remains abundant at several locations in more mesic regions of the shortgrass steppe of southeastern Colorado. Our numerous studies of the ecology/natural history and venom biochemistry/genomics make Desert Massasaugas one of the better-characterized species of rattlesnakes, and this summary examines the interplay of animal biology and venom biochemistry. Snakes were collected primarily on a private ranch in southeastern Colorado and were processed in the lab (morphometrics, venom extraction, and PIT-tagging; 12 snakes were also implanted with radiotransmitters for telemetry studies). Massasaugas were radiotracked for two years during the active season to analyze spatial ecology and habitat use. Venoms were subjected to a variety of biochemical and proteomic analyses, and two snakes were sacrificed for transcriptomics studies. Massasaugas make strongly directional migratory movements that are resource-driven. Abundant prey (lizards, centipedes, rodents) and favorable thermoregulatory sites occur in the summer (sand hills) habitat, while stable hibernacula exist in the winter (shortgrass) habitat. Long-term mark/recapture studies indicate that Desert Massasaugas are abundant, but short-lived, with average adult survivorship of <4 years. Proteomic and genomic analyses indicate that a crotoxin homolog, characteristic of other type II rattlesnake venoms, is not expressed in this species, but genes for 5 isoforms of three-finger toxins (3FTXs) are present. Unlike most viper venoms, only one isoform of an acidic PLA_2 is present in venom of *S. t. edwardsii*. The Desert Massasauga is one of only a few species of viperids demonstrated to possess genes for 3FTxs, a protein family that includes the potent α-neurotoxins of elapids and several taxon-specific neurotoxins of some rear-fanged snakes. However, 3FTxs do not appear to be expressed in the venom. Based on 2D SDS-PAGE, over 100 proteins comprise this venom, and serine proteinases (thrombin-like, kallikrein-like) are abundant components that may contribute to high venom lethality in mice and lizards. The Desert Massasauga in Colorado is an excellent model species for evaluating influences of numerous ecological factors on venom evolution, and continuing studies are investigating population levels of venom and genetic variation.

INTRODUCTION

Snakes of the genus *Sistrurus* are characterized by the presence of nine enlarged scales on top of the head, a meristic character that distinguishes them unequivocally from all other rattlesnakes in the United States. Within the Massasauga (*Sistrurus catenatus*) complex, three subspecies have been distinguished morphologically: the Eastern Massasauga (*S. c. catenatus*), the Western Massasauga (*S. c. tergeminus*), and the Desert Massasauga (*S. c. edwardsii*) (Klauber 1936, Gloyd 1940, Gloyd 1955, Klauber 1956, Conant and Collins 1991). Klauber (1936) described only the eastern and western subspecies; Conant and Collins (1991) included snakes from extreme southeastern Colo-

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rado, the plains of central and southern New Mexico, and extreme southeastern Arizona. Gloyd (1955) reviewed the Massasaugas of the southwestern United States and included the only known specimen from Colorado (unspecified locality) as a Desert Massasauga. This specimen is now known to have been collected in 1882 by Mr. A. E. Beardsley in Baca County, Colorado, and it is listed as voucher #96-265 in the Colorado State Normal College (now University of Northern Colorado) museum register (Mackessy et al. 1996). Wright and Wright (1957) then described specimens from western Missouri and southeastern Nebraska to southeastern Arizona and extreme northern Mexico as the western subspecies. Massasaugas in Colorado were considered Western Massasaugas until Maslin (1965) described them as an intergrade between western and desert subspecies. While Maslin's classification of Massasaugas in Colorado has been considered valid since that time (e.g., Conant and Collins 1991), Maslin himself indicated that a more thorough investigation was needed and emphasized the need for more material. Maslin (1965) further noted that "scale characters of the Colorado population may be so distinctive that nomenclatural recognition of this biological entity might be justified". Based on results of a morphological study done at University of Northern Colorado (Hobert 1997) in which 345 Massasaugas from Colorado, Arizona, New Mexico, and Kansas were analyzed, the Massasaugas in Colorado should be considered Desert Massasaugas. Kubatko et al (2011) considered *S. c. edwardsii* and *S. c. tergeminus* to be closely related and distinct from *S. c. catenatus*, based on nuclear and mitochondrial DNA loci. More recently (2013), the International Commission on Zoological Nomenclature revised the names for Massasaugas, with Massasaugas occurring west of the Mississippi River referred to *Sistrurus tergeminus*. The Desert Massasauga is now be assigned to *Sistrurus tergeminus edwardsii*. It is recommended here that if the assignment of *S. tergeminus* is retained, subspecies distinction should also be retained for the Desert Massasauga, based on differential scale counts (above), diminutive size, venom characteristics (Milne and Mackessy, unpubl. data) and the distinct distribution/habitat that it occupies (relative to either *S. t. tergeminus* or *S. c. catenatus*). In this chapter, the Desert Massasauga will be referred to as *S. t. edwardsii*.

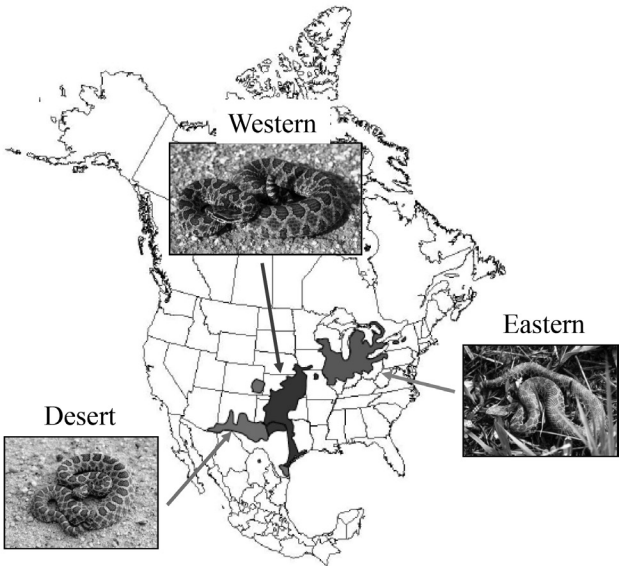


Figure 1. Distribution of the Massasauga Rattlesnake (*Sistrurus catenatus*) in North America. Note that ranges are not continuous over areas indicated for each of the subspecies. Reprinted from Mackessy 2005.

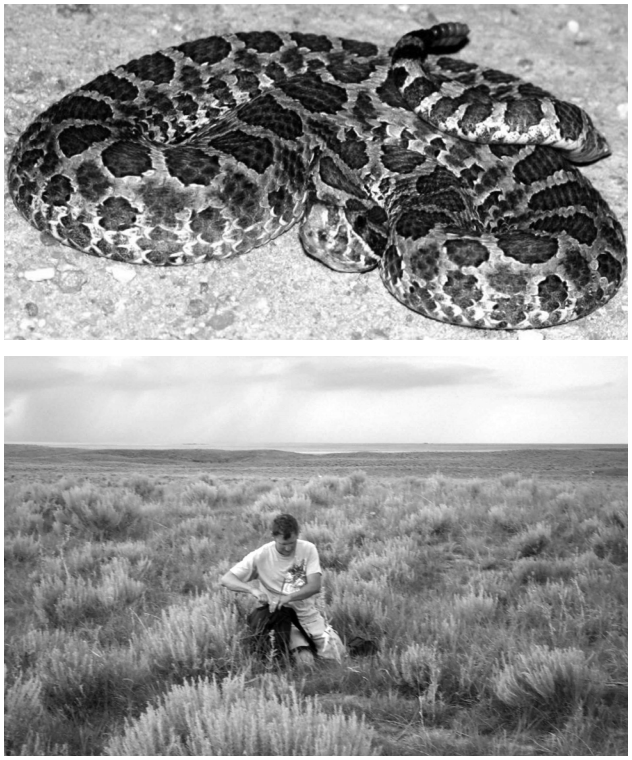


Figure 2. A. Desert Massasauga (*S. t. edwardsii*) from Lincoln County, Colorado. B. Andrew Wastell in the mixed grass-sandsage habitat utilized in summer by Desert Massasaugas in SE Colorado.

NATURAL HISTORY AND ECOLOGY OF THE DESERT MASSASAUGA

Distribution of the Desert Massasauga.—The Desert Massasauga (*S. t. edwardsii*) is a small rattlesnake that occurs in mesic to xeric habitat in the southwestern United States and northern Mexico (Fig. 1). Though much more tolerant of arid conditions than the mid-western and eastern subspecies (e.g., Reinert and Kodrich, 1982; Siegel, 1986),

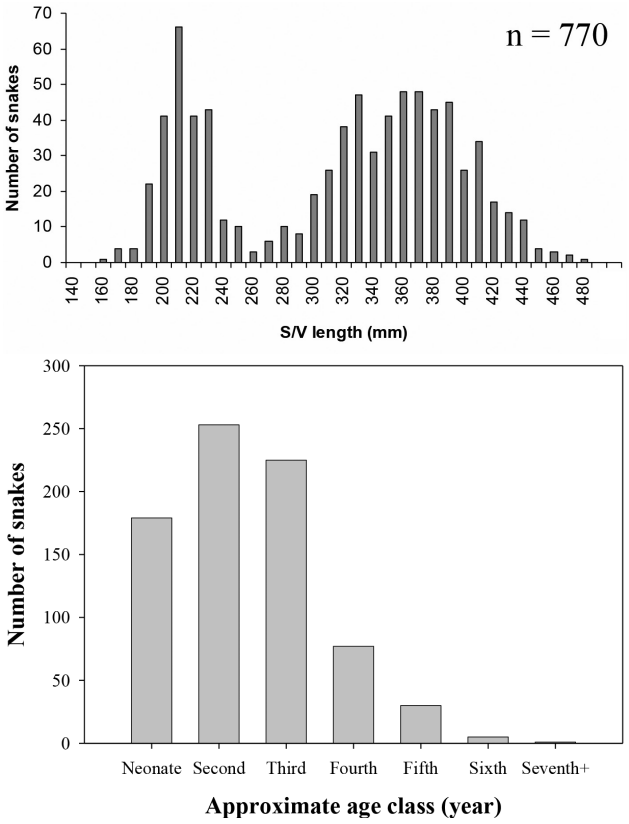


Figure 3. A. Size class distribution of *S. t. edwardsii* from Lincoln County, Colorado. B. Approximate age class frequencies for *S. t. edwardsii* from Lincoln County, Colorado. Age class determinations were derived from recapture-growth data. Both figures reproduced from Wastell and Mackessy, 2016.

it nonetheless appears to favor microhabitat with more abundant surface water within generally drier habitats, and it may be the most common snake species in these areas (Mackessy 2005; Wastell and Mackessy 2011). In 1994, we initiated a study of *S. t. edwardsii* in southeastern Colorado because species status in the state was poorly known at that time. Subsequently, we have identified several large populations and have worked extensively with one on a private ranch in Lincoln Co., CO (Hobert et al., 2004; Mackessy, 2005; Wastell and Mackessy, 2011; Wastell and Mackessy, 2016). We have collected natural history and ecology data for this species in Colorado for ~15 years, much of it on this private ranch. Over 1000 *S. t. edwardsii* have been captured, sampled, PIT-tagged and released at this study site, and a radiotelemetry study was also conducted here (Wastell and Mackessy, 2011).

Natural history.—*Sistrurus t. edwardsii* occurs in shortgrass steppe habitat below 1500 m elevation in SE Colorado (Fig. 2B), an environment characterized by relatively low rainfall, few trees, dominant sandsage (*Artemesia filifolia*) and several shortgrass species. Activity (see below) occurs primarily during the warmer months (April–October). *Sistrurus t. edwardsii* feed on a variety of prey, including lizards, small rodents, and centipedes (Holycross and Mackessy, 2002). Massasaugas in SE Colorado breed in either spring or in fall, and copulations have been observed in April and in September (Mackessy, 2005). Due to the small adult size, clutches are small (~5/clutch) and are born in late August–early September. Neonates associate with the female until the neonatal shed (about day 5–7), after which they disperse (Wastell and Mackessy, in review).

Demographics.—Desert Massasaugas are small rattlesnakes, and the maximum SVL recorded in Colorado (based on approx. 1200 specimens) was 490 mm (TL = 529 mm); Holycross (2002) reported a maximum TL of 588 mm for *S. t. edwardsii* in Arizona. In Colorado, average adult size is ~360 mm SVL, and snakes larger than 440 mm SVL are rarely encountered (Fig. 3). The adult sex ratio, based on 722 snakes from Lincoln Co., CO, is slightly male-biased (M:F = 1.07). Based on mark-recapture studies, most adult snakes are in the 3–4 year age classes (Fig. 3B; Wastell and Mackessy, 2016), though captive *S. t. edwardsii* have lived for over 20 years (pers. obs.). These observations suggest that survivorship of adults past year 4 is quite low, and indeed, larger/older snakes are very rarely seen.

Spatial ecology.—Desert Massasaugas in Colorado are active from late March to mid-October, and most snakes were encountered in spring or fall as they crossed a dirt road that bisects the two distinct habitat types used in this area. On 12 November, when surface temperatures were 12°C, a radio-tagged snake was found above ground next to a rodent burrow, which it used as a hibernaculum, so it is probable that snakes remain locally active if surface temperatures are sufficiently high. Snakes were found most commonly while migrating in April, September, and October, and were least commonly encountered from May through August, when they are utilizing summer forage habitat where they are extremely cryptic.

Ambient temperature affects above-ground activity of *S. t. edwardsii*. Desert Massasaugas also show seasonally dependent changes in daily activity patterns. During the spring and fall, when evening temperatures fall rapidly after sunset, Massasaugas were observed crossing roads in morning and late afternoon (indicating essentially crepuscular behavior). In the summer, when daytime temperatures become prohibitive to long diurnal movements in the open, Massasaugas adopt a nocturnal pattern of movement and are primarily active between 1900–2100 hrs. Before we initiated telemetry studies, we believed that this partitioning of activity was near absolute, as Massasaugas were never observed in the daytime in summer (May through August). However, observations of radio-tagged snakes firmly established that *S. t. edwardsii* spend a considerable

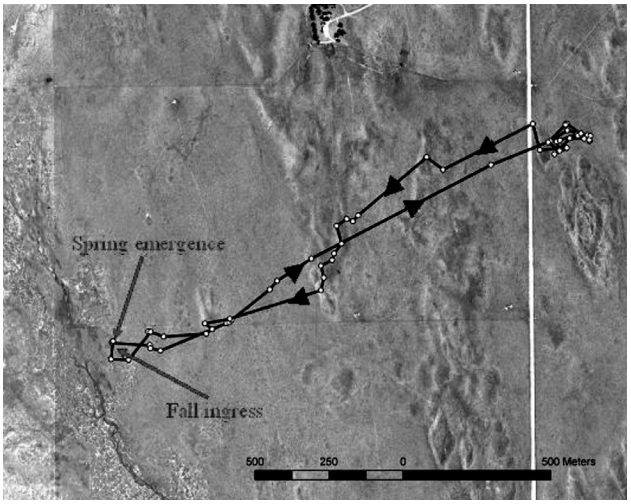


Figure 4. Representative migration path (based on radiotelemetry) of a *S. t. edwardsii* over the entire active season (overlain on a satellite image of the habitat), from spring emergence from the hibernaculum to ingress in the fall. Reproduced from Wastell and Mackessy, 2011.

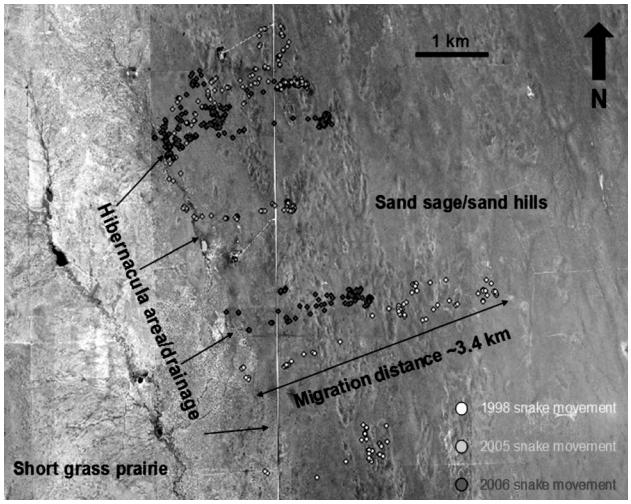


Figure 5. Summary map of all movements of 12 radioed *S. t. edwardsii* overlain on a satellite image of the habitat. Note that some snakes may travel well over 6 km while migrating from and to the hibernaculum. Reproduced from Wastell and Mackessy, 2011.

amount of time during the day above ground, but they are typically observed in resting coils at the base of sandsage, which provides cover for thermoregulation and predator avoidance (and perhaps avoids excess water loss). Most above-ground sightings of radio-tagged snakes occurred between 17 and 34°C, while below 13 and above 38°C, all observations were below ground (surface temperature in shade at snake position; Mackessy, 2005). They are highly cryptic in the sandsage-shortgrass steppe, and non-radio-tagged snakes were very rarely seen in the field when not crossing roads.

Sistrurus t. edwardsii in Colorado show long-distance movements in spring, which occur shortly after egress from hibernacula, and these long movements occur again in fall before ingress (Fig. 4). Snakes may migrate as far as 3.4 km from hibernacula, traveling nearly 7 km in a season, and the majority of snakes were encountered as they crossed a 5 km stretch of a dirt road (Fig. 5). This road roughly corresponds to the boundary between the two habitats, and sampling along this road increased encounter rate tremendously.

Sistrurus t. edwardsii in Colorado hibernate in refugia in hardpan soils characterized as shortgrass steppe. Vegetation of this area consists primarily of buffalo and grama grasses, and soils are highly compacted (Wastell and Mackessy, 2011). During the warmer months, from May to September, snakes forage in mixed-grass sand hills dominated by tall grasses and sandsage shrubs. Because snakes make long migratory movements in spring and fall between the hardpan and the sand hills, home range estimates are

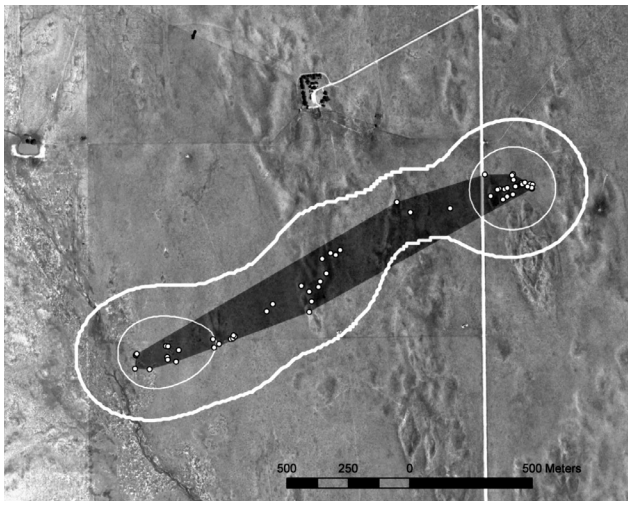


Figure 6. Representative seasonal activity range, home range, and core use areas for a radio-tracked *S. t. edwardsii* overlain on a satellite image of the habitat. White dots indicate individual localities; minimum convex polygon is shown as a black transparency (529.7 ha), 95% activity area (95% kernel densities; KD) is enclosed in a thick white line (598.1 ha), and 50% core areas (50% KD) are shown as thin white ovals (518.4 ha). Straight while lines are gravel roads. Reproduced from Wastell and Mackessy, 2011.

relatively large (MCPs 15–109 ha), but the majority of time is spent in much smaller regions, indicated by the 50% kernel density areas (Fig. 6).

Migration patterns.—In the summer, snakes make short,

Table 1. Analysis of movement patterns for Desert Massasaugas in southeastern Colorado using circular statistics. Z-values > 2.0 indicated that movements were statistically significant linear movements.

	Mean bearing (°)	# Movement segments/snake*	Z-value	*P* value
Summer movements	116.0 (9–247)	53.3	0.63	0.680
Spring movements (NE = 45°)	37.1 (19–65)	13.2	9.20	<0.001
Fall movements (SW = 225°)	207.0 (192–238)	16	8.06	<0.001

non-directional movements in the sand hills, which are related to foraging efforts (Table 1). In spring and fall, however, this pattern of movement is markedly different; snakes make long-distance, highly directional movements, migrating from and to the hibernaculum (Wastell and Mackessy, 2011). At first consideration, it is not apparent why snakes in this area should show such different seasonal movement patterns. Migration is energetically costly, and snakes were observed to move as much as 500 m in a 24 hr period. Migration is also potentially hazardous, as predators are abundant in the region, including Swainson's Hawks and other raptors, Long-tailed Weasels and other mammalian carnivores, ophiophagous snakes, and humans. An examination of the driving forces favoring these long migratory movements (up to 3 km in each direction) strongly indicates that they are resource driven. In the summer sandhill habitat, prey is abundant, and rodent burrows and sandsage are utilized as retreats for shelter and for thermoregulation. Female birthing sites also occur in this habitat, and gravid females utilized rodent burrows, typically at the base of sandsage, as birthing sites.

Snakes return to the compacted soils of the shortgrass steppe habitat in September and October. Prey is scarce in these areas, but the compacted soils favor stable hibernacula; additionally, subsurface structure is hypothesized to provide extensive below-frostline refugia near the water table; numerous sinkholes occur in the area utilized by *S. t. edwardsii* in winter, indicating that the belowground structure is extensive. Many other species of reptiles also hibernate in this same limited area, indicating that hibernation sites are generally uncommon, but highly localized

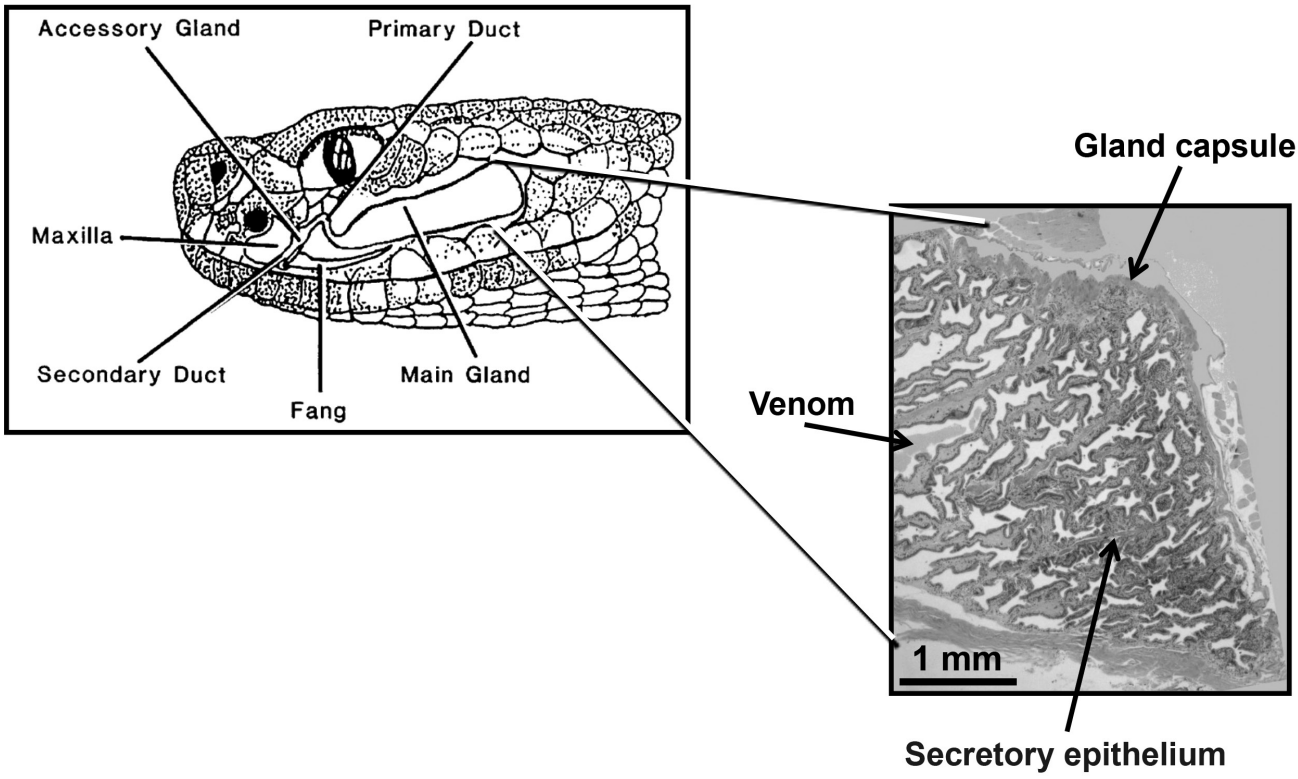


Figure 7. Venom apparatus of rattlesnakes. The micrograph shows the convoluted secretory epithelium and numerous ductules of *S. t. edwardsii* characteristic of snake venom glands generally. Sagittally sectioned gland tissue (5 µm) was stained with hematoxylin/eosin. Line drawing reproduced from Mackessy and Baxter, 2006.

and abundant here (Wastell and Mackessy, 2011). Egress is weather-dependent, but surface activity usually commences in mid-March, and most snakes have left the hibernation site by end of April.

VENOM PROTEOMICS AND TRANSCRIPTOMICS

Venom and the venom apparatus.—In collaboration with colleagues from Ohio, Spain, Singapore, and South America, we have investigated aspects of the venom proteome and venom gland transcriptome of Massasaugas from the Lincoln Co., Colorado population (Chapeaurouge et al., 2015; Doley et al., 2008, 2009; Gibbs and Mackessy, 2009; Pahari et al., 2007; Sanz et al., 2006;). Many aspects of venom biochemistry are well known for this species in Colorado, and this section summarizes some of this information.

Gland structure.—Like the venom glands of most vipers, the gland of *S. t. edwardsii* is filled with ductules and includes a basal lumen leading to a primary duct. Then there is a small but distinct accessory gland, and finally a secondary duct connected to the base of the fang (Fig. 7). The main gland is filled with a densely packed secretory epithelium where venom constituents are synthesized and exported to the ductules via exocytosis of granules and vesicles/exosomes containing venom (Mackessy, 1991; Ogawa et al., 2008). During deployment, venom in the lumen and ductules is pressurized by contraction of the compressor glandulae (a derivation of the adductor mandibulae) muscle (Kardong, 1973) and flows through the secondary duct, through the hollow fang and into prey tissues. After complete emptying of the gland (following venom extraction), venom replenishment takes approximately 10–12 days, a process dependent on temperature and on sympathetic outflow and noradrenergic innervation (Yamanouye et al., 1997; Kerchove et al., 2004; Luna et al., 2009).

Venom yields.—As with venomous snakes generally, venom yields (manual extraction) increase exponentially with size (Fig. 8), but because of small size, yields are relatively low for this species. Yields from neonates (<250 mm SVL; $N = 221$) averaged 6.6 µL and ranged from 2–22 µL, and adults (>350 mm SVL; $N = 368$) produced an average of 29.4 µL (range = 12–75 µL). The median lethal dose (LD_{50}) of venom from this species toward mice (0.6 µg/g) and toward lizards (0.39 µg/g) is quite low (Gibbs and Mackessy, 2009), so in spite of the low adult yields, *S. t. edwardsii* should be considered hazardous to humans.

Venom.—Venom is primarily a trophic adaptation that allows snakes to subdue prey chemically rather than mechanically, as is seen in boids generally and in many

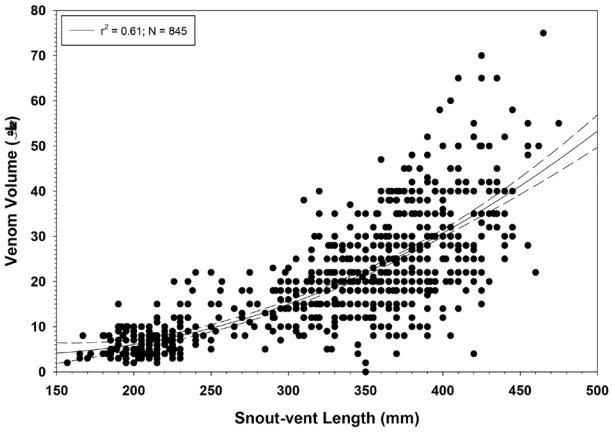


Figure 8. Venom yields from 845 *S. t. edwardsii* sampled in SE Colorado. Venom yields are generally lower than other rattlesnakes as a consequence of small size, with a maximum adult yield of 75 µL, corresponding to approximately 16.9 mg dry venom.

colubroid snakes, including some elapids (Shine and Schwaner, 1985). Venom is a complex mixture, often consisting of >100 protein/peptide components, as well as an undefined number of smaller organic and inorganic compounds (Mackessy, 2010). Although venom serves an obvious defensive function for many species, sometimes with highly specialized delivery mechanisms (i.e., spitting cobras), the trophic role of venoms appears to be primarily responsible for the elaboration of diverse venom protein families that facilitate prey handling (Mackessy, 1988, 2010) and also allow prey recovery by strike-and-release predators such as vipers (Saviola et al., 2013). In many cases, venom composition appears linked to specific pharmacological attributes of "preferred" prey (e.g., da Silva and Aird, 2001; see below also), and this predator-prey interaction may largely govern evolution of venom protein diversity via gene duplication, followed by subfunctionalization and neofunctionalization (Ohno, 1970; Fry et al., 2008; Casewell et al., 2013; Mackessy and Castoe, 2015). There has been considerable discussion about precisely what constitutes a venom (Mackessy, 2002; Kardong, 2012 and discussions therein; Nelsen et al., 2014), but there is no question that *S. t. edwardsii* produces a complex oral secretion, delivered via a specialized apparatus, which is lethal when injected into animal tissues (a basic definition of venom; see also Weinstein, 2015). Whether some of the proteins and peptides found in this venom constitute "toxins" is still a matter of debate, but probably should not be. Even compounds that by themselves are non-toxic, such as purine nucleosides, may effectively contribute to prey immobilization (Aird, 2002).

Venom Analysis.—Most analyses of venoms have focused on proteins and peptides, as these compounds generally comprise >90% of the dry weight. Classically, SDS-PAGE

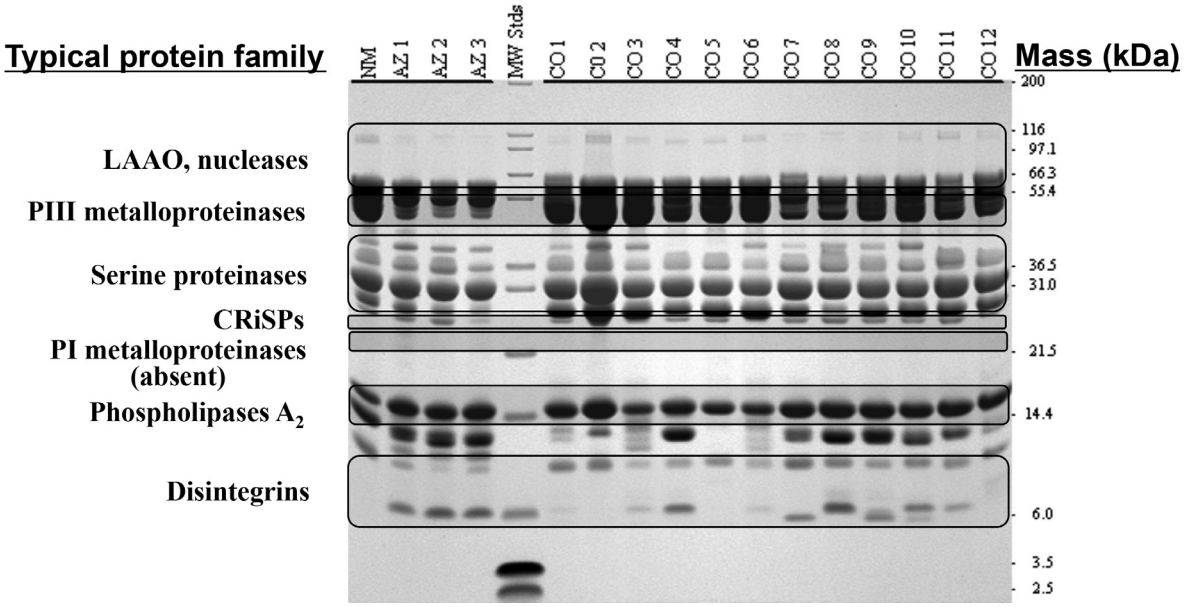


Figure 9. One-dimensional SDS-PAGE of venoms from *S. t. edwardsii* sampled in New Mexico (NM), Arizona (AZ), and SE Colorado (CO). Note that all samples show very similar banding patterns, and no geographic variation is apparent. Protein families typical of rattlesnake venoms and size classes are shown on the left. Molecular standard (MW Stds) masses are indicated on the right (in kilodaltons).

(sodium dodecyl sulfate polyacrylamide gel electrophoresis), bioassays, and enzyme analyses have been used to evaluate venoms, as these approaches are rapid, are reasonably sensitive, require a minimum of specialized equipment, and allow a moderately high-throughput approach. One-dimensional SDS-PAGE can provide a molecular fingerprint of many venom samples on one gel, and because protein families are well known for rattlesnakes, bands can be assigned to specific activities with a high degree of certainty (Fig. 9). For *S. t. edwardsii* venoms, 18–22 protein bands are visible, and banding patterns between venoms from snakes originating in Arizona, New Mexico, and Colorado are generally quite similar; no geographic differences in composition are observed. Similarly, venoms can be assayed for a variety of enzyme activities, which are proportional to abundances of the proteins responsible (Mackessy, 2008).

As proteomic approaches became more commonly applied to snake venoms (e.g., Calvete et al., 2006), much more detailed compositional data could be obtained from them. A venom proteomic, or venomic, approach typically utilizes reversed phase HPLC (high pressure liquid chromatography), mass spectrometry and/or two-dimensional electrophoresis to provide much greater sensitivity of detection. In particular, mass spectrometry, combined with various HPLC techniques, has become the approach of choice to define venom proteomes. Two-dimensional electrophoresis (Fig. 10) provides a detailed visual representation of venom proteins, and for *S. t. edwardsii* venom, over 100 individual protein spots are apparent; however, the majority of these proteins belong to only 8 different protein families. Combined with in-gel trypsin digest of individual protein spots followed by LC-MS or MALDI-TOF MS

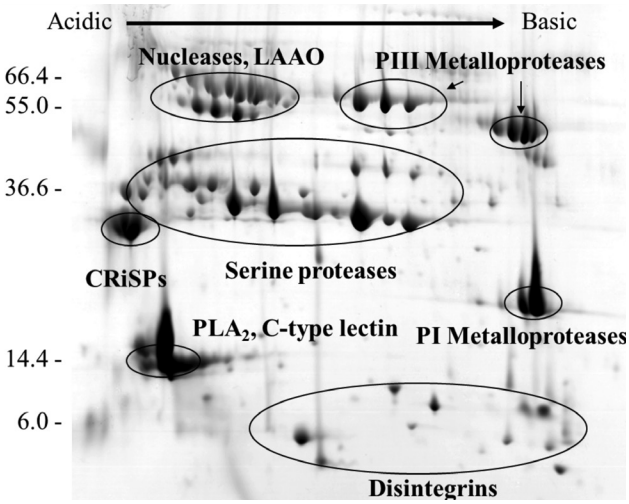


Figure 10. Two-dimensional SDS-PAGE analysis of *S. t. edwardsii* venom sampled in SE Colorado. Tentative identifications of spots are indicated; over 100 individual spots, many of them isoforms within a family, are observed. Protein pI increases (from acidic to basic) from left to right, and approximate protein masses (in kilodaltons) are indicated on the left.

analysis of resultant peptides, unequivocal assignment of protein identities can be made. A limitation of this method is that it is dependent on specialized equipment. It is expensive and laborious, and in general is relatively low throughput. More recent methods, using more sensitive LC-MS-MS instruments, are largely overcoming these limitations (i.e., Rokyta et al. 2015), but cost still represents a barrier to analysis of large numbers of samples.

A relatively low-cost approach involves using 1D SDS-PAGE (Fig. 9) and MALDI-TOF MS (Fig. 11) on the same samples, which can provide reasonable coverage of venom proteome complexity, although this combination is not as sensitive or complete as a venomics approach. However, to understand the biological roles of venoms to the snakes, a highly sensitive approach may not be necessary. For example, among many rattlesnakes and other vipers, venoms can be classified into two basic functional types: type I venoms, which have high levels of tissue-damaging metalloproteinases, but are relatively less toxic, and type II venoms, which are highly toxic but which typically show low to exceptionally low metalloproteinase activity (Mackessy, 2008, 2010). Further, if venom proteomic complexity is collapsed to reflect relative abundances of major venom protein families, it becomes clear that this trend occurs generally in vipers as well as other venomous snakes (Fig. 12). High lethal toxicity in viper venoms is typically tied to the occurrence of high levels of the presynaptic neurotoxin crotoxin (and homologs such as Mojave toxin; see Gren et al., this volume), while in non-vipers, high toxicity results primarily from the action of post-synaptically-active 3FTxs. Yet another approach involves utilizing several different mass spectrometry methods on the same sample; applied to *S. t. edwardsii* venom, ESI- and MALDI-based approaches were complementary, with each detecting a subset of unique peptides and proteins and increasing sequence coverage of individual proteins (e.g., Chapeaurouge et al., 2015). This combined approach is recommended if one is interested in determining more completely the proteome of venoms with both abundant and rare protein components.

In collaboration with R.M. Kini's lab in Singapore, we determined the venom gland transcriptome of *S. t. edwardsii*, again from the same source population in Lincoln Co., Colorado (Pahari et al., 2007). This expressed sequence tag (EST)-based transcriptome identified 11 protein families in the venom gland, including low-abundance transcripts for three-finger toxins, which are typical and highly expressed

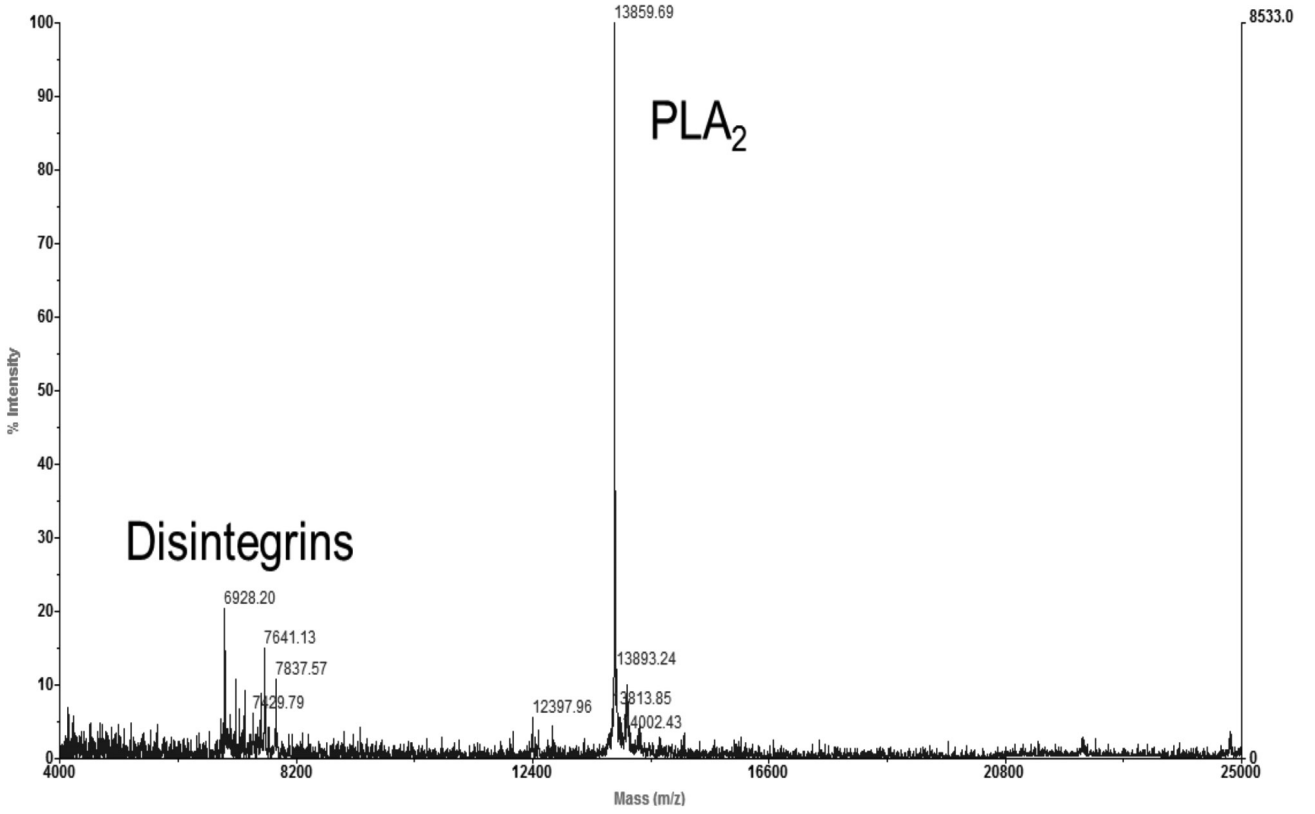
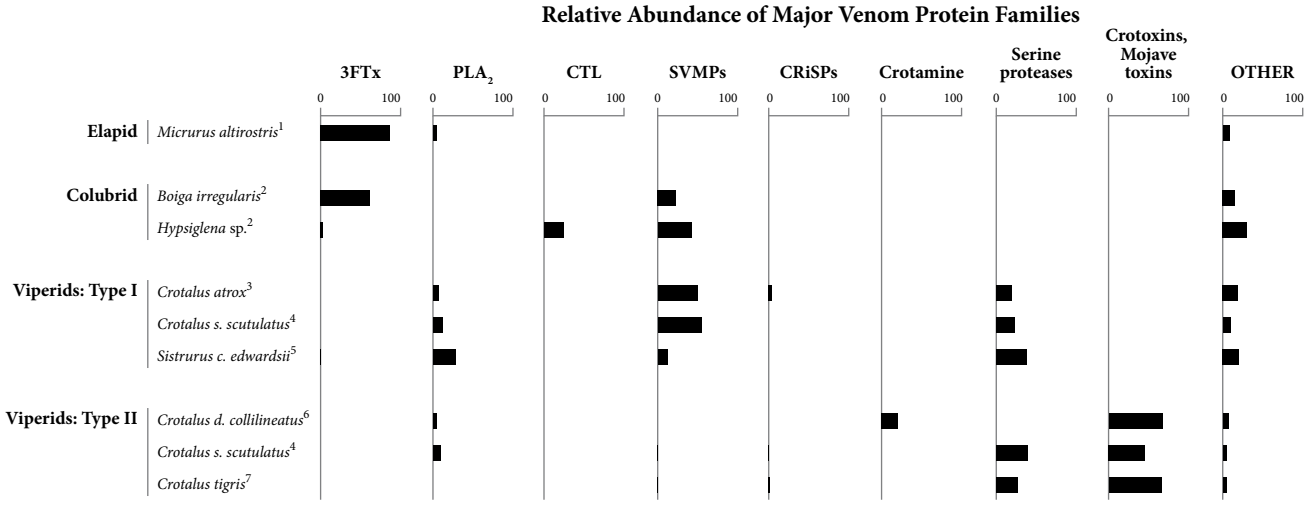


Figure 11. Representative mass spectrum (MALDI-TOF) of *S. t. edwardsii* venom sampled in SE Colorado. A mass window of 4-25 kDa is shown; note that this sample shows low complexity in this mass range. *Sistrurus t. edwardsii* venom from these populations contains only a single dominant phospholipase A_2 (PLA_2) with a mass of 13.859 kDa, an unusual trait for a viperid venom.



[1] Correa-Netto et al., 2011; [2] McGivern et al., 2014; [3] Calvete et al., 2009; [4] Massey et al., 2012; [5] Sanz et al., 2006; [6] Boldrini-França et al., 2010; [7] Calvete et al., 2012

Figure 12. Comparative proteomes of elapid, colubrid, and viperid snakes, showing the predominance of venom metalloproteinases in type I venom compared to the abundant presynaptic neurotoxins (crotoxin, Mojave toxin) in type II rattlesnake venoms. Reproduced from Modahl et al., 2015.

proteins in elapid and some rear-fanged snake venoms (e.g., Mackessy, 2010). When compared with the proteome of the same venom (Sanz et al., 2006), some discrepancies are observed (Table 2), and relative abundances of protein families are different. At about the same time, a study of the transcriptome of *Lachesis muta* also revealed the presence of low-abundance transcripts of 3FTxs (Junqueira-de-Azevedo et al., 2006), and since this time, 3FTx transcripts have been found in low abundance in many viper venom gland transcriptomes. However, they are rarely detected in the venom proteome, and are not apparent in the proteome of *S. t. edwardsii*, which begs the question of why this potent toxin family is not expressed at significant levels even though transcripts are produced.

BIOCHEMICAL ECOLOGY OF VENOM

Relation of venom composition to diet.—Venoms are trophic adaptations that are likely central to the successful radiation of colubroid snakes (Savitsky, 1980; Kochva, 1987); therefore, one might expect an obvious link between diet and venom composition. This relationship has been advanced for many species (e.g., da Silva and Aird, 2001; Mackessy, 1988; Mackessy et al., 2003, 2006; Barlow et al., 2009; Richards et al., 2012), and the connection seems clear for species such as the Brown Treesnake (*Boiga irregularis*), a rear-fanged venomous species that feeds primarily on birds and lizards in its native range (Greene, 1997; Mackessy et al., 2006). Venom from *B. irregularis* contains a large amount (~10% dry weight) of a postsynaptic dimeric three-finger toxin that also shows taxon-specific toxicity; this toxin is rapidly lethal to birds and lizards, but is essentially non-toxic to mammals (Pawlak et al., 2009). For other species, the trophic connection is not as clear: for example, the Mojave Rattlesnake (*Crotalus s. scutulatus*) in Arizona has populations which produce both type I and type II venoms, which differ significantly in composition (Massey et al., 2012; see also Fig. 12), but diet does not seem to differ significantly between these populations (though specific diets in these populations need detailed analyses). An answer to the varying expression of these major venom

Table 2. Comparison of venom proteome and venom gland transcriptome protein family compositions[1]. CRISP, cysteine-rich secretory protein; PP, potentiating peptide.

Protein Family	% in Proteome	% in Transcriptome
Disintegrin	0.9	-
Three finger toxin	-	0.8
Ku-wap-fusin	-	0.3
C-type bradykinin PP	<0.1	0.3
Kunitz-type inhibitor	<0.1	-
Nerve growth factor	<0.1	6.7
Phospholipase A_2	13.7	28.3
CRISP	10.7	7.9
Serine proteinase	24.4	37.9
C-type lectin	<0.1	1.4
Metalloproteinase	48.6	12.4
L-amino acid oxidase	2.5	3.7
Phosphodiesterase	-	0.3

[a] Adapted from Sanz et al., 2006 and Pahari et al., 2007.

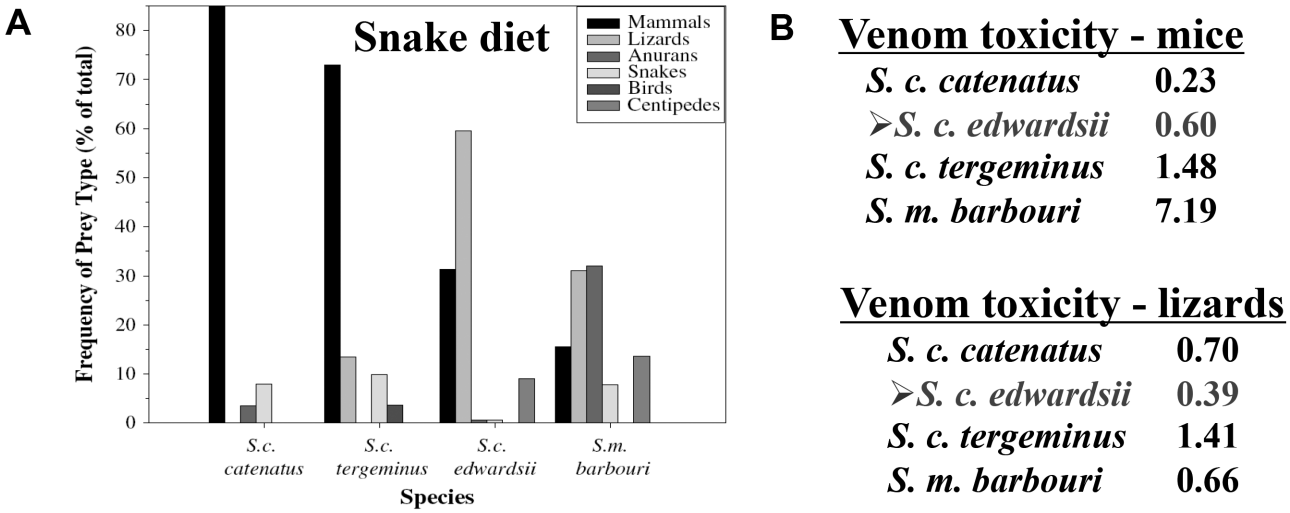


Figure 13. A. Frequency of prey types in the diets of four *Sistrurus* taxa. Mammals predominate in the diet of *S. c. catenatus*, while lizards make up the largest proportion of the *S. t. edwardsii* diet. B. Comparative toxicity of venoms of four taxa of *Sistrurus*. *Sistrurus c. catenatus* and *S. t. edwardsii* venoms are highly toxic to both mice and lizards, while *S. m. barbouri* venom shows low toxicity toward mice and high toxicity to lizards. Both reproduced from Gibbs and Mackessy, 2009.

phenotypes in *C. scutulatus* may provide important insight into the relationship between diet and venom composition.

For the genus *Sistrurus*, a relationship between lethal toxicity and diet has apparently evolved differently among different taxa. High lethal toxicity is seen toward lizards from venoms of *S. t. edwardsii* and *S. miliarius barbouri*; both of these species include lizards as a significant part of their diets (Fig. 13A), whereas the two other taxa analyzed (*S. c. catenatus* and *S. t. tergeminus*) took lizards rarely (Gibbs and Mackessy, 2009). Conversely, the three taxa of Massasaugas include small mammals as a moderate to near-exclusive part of their diet, and their venoms were 7–10 times more toxic to mammals than venom of *S. m. barbouri*, which rarely takes mammals (Fig. 13B). Among vipers, which possess some of the most complex venoms among vertebrates (Gans and Elliott, 1968), evolutionary patterns linking chemical predation and prey type are complex and multifactorial, and these patterns are likely further confounded by the potential for evolution of prey resistance among mammals (e.g., Neves-Ferreira et al., 2010).

Concluding remarks.—The biology and venom biochemistry of the Desert Massasauga (*S. t. edwardsii*) from southeastern Colorado have been subjected to extensive analysis, and studies of this diminutive species have provided considerable information about habits of these specialized rattlesnakes (Mackessy, 2005; Wastell and Mackessy, 2011, 2016). In addition, *S. t. edwardsii* has provided evidence supporting interesting mechanisms leading to the generation of venom complexity (Doley et al., 2008). The Desert Massasauga is therefore an excellent model species for evaluating influences of numerous ecological factors on venom evolution, and continuing studies are investigating population levels of venom and genetic variation. At present, populations of *S. t. edwardsii* in Colorado appear stable, and they occur away from population centers, but the effects of global climate changes, particularly if desertification of grassland habitats occurs, although difficult to predict, will likely impact Massasaugas negatively.

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