# Functional basis of a molecular adaptation: Prey-specific toxic effects of venom from rattlesnakes

H. Lisle Gibbs [a,*], Stephen P. Mackessy [b]

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[b]

2006; Pahari et al., 2007; Gibbs and Rossiter, 2008), but functional characterization of this variation, in terms of effects on prey, still is poorly characterized. In general, the working hypothesis is that the high level of variation in venom at the inter- or intraspecific level (for a review see Chippaux et al., 1991) allows snakes to specialize on

unpublished sources (Holycross and Mackessy, 2002; Farrell and May, unpublished data). Prey items were classified into six categories (mammals, lizards, anurans, snakes, birds, centipedes) and plotted as percent of total diet for all samples for each taxon collected across all populations.

We determined the toxicity of venoms towards three different animals which are representative of major prey classes (Mammalia, Reptilia, and Amphibia) in diets (see above): NSA mice (obtained from UNC Animal Facility breeding stock), wild-caught Brown Anoles ( ) from Florida (purchased from Quality Pets, Florida) and wild-caught Northern Leopard Frogs ( ) from Ohio. Our goal in this study was to gain a broad picture of toxicity across classes of prey that characterize diet variation in these snakes. We recognize that in the wild, prey on a diversity of species in each of these general categories, and so our assumption is that patterns of toxicity we observe for each of these ''types'' of prey will be broadly representative of the response of other types of small mammals, lizards and frogs. This assumption could be tested in future studies but for logistic reasons was beyond the scope of this work.

We measured $LD_{50}$s for each venom–prey combination using the general procedures outlined in Munekiyo and Mackessy (1998) and Mackessy et al. (2006). Briefly, venom doses were delivered intraperitoneally (IP) in sterile saline, with doses adjusted to individual animal body masses. Three animals per dose were utilized, and all animals were monitored for 24 h. Lethality was expressed as micrograms venom per gram body mass (=mg/kg) producing 50% mortality after 24 h and was calculated (along with 95% confidence intervals) from the raw mortality-dose data using the Trimmed Spearman–Karber (TSK) program version 1.5 (U.S. Environmental Protection Agency, 1990). Our methods make a careful attempt to minimize the number of animals used in these assays. All procedures with vertebrates have been evaluated and approved by the University of Northern Colorado-IACUC (protocol #9401).

Initial analyses identified toxicity of venom to mammals as a key axis along which whole venom toxicity varies in this group. To gain a broader evolutionary perspective on the evolution of venom toxicity to mammals

crotoxin homologs in venoms was based on comparison with venom (type A) from , which contains Mojave toxin (Mackessy, 2008) and the proteomic data of Sanz et al. (2006).

by approximately one-half. Among rattlesnakes as a whole, both values represent extremes, with the $LD_{50}$ value for being among the top three most toxic values, and the value is the least toxic that is observed (Fig. 2d).

When dose equivalents are used as a measure of toxicity, the patterns are generally the same, although the loss of toxicity to mammals in is more striking than the gain in

support a "trade-off" model of venom function implied by some models of venom evolution (e.g. Daltry et al., 1996), whereby an increase in the overall toxicity of venom towards a particular prey class is correlated with a decline in toxicity towards a different type of prey. Rather they suggest that within this group of closely related snakes that

None declared.


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