Is the Venom Related to Diet and Tail Color During *Bothrops moojeni* Ontogeny?

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Snakes are strictly carnivorous reptiles, and many of them feed upon large prey which they swallow whole. Juveniles and adults, however, may exhibit a significant difference in body size, which can lead to an ontogenetic dietary shift (see Mushinsky, 1987). In this regard, many species of snakes feed on anurans and lizards while juveniles, and on birds and mammals as adults (e.g., Sexton, 1956-1957; Saint Girons, 1980). Moreover, juveniles of some snakes use the conspicuous tip of the tail to lure ectothermic prey (Greene and Campbell, 1972; Heatwole and Davison, 1976), losing this feature with growth, when the diet changes to endotherms (Neill, 1960; Henderson, 1970; Murphy et al., 1978; Jackson and Martin, 1980). Nevertheless, some sub-adult and adult snakes, always males, retain the conspicuous color of the tail (see Burger and Smith, 1950).

The occurrence of ontogenetic changes in diet and caudal luring are well documented for the family Viperidae, a group of snakes in which venom has an important role in prey capture (e.g., Mushinsky, 1987; Meier and Stocker, 1991). Because venom properties may vary ontogenetically (Fiero, 1972; Theakston and Reid, 1978; Gutiérrez et al., 1980; Lomonte et al., 1983; Meier, 1986; Furtado et al., 1991) it has been proposed that such variation could be caused by differences in the feeding habits of juveniles and adults (Gans and Elliot, 1968; Szazma, 1991).

To evaluate the possible specificity between venom and prey, we investigated the toxicity of juvenile and adult *Bothrops moojeni* venom in frogs and mice, which
represent the preferred prey of juveniles and adults, respectively. *Bothrops moojeni* is a large neotropical pit viper, reaching 130 cm in snout-vent length, in central and southern Brazil (Campbell and Lamar, 1989). Although dietary information is scant, field data revealed amphibians and reptiles in gut content of juveniles, while mammals and birds are more often found in adults (A. S. Abe and P. R. Manzani, unpubl. obs.). Furthermore, *B. moojeni* belongs to a genus in which an ontogenetic shift of diet from ectotherms to endotherms is well documented (e.g., Sexton, 1956–1957; Sazima, 1991; Sazima, 1992; Gasparini et al., 1993; Martins and Gordo, 1993). Juvenile *B. moojeni* also have conspicuous tail tips (Leloup, 1975) employed to lure ectothermic prey (pers. obs.). Thus, the relationship between venom composition, accessed by electrophoresis, and change in tail color was also investigated.

Snakes were collected at the municipalities of Aracatuba (21°38'S; 50°25'W) and Pereira Barreto (20°38'S; 51°06'W) São Paulo, Brazil. Three size classes were defined: juveniles (<450 mm SVL), sub-adults (450–840 mm SVL), and adults (>840 mm SVL) (see Leloup, 1975). Venoms of 48 juveniles and 8 adults were manually extracted, pooled, and immediately vacuum dried and stored at -20°C for later use in the toxicity tests. For electrophoresis, fresh samples of individual venoms (4 juveniles, 6 sub-adults, and 4 adults) were collected in glass capillaries and stored frozen.

Toxicity of venoms from juvenile and adult *B. moojeni* was determined in outbred Swiss-Webster mice (18–22 g) and juvenile bullfrogs (*Rana catesbeiana*, 5 to 10 g). For mice, five dose levels (2.4, 3.6, 5.4, 8.1 and 12.15 mg venom per kg body weight), were utilized in the toxicity tests, both for juvenile and adult venoms. In frogs, the dose levels employed were: 17.5, 26.25, 39.37, 59.06 and 88.59 mg venom per kg body weight for juvenile venoms, and 35, 49, 68.6, 96.04 and 134.45 mg venom per kg body weight for venoms of adult snakes. Venom was diluted in 0.9% saline and injected intra-peritoneally in six animals at each dose level. The volume injected was 0.5 ml for mice and 12.15–108.57 mg venom (3.46–5.75) (32.94–66.36)

The results of toxicity tests are shown in Table 1. LD₅₀ values for adult and juvenile venoms were similar in mice and the adult/juvenile ratio was 1.06. On the other hand, a marked difference in LD₅₀ values was found between adult and juvenile venoms in frogs. The juvenile venom toxicity was nearly twice that of adults with an adult/juvenile ratio of 1.86. Electropherograms revealed qualitative differences in the venom of snakes of different sizes (see Furtado et al., 1991). There was a trend towards reduction in the number of bands with an increase in body size, particularly bands of high molecular weight (above 66,000 M.W.). Electropherograms of sub-adult venoms were rather variable and exhibited a transitional pattern between juveniles and adults; some samples containing fractions of juvenile venoms, while other electropherograms were closer to the adult pattern.

The electrophoretic pattern of a venom was not associated with either sex or tail color. The greater variation of the pattern reported for sub-adult samples is likely to be only due to transitional stage of this size group. Therefore, the electrophoretic change of *B. moojeni* venom seems to be related only to the stage of development of the animal, and is independent of tail color. The loss of high molecular weight fractions of the venom during ontogeny was previously reported for others *Bothrops* species (Meier, 1986; Furtado et al., 1991). In *B. moojeni* this feature was related with the loss of high toxicity of venom on anurans. Therefore, high molecular weight fractions could be responsible for the specific toxicity of juvenile venom on anurans.

*Bothrops moojeni* has a wide geographic distribution in a seasonal climate (Campbell and Lamar, 1989). Recruitment in this species occurs during the warm and rainy season (Leloup, 1975), which is also favorable for breeding activities of tropical anurans (e.g., Duelman and Trueb, 1985). *Bothrops moojeni* recruitment appears to be synchronized with high food (frogs) availability (see Seigal and Ford, 1987, Sazima, 1992, for similar cases), whose predation is favored by tail luring. Such strategies may reduce both cost and risk of active foraging for small and vulnerable snakes (Mushinsky, 1987). The overall result of the synergistic interaction of tail luring, food availability, time of birth, and high venom toxicity in anurans seems to maximize the capture of prey by juveniles. Thus, among the two main biological roles of the venom, initiation of digestion and immobilization/death of prey (e.g., Gans and Elliot, 1968), killing prey appears to be the most important role in juvenile snakes, since they possess limited resources to withstand long fasting periods, such as aestivation (see Hirth, 1966). The capture of a prey once located by juveniles could be also ensured by holding the prey after the strike (Mackessy, 1988), a strategy that we have observed in captivity for juvenile *B. moojeni* feeding on anurans and newborn mice. On the other hand, adult snakes that handle potentially retaliative prey

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<th>Mice</th>
<th>Frogs</th>
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<tr>
<td>Juvenile venom</td>
<td>4.54</td>
<td>45.02</td>
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<tr>
<td>(3.46–5.75)</td>
<td>(32.94–66.36)</td>
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<tr>
<td>Adult venom</td>
<td>4.8</td>
<td>83.7</td>
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<tr>
<td>(3.65–6.19)</td>
<td>(68.15–108.57)</td>
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Table 1. Toxicity (LD₅₀) of the venom of adult and juvenile *B. moojeni* in mice and frogs (mg/kg). 95% Confidence limits in parenthesis.
commonly release them after rapid strike, and then locate the dead prey by visual, olfactory, and vomeronasal cues (Radcliffe et al., 1980; Kardong, 1986).

The toxicity of adult and juvenile *B. moojeni* venoms, in mice, did not change substantially with snakes’ growth and ontogenetic change of diet. Adult *Bothrops* had greater amount of venom in their glands (Furtado et al., 1991), and injected a greater volume of it into their prey (Puorto et al., 1993), a fact that may improve prey capture. As the snakes grow larger mammalian prey are taken (see Greene, 1992; Sazima, 1992), and digestion became more troublesome because of the greatly reduced surface to volume ratio (Pough, 1983; Pough and Groves, 1983). Such an increase in prey digestive resistance is accompanied by a concurrent increase in venom proteolytic activity in *B. moojeni* (Furtado et al., 1991), which speeds the rupture of visceral tissues and increases the prey surface area available to stomach enzymes (Gans, 1961; Thomas and Pough, 1979; Pough and Groves, 1983). The improvement of prey digestion may reduce the time in which snakes suffer reduced locomotion and defense capabilities (Ford and Shuttlesworth, 1986) and may allow them to spend more time in other activities such as foraging and reproduction. Digestion by juveniles is believed not to be problematic because they take small prey (in absolute terms) which are readily digested (Mackessy, 1988).

*Bothrops moojeni* venom changes are consistent with an optimization of the venom effects toward a specific prey in a specific stage of life. Lethality could be more important (selective) for juveniles than adults, while for adults the pressure could arise from problems in digesting large prey (see Mackessy, 1988, for a similar case in *Crotalus viridis*). Furthermore, the wide occurrence of conspicuous tail color in *Bothrops*, frequently associated with tail luring, and the ontogenetic shift in diet (Sazima, 1992) suggests that the sort of venom variation reported here for *B. moojeni* could be a widespread feature among the genus *Bothrops*.

**Acknowledgments.**—We wish to thank R. Wirz and D. Fontanello from Instituto de Pesca, who kindly supplied the froglets used in this study. An earlier version of this manuscript was largely improved by the comments of David L. Hardy, Sr. and an anonymous reviewer. D. V. A. and A. S. A. were supported by CNPq grant.

**Literature Cited**


Accepted: 25 January 1996.