

## RELATIONSHIP OF VENOM ONTOGENY AND DIET IN *BOTHROPS*

DENIS V. ANDRADE AND AUGUSTO S. ABE

*Departamento de Zoologia, Universidade Estadual Paulista, c. p. 199, 13506-900, Rio Claro,  
São Paulo, Brazil*

**ABSTRACT:** We studied ontogenetic changes in venom toxicity of the pitvipers *Bothrops jararaca* and *B. alternatus* in order to evaluate the relationship between venom action and diet. Toxicity tests ( $LD_{50}$ ) were performed for the venoms of adult and juvenile snakes on mice and bullfrog froglets, which represented endothermic and ectothermic prey respectively. The venom of juveniles of *B. jararaca*, but not of *B. alternatus*, had a higher toxicity on anurans than that of adults. This finding is consistent with the feeding habits of these two species, because juveniles of *B. jararaca* feed mainly on small anurans and lizards, shifting to endothermic prey at maturity, whereas *B. alternatus* preys mainly on endotherms throughout its life. Venom toxicity in endotherms was higher for adults of *B. jararaca* compared to juveniles, a feature not observed for *B. alternatus*. It is proposed that prey death/immobilization is the main function of the venom of juvenile snakes. As the snake grows, the digestive role of venom may become increasingly important, because adults prey upon large and bulky prey. The importance of adult venoms in prey digestion is reflected in their higher proteolytic activity.

*Key words:* *Bothrops*; Electrophoresis; Venom ontogeny; Venom specificity; Viperidae

SNAKES are strictly carnivorous and always ingest their prey whole, and for many species feeding episodes occur sporadically on relatively large animals (Greene, 1983, 1997). Juvenile and adult snakes often show differences in body size and other behavioral, morphological, and physiological characteristics that may lead to an ontogenetic shift in diet (Mushinsky, 1987). In a number of snakes, such dietary shifts are characterized by juveniles preying primarily upon ectotherms while adults feed mainly on endotherms (Mushinsky, 1987; Saint-Girons, 1980; Sexton, 1956–1957).

Diet shifts are widespread among viperids, a group of snakes in which prey capture is largely dependent on a parenteral venom injection (Greene, 1992). Viperid venoms are among the most complex and variable substances secreted by vertebrates (Gans and Elliot, 1968), varying specifically (Bernadsky et al., 1986; Tan and Ponudurai, 1990a,b; Wüster and Thorpe, 1991), geographically (Daltry et al., 1996; Glenn et al., 1983; Jayanthi and Gowda, 1988), seasonally (Gubensek et al., 1974; Ishii et al., 1970), and ontogenetically (Dempfle et al., 1990; Lomonte et al., 1983; Meier, 1986). It has been proposed

that ontogenetic variation could be related to differences between the feeding habits of juvenile and adult snakes (Gans and Elliot, 1968; Sazima, 1991). In this study, we tested the association between venom ontogeny and diet for two species of the pitviper genus *Bothrops*. This genus is composed of species that change their diet during growth (Martins and Gordo, 1993; Sazima, 1991, 1992; Sexton, 1956–1957) and that feed mainly on endotherms throughout their life (Andrade, 1995; M. Martins, personal communication), making it possible to examine the relationship between the ontogeny of venom and diet.

To evaluate the possible correlations between venom toxicity and prey type, we tested venoms of juveniles and adults of *Bothrops* for their effectiveness in frogs and mice, which represent ectothermic and endothermic prey, respectively. Among *Bothrops* exhibiting ontogenetic shifts in diet, we studied the venom of *B. jararaca*, a medium-sized (approximately 100 cm snout–vent length: SVL), relatively slender pitviper widespread in southeastern Brazil (see Sazima, 1992, for natural history). Among *Bothrops* that feed on endotherms throughout life, we investigated the venom of *B. alternatus*, a large, heavy-

bodied snake growing up to 160 cm SVL (Andrade, 1995; Campbell and Lamar, 1989).

## MATERIALS AND METHODS

### Snakes and Venom Samples

Individuals of *B. jararaca* were collected at several localities in São Paulo State, southeastern Brazil. Specimens of *B. alternatus* were collected in Botucatu, São Paulo. Venoms from juveniles of *B. jararaca* and *B. alternatus* were obtained mainly from the offspring of females kept in captivity.

Venom samples of *B. jararaca* were collected from juveniles (<45 cm SVL), sub-adults (45–80 cm SVL), and adults (>80 cm SVL). These age classes reflect approximately the size at which diet changes in this species, with juveniles feeding mainly on ectotherms, adults on endotherms, and sub-adults making the transition between both diets (Andrade et al., 1996). For comparative purposes, the same size-based separation was applied to *B. alternatus*, regardless that its diet does not change.

Venoms were extracted manually, pooled, and immediately vacuum dried and stored at -20 C. Storage never exceed 10 mo after collection. For all toxicity tests, venom solutions were prepared immediately before use.

### Toxicity Tests

We determined lethal toxicity of the venoms in outbred Swiss-Webster mice (18–22 g) and in juvenile bullfrogs (*Rana catesbeiana*, 5–10 g). We used five dose levels in each toxicity assay. Dried venom was diluted in 0.9% saline and injected intraperitoneally (i.p.) in six animals at each dose level. The volume injected was 0.5 ml for mice, but in frogs it was adjusted to individual masses (0.025 ml of venom solution for 1 g of frog) due to their larger range of masses. Toxicity was expressed as lethal dose 50% (LD<sub>50</sub>) estimated using the probit analysis following Finney (1971). We considered mortality recorded up to 48 h treatment for mice and 72 h for frogs. All experiments with frogs were performed in a climatic chamber at 25 C

TABLE 1.—Toxicity (LD<sub>50</sub>) of the venoms of adults and juveniles of *Bothrops jararaca* and *B. alternatus* in mice and frogs (mg/kg); 95% confidence limits in parentheses.

	Mice	Frogs
<i>Bothrops jararaca</i>		
Juvenile venom	5.88 (4.6–7.31)	53.64 (44.04–65.99)
Adult venom	1.74 (1.26–2.2)	91.44 (73.11–114.79)
<i>Bothrops alternatus</i>		
Juvenile venom	4.54 (3.56–5.86)	79.11 (65.03–99.2)
Adult venom	4.69 (3.49–6.44)	77.53 (63.73–97.22)

to prevent temperature effects on metabolic rate (Witford, 1973) which could affect the action of the venom.

## RESULTS

Venoms from adults of both *B. jararaca* and *B. alternatus* as well as juveniles of *B. alternatus* did not differ markedly in their LD<sub>50</sub> values in frogs (Table 1). Conversely, the toxicity of juvenile venom from *B. jararaca* in frogs was nearly twice that observed for the venom of adults. For mice, the venom of the adults of *B. jararaca* was about 3.4 times more lethal than that of juveniles, whereas for *B. alternatus*, the toxicity of the venoms was similar in juveniles and adults.

## DISCUSSION

Toxicity data indicate that the venom of juveniles of *B. jararaca* is especially efficient on anurans. As the snake grows and its diet changes, the venom of this species loses about 70% of its toxicity upon the preferred prey of the juvenile phase. Similarly, in *B. moojeni*, which also changes its diet from ectotherms to endotherms during ontogeny, the toxicity of the juvenile venom is about 86% higher for ectotherms than that of adults (Andrade et al., 1996). Thus, in *B. jararaca* (this study), *B. moojeni* (Andrade et al., 1996), and possibly other species of *Bothrops* that possess an ontogenetic shift in diet, the toxicity of the venom of juveniles correlates with their feeding habits. In contrast, in *B. alternatus*, which feeds on endotherms at any

size, the venom of juveniles lacks an increased toxicity on ectothermic organisms. Further investigation may reveal that this pattern extends to other heavy-bodied species of *Bothrops*, like *B. cotiara* and *B. fonscai*, possessing ontogenetically fixed diets.

For mice, the venom toxicity of adults of *B. jararaca* is 2.3 times higher than that of juveniles, reflecting a diet change to endothermic prey. For *B. alternatus*, which feeds on endotherms both as juvenile and adult, the venom of juveniles is expected to have a higher toxicity on endotherms to compensate for its smaller volume. However, we found no difference in the toxicity of juvenile venom from *B. alternatus* and that of adults. Therefore, a clear relationship between venom toxicity in endotherms and feeding habits is not apparent in the species of *Bothrops* presently studied. In general, the amount of venom spent by a snake to capture an endothermic prey item is small (Hayes, 1991; Hayes et al., 1995) compared to the quantity produced by an adult or even a juvenile snake (Furtado et al., 1991). Thus, it is possible that, in endotherms, a clear adaptive relationship between the prey capture role of the venom and diet may be disguised by functions other than prey killing. In this regard, alternative functions for venom (discussed below) may acquire increasing importance as the snake grows.

Juvenile snakes are constrained by body size to feed on small prey (in absolute terms), which are not as difficult to digest as the larger prey of adults (Andrade et al., 1997). Juveniles, however, may have limited resources to withstand long food deprivation periods, such as aestivation (Hirth, 1966). Therefore, venoms of juvenile snakes are thought to be under strong selective pressure to ensure prey capture (Mackessy, 1988), which may explain the high toxicity of juvenile venoms of *B. jararaca* (this study) and *B. moojeni* (Andrade et al., 1996) on anurans. In the case of juveniles of *B. alternatus*, a high toxic specificity on their preferred prey could be redundant, because the venom already has a high toxicity for endotherms.

As the snakes grow, larger mammalian

prey are taken (Greene, 1992; Sazima, 1992), and digestion becomes more difficult due to the greatly reduced surface to volume ratio of the prey (Pough, 1983; Pough and Groves, 1983). At this point, the selective pressures acting on venom composition may shift, and its function in prey digestion may become increasingly important. Accordingly, increased prey digestive resistance leads to a concurrent increase in venom proteolytic activity (Andrade et al., 1996; Mackessy, 1988), a feature also observed for *B. jararaca* and *B. alternatus* (Furtado et al., 1991). As the venom is injected into the prey's body, higher proteolytic activity associated with injection of a greater volume (Hayes et al., 1995) could accelerate the rupture of tissues, increasing the prey surface area exposed to stomach acids and enzymes (Thomas and Pough, 1979). Enhancement of prey digestion may reduce time and energy allocation, shortening the period in which snakes have their capacity for locomotion and defense decreased (Ford and Shuttlesworth, 1986), and lowering the high cost of prey digestion observed for sit-and-wait foraging snakes such as *Bothrops* (Andrade et al., 1997). Therefore, venom changes in *Bothrops* are consistent with an optimization of the venom action towards specific prey at specific stages of life. It appears that venom from *Bothrops* is specialized to kill prey for juveniles, and to aid in prey digestion for adults. This conclusion reinforces the ecological interpretation of venom ontogeny first envisaged by Kardong (1986) and further experimentally confirmed by Mackessy (1988) in *Crotalus viridis*.

The difference in toxicity between the venoms of adults of *B. moojeni* (Andrade et al., 1996) and *B. jararaca* in endothermic prey may also involve the prey capture role of venom. Although an ontogenetic shift in diet is observed in both species, adults of *B. jararaca* have a specialized diet preying exclusively upon endotherms (Sazima, 1992; Sazima and G. Puerto, unpublished data), while adults of *B. moojeni* include a larger variety of prey items in their diet, occasionally preying upon ectotherms (A. S. Abe and P. R. Manzani,

unpublished data). Thus, the increased toxicity of adult venom of *B. jararaca* in mice may reflect, at least partially, the specialized feeding habits of adults, while the more generalist adults of *B. moojeni* have a venom able to act on a broader spectrum of prey, but with lower specificity.

Snake venoms have long been a subject of great research interest; however, due to their medical importance, emphasis has been devoted mainly to the biochemical and pharmacological properties of the venoms. The contribution of such approaches to the understanding of venom system evolution and its relevance to snakes in their natural environment is limited and may even be misleading (Kardong, 1996). On the other hand, a few studies have examined snake venoms in an ecological/evolutionary framework seeking to understand the functional significance of their complexity, diversity, and variability to snake's survival. In this context, tests of venom toxicity on specific prey should be considered as a promising tool to examine further the biological role of venoms and their importance in the ecology and evolution of venomous snakes. Nonetheless, it is clear that snake venoms serve diverse roles (e.g., digestion, defense), such that adaptive relationships will require an integrated view of venom function.

*Acknowledgments.*—This study was a part of the M.Sc. thesis by D. V. Andrade, who thanks his thesis committee, I. Sazima and M. F. D. Furtado, for their valuable suggestions. An early version of this manuscript was largely improved by the comments of M. Martins and I. Sazima, both of whom also provided unpublished information about natural history of *Bothrops*. We thank R. Wirz and D. Fontanello from the Instituto de Pesca for the donation of the froglets, J. Jim from the Centro de Estudo de Venenos e Animais Peçonhentos (CEVAP), and W. Fernandes and M. F. D. Furtado from the Instituto Butantan for snakes and venom donation. The first author was supported by a Graduate Research Grant from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

#### LITERATURE CITED

- ANDRADE, D. V. 1995. Variação ontogenética do veneno em serpentes do gênero *Bothrops* (Squamata, Viperidae). Dissertação de Mestrado, Universidade Estadual Paulista, Rio Claro, São Paulo, Brasil.
- ANDRADE, D. V., A. S. ABE, AND M. C. DOS SANTOS. 1996. Is the venom related to diet and tail color during *Bothrops moojeni* ontogeny? *Journal of Herpetology* 30:285–288.
- ANDRADE, D. V., A. P. CRUZ-NETO, AND A. S. ABE. 1997. Meal size and specific dynamic action in the rattlesnake, *Crotalus durissus* (Serpentes, Viperidae). *Herpetologica* 53:485–493.
- BERNADSKY, G., A. BDOLAH, AND E. KOCHVA. 1986. Gel permeation patterns of venoms from eleven species of the genus *Vipera*. *Toxicon* 24:721–725.
- CAMPBELL, J. A., AND W. W. LAMAR. 1989. *The Venomous Reptiles of Latin America*. Cornell University Press, Ithaca, New York, U.S.A.
- DALTRY, J. C., W. WÜSTER, AND R. S. THORPE. 1996. Diet and snake venom evolution. *Nature* 379:537–540.
- DEMPFLE, C. E., R. KOHL, J. HARENBERG, W. KIRSCHSTEIN, D. SCHLAUCH, AND D. L. HEENE. 1990. Coagulopathy after snake bite by *Bothrops neuwiedii*: case report and results of *in vitro* experiments. *Blut* 61:369–374.
- FINNEY, D. J. 1971. *Probit Analysis*, 3rd ed. Cambridge University Press, Cambridge, U.K.
- FORD, N. B., AND G. A. SHUTTLESWORTH. 1986. Effects of variation in food intake on locomotory performance of juvenile garter snakes. *Copeia* 1986:999–1001.
- FURTADO, M. F. D., M. MARUYAMA, A. S. KAMIGUTI, AND L. C. ANTONIO. 1991. Comparative study of nine *Bothrops* snake venoms from adult female and their offspring. *Toxicon* 29:219–226.
- GANS, C., AND W. B. ELLIOT. 1968. Snake venoms: production, injection and action. *Advances in Oral Biology* 3:45–51.
- GLENN, J. L., R. C. STRAIGHT, M. C. WOLFE, AND D. L. HARDY, SR. 1983. Geographical variation in *Crotalus scutulatus scutulatus* (Mojave rattlesnake) venom properties. *Toxicon* 21:119–130.
- GREENE, H. 1983. Dietary correlates of the origin and radiation of snakes. *American Zoologist* 23:431–441.
- . 1992. The ecological and behavioral context for pitviper evolution. Pp. 107–117. *In* J. A. Campbell and E. D. Brodie, Jr. (Eds.), *Biology of the Pitvipers*. Selva, Tyler, Texas, U.S.A.
- . 1997. *Snakes: the Evolution of Mystery in Nature*. University of California Press, Berkeley, California, U.S.A.
- GUBENSEK, F., D. SKET, V. TURK, AND D. LEBEZ. 1974. Fractionation of *Vipera ammodytes* venom and seasonal variation of its composition. *Toxicon* 12:167–171.
- HAYES, W. K. 1991. Ontogeny of striking, prey handling and envenomation behavior of prairie rattlesnakes (*Crotalus v. viridis*). *Toxicon* 29:867–875.
- HAYES, W. K., P. LAVÍN-MURCIO, AND K. V. KARDONG. 1995. Northern Pacific rattlesnake (*Crotalus viridis oregonus*) meter venom when feeding on prey of different sizes. *Copeia* 1995:337–343.
- HIRTH, H. F. 1966. Weight changes and mortality of three species of snakes during hibernation. *Herpetologica* 22:8–12.
- ISHII, A., T. ONO, AND T. MATUHASHI. 1970. Electrophoretic studies on Habu snake venom (*Trimeresurus flavoviridis*), with special reference to the

- consecutive venom collection. *Japanese Journal of Experimental Medicine* 40:141-149.
- JAYANTHI, G. P., AND T. V. GOWDA. 1988. Geographical variation in India in the composition and lethal potency of Russell's viper (*Vipera russelli*) venom. *Toxicon* 26:257-264.
- KARDONG, K. V. 1986. Predatory strike behavior of the rattlesnake, *Crotalus viridis oreganus*. *Journal of Comparative Psychology* 100:304-314.
- . 1996. Snake toxins and venoms: an evolutionary perspective. *Herpetologica* 52:36-46.
- LOMONTE, B., J. A. GENE, J. M. GUTIÉRREZ, AND L. CERDAS. 1983. Estudio comparativo de los venenos de serpiente cascabel (*Crotalus durissus durissus*) de ejemplares adultos y recién nacidos. *Toxicon* 21:379-384.
- MACKESSY, S. P. 1988. Venom ontogeny in the Pacific rattlesnakes *Crotalus viridis helleri* and *C. v. oreganus*. *Copeia* 1988:92-101.
- MARTINS, M., AND M. GORDO. 1993. *Bothrops atrox* (common lancehead): diet. *Herpetological Review* 24:151-152.
- MEIER, J. 1986. Individual and age-dependent variations in the venom of the ferdelance (*Bothrops atrox*). *Toxicon* 24:41-46.
- MUSHINSKY, H. R., 1987. Foraging ecology. Pp. 1624-1629. In R. A. Seigel, J. T. Collins, and S. S. Novak (Eds.), *Snakes: Ecology and Evolutionary Biology*. MacMillan, New York, New York, U.S.A.
- POUGH, F. H. 1983. Amphibians and reptiles as low-energy system. Pp. 141-188. In W. P. Aspey and S. I. Lustick (Eds.), *Behavioral Energetics: the Cost of Survival in Vertebrates*. Ohio State University Press., Columbus, Ohio, U.S.A.
- POUGH, F. H., AND J. D. GROVES. 1983. Specialization of the body form and food habits of snakes. *American Zoologist* 23:443-454.
- SAINT-GIRONS, H. 1980. Selective modifications in the diet of vipers (Reptilia: Viperidae) during growth. *Amphibia-Reptilia* 1:127-136.
- SAZIMA, I. 1991. Caudal luring in two neotropical pitvipers, *Bothrops jararaca* and *Bothrops jararacussu*. *Copeia* 1991:245-248.
- . 1992. Natural history of the jararaca pitviper, *Bothrops jararaca*, in Southeastern Brazil. Pp. 199-216. In J. A. Campbell and E. D. Brodie, Jr. (Eds.), *Biology of the Pitvipers*. Selva, Tyler, Texas, U.S.A.
- SEXTON, O. J. 1956-1957. The distribution of *Bothrops atrox* in relation to food supply. *Boletín del Museo de Ciencias Naturales* 2:47-54.
- TAN, N. H., AND G. PONNUDURAI. 1990a. A comparative study of the biological properties of Australian elapid venoms. *Comparative Biochemistry Physiology* 97C:99-106.
- . 1990b. A comparative study of the biological properties of venoms from snakes of the genus *Vipera* (true adders). *Comparative Biochemistry Physiology* 96B:683-688.
- THOMAS, R. G., AND F. H. POUGH. 1979. The effects of rattlesnake venom on the digestion of prey. *Toxicon* 17:221-228.
- WITFORD, W. G. 1973. The effects of temperature on respiration in Amphibia. *American Zoologist* 13:505-512.
- WÜSTER, W., AND R. S. THORPE. 1991. Asiatic cobras: systematics and snakebite. *Experientia* 47:205-209.

Accepted: 15 March 1998

Associate Editor: Allison Alberts