

Venom Function in the Puerto Rican Racer, *Alsophis portoricensis* (Serpentes: Colubridae)

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***Alsophis portoricensis* is an opisthomegadont, colubrid snake that normally employs the secretion of its Duvernoy's gland to subdue its prey. We studied the effect of Duvernoy's gland secretion of *A. portoricensis* on prey digestion by comparing digestion of various structures of normally ingested lizards to that of nonvenomated lizards force fed to snakes. Faster digestion rates were recorded for the skin, forelimbs, liver, and lungs of envenomated anoles when compared to nonvenomated lizards. Also, overall digestion of envenomated anoles was faster than that of nonvenomated lizards.**

DURING ophidian evolution, two major methods of immobilizing prey have arisen: constriction and venom, both of which presumably reduce the risk of the struggling prey escaping or injuring the snake during ingestion (Gans, 1961; Shine and Schwaner, 1985). Venom has several possible functions: (1) immobilization and/or killing of prey, (2) accelerating digestion, and (3) predator deterrence (Minton and Minton, 1969; Gans, 1978).

Although popularly considered nonvenomous, it has become evident that some colubrids are at least mildly venomous (Willard, 1967; Morris, 1985; Nichols, 1986), and a few can even cause human fatalities (FitzSimons and Smith, 1958; Pope, 1958; Mittleman and Goris, 1978). Minton (1990) has recently reviewed human envenomation by colubrids.

To characterize a secretion as a venom implies that its toxicity is functional and not incidental. Secretions of Duvernoy's gland vary from those that produce hardly discernible physiological effects to those that are drastically toxic (Mittleman and Goris, 1978; Jansen, 1987). Those secretions producing minimal effects might not be considered venoms; however, the term has been applied to the oral secretions of even such apparently nonvenomous snakes as garter snakes (*Thamnophis*), hog-nosed snakes (*Heterodon*) (Minton, 1987), and the boid *Epicrates inornatus* (Minton and Weinstein, 1987). It is in this broad sense that the term venom is used in this study.

The primary morphologic indicator of toxicity in colubrids is the presence of Duvernoy's gland (McKinstry, 1978, 1983). This branched, tubular, salivary, sero-mucous or serous gland "is located in the postocular region, reaching the angle of the mouth posteriorly and the level

of the eye dorsally [anteriorly]" (Kochva, 1978). The secretion from Duvernoy's gland enters the oral cavity via a single duct that generally opens in the oral mucosa near the posterior maxillary teeth (Taub, 1967). Duvernoy's gland is regarded as a characteristic unique to colubrid snakes (Taub, 1966).

Gans (1978) considered that the primary function of the secretion of this gland may not be killing but immobilization of prey and, perhaps, aiding in digestion. However, he proposed that venom glands initially evolved in presnakes to secrete substances for lubricating the food, for conditioning dental surfaces, and for cleaning away material that might adhere to the teeth. Later, in the evolution of snakes, these secretions became more complex and facilitated prey immobilization, digestion, and predator deterrence (Gans, 1978). Savitzky (1980) also proposed that the Duvernoy's gland evolved to produce venomous secretions that allowed colubrid snakes to immobilize prey.

The Puerto Rican racer, *Alsophis portoricensis*, is the second largest Puerto Rican snake. It is an opisthomegadont xenodontine colubrid (Dowling, 1978), with a serous Duvernoy's gland (Taub, 1967). The occurrence of venom in *A. portoricensis* appears to have been first commented upon in 1961 by Hegeman. Heatwole and Banuchi (1966) reported on a bite from *A. portoricensis* that resulted in swelling, itching, pain, and discoloration of the bitten arm, complicated by secondary infection. Both of us have experienced bites on several occasions that produced a burning sensation, itching, swelling, bleeding with impaired coagulation, and pain.

Because a digestive function of venom has been found for the highly venomous viperid snake *Crotalus atrox* (Thomas and Pough, 1979),

our objective in this study was to assess the effect of Duvernoy's gland secretion on digestion of an opisthomedont snake, *A. portoricensis*.

MATERIALS AND METHODS

Most (84%) of the 32 adult snakes used during this study were collected at two localities: Caja de Muertos Island Natural Reserve (14 snakes) and the Cambalache Forest Reserve (13 snakes). The remaining five snakes were collected at different sites in northern Puerto Rico. All animals were collected from 7 July 1988 to 3 Dec. 1989. Caja de Muertos, off the south central coast of Puerto Rico, is an area of Subtropical Dry Forest, whereas Cambalache is an area of Subtropical Moist Forest (Ewel and Whitmore, 1973).

The snakes were housed individually in plastic reptile cages (61 cm wide $\times$ 31 cm high $\times$ 32 cm deep) with newspaper substrates and water ad libitum. The snakes were maintained on a natural photoperiod at a mean diurnal temperature of 29.6 C (SD = 2.31, range = 22–34.5 C, n = 375). Mean body temperature was 29.0 C (SD = 2.26, range = 21.5–33.7 C, n = 375). *Anolis cristatellus*, one of the three most common anoles in Puerto Rico and a lizard upon which *Alsophis* feeds in the wild (pers. obs.), was used as the prey item in all experiments. Snakes were weighed and anoles measured and weighed prior to the beginning of the feeding experiments. Testing was conducted in the cages during daytime. One day after the feeding experiments, snakes were offered anoles that they were allowed to ingest and digest normally. Prey included mostly *A. cristatellus*, but occasionally, depending on availability, snakes were also offered other species of local anoles. If a snake failed to eat for two consecutive weeks, it was excluded from the study until normal feeding returned.

We observed 35 feeding sequences of *A. portoricensis* on live prey. To standardize hunger level, snakes were tested not less than six days after the previous meal. Snakes were forced to regurgitate their anoles by palpation six hours after ingestion. Regurgitated anoles were preserved in 10% formalin for further examination. The extent of digestion was quantified by three independent observers using a digestion scoring system (Table 1). Modal scores were used as the score for a given structure. Twice we could not palpate any compact mass in the snake's stomach after six hours. In these two instances anoles were given a score of zero for

TABLE 1. DIGESTION SCORING SYSTEM USED FOR *Anolis cristatellus*.^a

Structures	Score for each structure for condition indicated		
	2	1	0
Skin (% digested)	0–33	34–66	>66
Head	Intact	Partly digested	Absent
Body wall	Closed	Partly open (small cavity)	Open
Forelimbs	Both present	One absent	Both absent
Hindlimbs	Both present	One absent	Both absent
Digestive tract	Continuous	Remnants	Absent
Liver	Intact	Partly digested	Absent
Lungs	Intact	Partly digested	Absent

^a A recently killed anole has a score of 16 with this system, and the score decreases progressively with extent of digestion.

all structures. We determined total digestion score by summing the score for all structures and we compared it to the total score of nonenvenomated anoles (see below).

To assess the role of *A. portoricensis* venom in prey digestion, we force fed captive snakes with *A. cristatellus*. Lizards were lubricated very lightly with Pam cooking spray and, with the aid of 25 cm long blunt forceps, pushed into the snakes' stomach. We shielded the snakes' teeth with a plastic or rubber dam before introducing lizards so that the anoles were not accidentally envenomated. We forced the snakes to regurgitate the prey after six hours.

To test whether the teeth punctures in the lizards occurring during natural feeding conditions provided a pathway that significantly augmented the penetration of digestive enzymes into the lizard's body (Gans, 1961), we made two rows of 8–9 punctures on either side of the dorsal midline of nine lizards ("puncture" experiments). The perforations (1 mm deep) were made with sterilized sewing needles. Again, snakes were forced to disgorge prey after six hours. Results obtained were compared to those from the force-feeding experiments (Table 2).

Statistical procedures follow Sokal and Rohlf (1981) and Zar (1984). Nonparametric statistics (Mann-Whitney U-test [two-tailed], Spearman's

TABLE 2. MANN-WHITNEY U-TEST FOR DIFFERENCES IN EXTENT OF DIGESTION BETWEEN FORCE-FEEDING (F) AND PUNCTURE EXPERIMENTS (P). E = experiment. Total digestion score was determined by summing the score for all structures. See Table 1 for digestion scoring system.

Structure	E	Mean ($\pm$ SD)	n	U	P
Skin	P	1.22 ($\pm$ 0.67)	9	0.865	0.387
	F	1.44 ($\pm$ 0.65)	36		
Head	P	1.0 ($\pm$ 0.0)	9	-0.014	0.989
	F	1.0 ($\pm$ 0.24)	36		
Body wall	P	1.22 ($\pm$ 0.83)	9	0.284	0.777
	F	1.31 ($\pm$ 0.82)	36		
Forelimbs	P	1.78 ($\pm$ 0.44)	9	0.624	0.533
	F	1.92 ($\pm$ 0.28)	36		
Hindlimbs	P	2.0 ($\pm$ 0.0)	9	-0.014	0.989
	F	2.0 ($\pm$ 0.0)	36		
Digestive tract	P	1.89 ($\pm$ 0.33)	9	-0.014	0.989
	F	1.89 ($\pm$ 0.32)	36		
Liver	P	1.44 ($\pm$ 0.53)	9	0.114	0.910
	F	1.47 ($\pm$ 0.51)	36		
Lungs	P	1.78 ($\pm$ 0.44)	9	0.596	0.551
	F	1.89 ($\pm$ 0.40)	36		
Average ($\pm$ SD) digestion score					
Force feeding vs puncture		12.92 ($\pm$ 2.10)	36	0.894	0.371
		12.33 ($\pm$ 2.12)	9		

rank correlation coefficient) and goodness of fit tests (G-test of independence) were utilized for data analysis. All statistical tests were performed using the STATISTIX (Version 3.0) software (Analytical Software, 1989). Statistical significance was determined at level $P \leq 0.05$.

RESULTS

Alsophis portoricensis injects its venom by chewing its prey (Thomas and Prieto-Hernández, 1985; pers. obs.). While holding prey items, snakes repeatedly embedded the teeth. These chewing motions did not appear to facilitate swallowing because the prey remained stationary but instead may have helped introduce Duvernoy's gland secretion into the prey (Jansen and Foehring, 1983).

There were no statistical differences in the degree of digestion of any structure nor in the average digestion scores (Table 2) between force-feeding and puncture experiments. Therefore, we combined the data for both experimental treatments to increase statistical power. We then compared prey digestion scores between force-fed and normally fed snakes.

Force-fed lizards had higher digestion scores for the skin, forelimbs, liver, and lungs than normally ingested animals. Average extent of digestion was significantly higher for envenomated anoles than for nonenvenomated lizards (Table 3). Because the data could not be normalized by any transformation to use an analysis of covariance, we plotted total digestion scores against ln-transformed anole/snake mass ratio to examine the effect of mass ratio on the rate of digestion for normal- and force-feeding (pooled) experiments. Curves were fitted to the data using the locally weighted scatterplot smoothing method (Cleveland, 1979, 1981), a graphing method that does not presuppose the shape of the function. For envenomated prey, a low mass ratio was associated with a faster digestion rate, but for nonenvenomated lizards, the relationship between mass ratio and digestion score was weaker (Fig. 1).

There were significant positive correlations between the percentage of skin digested and the occurrence of cavities in the anole body wall and between cavities in the body wall and the extent of digestion of the digestive tract, liver, and lungs (Table 4). These observations suggest

TABLE 3. MANN-WHITNEY U-TEST FOR DIFFERENCES IN EXTENT OF DIGESTION BETWEEN NORMAL-FEEDING (N) AND FORCE-FEEDING EXPERIMENTS (F') (POOLED). E = experiment. Total digestion score was determined by summing the score for all structures. See Table 1 for digestion scoring system.

Structure	E	Mean ($\pm$ SD)	n	U	P
Skin	N	1.03 ($\pm$ 0.79)	35	1.979	0.048
	F'	1.40 ($\pm$ 0.65)	45		
Head	N	0.86 ($\pm$ 0.43)	35	1.062	0.288
	F'	1.0 ($\pm$ 0.21)	45		
Body wall	N	1.11 ($\pm$ 0.80)	35	0.955	0.339
	F'	1.29 ($\pm$ 0.82)	45		
Forelimbs	N	1.40 ($\pm$ 0.65)	35	3.147	0.002
	F'	1.89 ($\pm$ 0.32)	45		
Hindlimbs	N	1.77 ($\pm$ 0.60)	35	1.086	0.277
	F'	2.0 ($\pm$ 0.0)	45		
Digestive tract	N	1.71 ($\pm$ 0.57)	35	0.941	0.347
	F'	1.89 ($\pm$ 0.32)	45		
Liver	N	0.97 ($\pm$ 0.45)	35	3.370	0.001
	F'	1.47 ($\pm$ 0.50)	45		
Lungs	N	1.34 ($\pm$ 0.68)	35	3.298	0.001
	F'	1.87 ($\pm$ 0.40)	45		
Average ($\pm$ SD) digestion score					
Normal vs force feeding (pooled)		10.20 ($\pm$ 3.83)	35	3.525	0.0004
		12.80 ($\pm$ 2.10)	45		

that, once the body wall of the prey was ruptured, action of digestive enzymes directly on these internal structure accelerated their digestion.

DISCUSSION

Alsophis portoricensis represents a fairly early stage in the evolution of a venom delivery system. Our data show that *A. portoricensis* venom has a marked digestive function, which suggests that, in at least some snake lineages, a function for digestion of prey was acquired with the evolutionary elaboration of fangs.

Hegeman (1961) found that the hyaluronidase, total protein content, proteolytic, and hemolytic activities of Duvernoy's gland secretion of *A. portoricensis* are intermediate between those of *Crotalus atrox* and the nonvenomous water snake *Nerodia sipedon* (= *Natrix sipedon*) (Colubridae).

Vest (1981) injected laboratory mice with re-constituted Duvernoy's gland secretion of *T. elegans* and found that it caused both localized hemorrhage at the injection site and systemic

hemorrhage. Lungs were the first organs affected, followed by the diaphragm, mesentery, stomach lining, and liver. Jansen (1987) showed that the secretion of Duvernoy's gland of *T. elegans* has myonecrotic and hemorrhagic effects as well. Thomas and Prieto-Hernández (1985) demonstrated that laboratory mice that had been bitten by *A. portoricensis* showed a limited bleeding in the subcutaneous area (although subcutaneous hematomas extended beyond the area of the bite to the inguinal and abdominal regions), whereas *A. cristatellus* died due to hemorrhaging into the lungs. Rosenberg et al. (1985) found that nonlethal doses of the Duvernoy's gland secretion of *Malpolon monspessulanus*, *Spalerosophis diadema*, and *Thamnophis sirtalis* injected into laboratory mice caused paralysis and impaired breathing, whereas lower doses greatly reduced mobility. Therefore, the venom of these species may act to weaken but not necessarily to kill struggling prey.

By accelerating digestion, venoms may allow snakes to expand foraging activities (Mackessy, 1988) or to consume more prey per unit time. At least in captivity, many colubrids feed more

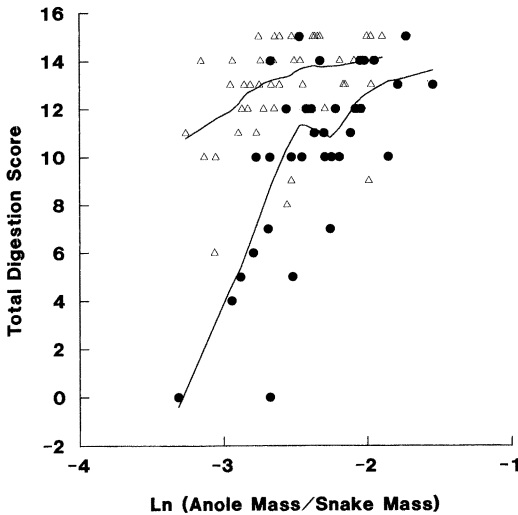


Fig. 1. Relationship between total digestion score and (ln-transformed) anole/snake mass ratio for normal- (closed circles) and force-feeding (open triangles) experiments.

often than either some elapids, or boids (Banks, 1985). Also, if a snake empties its stomach more rapidly, it can escape faster from predators (Garland and Arnold, 1983) or move faster when pursuing prey.

Thomas and Pough (1979) clearly demonstrated that the venom of *C. atrox* also accelerated the process of digestion. In particular, viperid venoms appear to weaken the gut and body wall of the prey so that these structures are ruptured. More recently, Pough and Groves (1983) showed that viperid snakes are specialized for the ingestion of very large prey.

A digestive function of snake venoms was proposed by Zeller (1948), Gans (1978), Ivanov and Ivanov (1979), and Kardong (1980). Kochva et al. (1983) suggested that oral glands of reptiles first produced hydrolytic enzymes that were already secreted by the pancreas and, therefore, had a digestive function.

Heavy necrosis at the bite site of vipers and colubrids does not itself contribute to prey death but is attributable to the "primitive digestive properties of snake venoms" (Zeller, 1948). In this regard, the tribe Alsophiini (Dowling, 1978) appears to be the base group of the xenodontinae, and *Alsophis* is believed to be among the more primitive colubrids (Maglio, 1970; Marx and Rabb, 1972; Rabb and Marx, 1973).

Snakes are specialized to ingest large prey relative to their size (Gans, 1961) and to swallow

TABLE 4. SPEARMAN'S RANK CORRELATIONS BETWEEN DEGREE OF DIGESTION OF VARIOUS *Anolis cristatellus* ORGANS AND STRUCTURES.

Comparisons	r	df	P
% Digested skin and body wall cavities	0.76	78	<0.0001
Body wall cavities and digestive tract	0.46	78	<0.0001
Body wall cavities and liver	0.29	78	0.0084
Body wall cavities and lungs	0.42	78	0.0001

them whole, without mastication. If, when colubrid snakes radiated, there was competition for prey between henophidian and colubrid snakes or prey was seasonally available, a higher digestive rate could have been selectively advantageous (Kardong, 1980).

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Systematics, Evolution, and Biogeography of *Notropis chlorocephalus* and *N. lutipinnis*

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Samples collected from throughout the ranges of the greenhead shiner, *Notropis chlorocephalus*, and the yellowfin shiner, *N. lutipinnis*, were examined electrophoretically for patterns of variation at 39 presumptive gene loci. Within *N. lutipinnis*, statistically significant genic heterozygosity occurred at 18 of the 23 polymorphic loci, whereas only three of 13 polymorphic loci exhibited significant variation in *N. chlorocephalus*.

A previous hypothesis of a single, polymorphic species inclusive of two subspecies and intermediate, intergrade populations in the Santee-Broad River is investigated. Patterns of allozyme variation are inconsistent with the hypothesis of intergradation. When considered together with morphological variation in fin color and pharyngeal dentition within a phylogenetic perspective, the evidence indicates that *N. chlorocephalus* is a diagnosable and monophyletic taxon. The presently recognized taxonomic species *N. lutipinnis* is a paraphyletic composite of three diagnosable and two undescribed monophyletic entities. The forms from the Santee-Broad and Pee Dee rivers are more closely related to *N. chlorocephalus* than to the taxon to which they are presently assigned. The overall pattern of divergence depicted in the phylogeny is congruent with the progressive dismemberment of a prehistoric northeasterly flowing "White's River" that has given rise to current drainage configurations and species distributions along the Atlantic Slope.

THE validity and level of reproductive isolation of species thought to exchange genes with other species has been a subject of considerable debate, especially with regard to fishes of the family Cyprinidae (Gilbert, 1961; Buth and Mayden, 1981; Dowling and Moore, 1984, 1985). To some degree, controversies of this nature are based upon the investigator's particular species concept. In most instances, reasons for questioning the evolutionary status of a species have been founded on the existence of clines in morphological traits leading an investigator to invoke hybridization or intergra-

dation. Few genetic studies have corroborated the congruence or predictability of morphological clines with patterns of gene flow, and many clines explained by hypotheses of intergradation remain untested. The evolutionary status of the largely Atlantic Slope species *Notropis lutipinnis* and *N. chlorocephalus* has recently been the subject of such speculation.

The greenhead shiner, *N. chlorocephalus*, and the yellowfin shiner, *N. lutipinnis*, belong to the *N. rubricroceus* species group of the subgenus *Hydrophlox* (Swift, 1970). *Notropis chlorocephalus* is endemic to the Santee-Catawba River drain-