# FACTORS THAT INFLUENCE VENOM EXPENDITURE IN VIPERIDS AND OTHER SNAKE SPECIES DURING PREDATORY AND DEFENSIVE CONTEXTS

William K. Hayes[1], Shelton S. Herbert[1], G. Curtis Rehling[1], and Joseph F. Gennaro[2]

Abstract: In this paper we review the many factors that can influence the quantity of venom snakes expend when feeding or defending themselves. In addition to data from prior studies, we draw conclusions from recent unpublished data and from the clinical findings of snakebite cases. Three families of venomous snakes (atractaspidids, elapids, and viperids) possess an advanced venom apparatus that has evolved to effectively deliver large quantities of venom during a brief envenomating strike. Experimental data support the hypothesis that snakes are capable of making decisions on how to allocate or "meter" their venom, deploying more in some circumstances and less in others. Snakes are not unique in this ability, however, as a number of invertebrates have independently evolved mechanisms for metering and conserving their venom. The amount of venom expended by snakes is presumably acted on by natural selection and may vary with respect to both intrinsic and extrinsic circumstances of the strike. The optimal amount delivered may be influenced by the multiple needs for venom to immobilize and/or kill prey, to facilitate chemosensory relocation of prey released after being bitten, to enhance prey digestion, and to defend against attack by potential predators and antagonists. For both predatory and defensive contexts, snake size (and the corresponding supply of venom) appears to be the most important determinant of venom expenditure during a bite, with larger snakes usually delivering much more venom. Hunger, size of prey, and species of prey, however, may contribute to metering decisions made by snakes during feeding. Compared to predatory strikes, defensive bites involve greater variation in venom expenditure. When biting models of human limbs, snakes can inject larger doses of venom than are ordinarily delivered into mice. Some snakes have the capacity to deliver multiple bites without severely depleting their venom reserves. In contrast to predatory strikes, the duration of fang contact appears to be an important determinant of envenomation success during defensive bites. Dry defensive bites occur, and may represent metering decisions by the snake or the consequences of kinematic constraints on venom delivery. Whereas viperid snakes typically strike and quickly release targets for both predatory and defensive bites, elapids are more inclined to hold after biting, which provides opportunity for delivery of more venom. Some elapids (e.g., spitting cobras) have a more sophisticated delivery system that allows them to repeatedly spit small fractions of their venom for defensive purposes. Certain members of another family, the colubrids, have a toxic Duvernoy's secretion that is part of a less-developed venom apparatus. Although some colubrids can deliver significant amounts of venom, they are much less effective in doing so than members of the truly venomous snake families. Several envenomation strategies of snakes have important implications for the severity of envenomation in humans.

## Introduction

The venom and venom apparatus of elapid, viperid, and atractaspidid snakes have been studied in considerable detail. The venom, a biochemically complex mixture of liquids comprised largely of toxic proteins, is synthesized and stored within the paired venom glands (e.g., Tu, 1977, 1982, 1991; Elliott, 1978; Gans, 1978; Kochva, 1987; Chippaux et al., 1991; Aird, 2002). Under pressure from associated muscles, the glands expel their contents through the venom ducts to a pair of hollow-tipped fangs (Rosenberg, 1967; Haas, 1973; Kochva, 1978; Mackessy, 1991; Kardong and Lavin-Murcio, 1993; Young et al., 2000, 2001; Young and Zahn, 2001). The venom, when injected (or sprayed, as in certain species of cobras) into the tissues of another organism, exerts toxic and often lethal effects. Although variable in effectiveness, the venom apparatus and kinematics of biting have evolved to deliver large quantities of venom during a brief period of fang contact (e.g., Gans, 1961; Kardong, 1982; Kochva, 1987; Hayes, 1992a; Kardong and Lavin-Murcio, 1993; Kardong et al., 1997a; Kardong and Bels, 1998). The actual amount of venom expended is under control of the central nervous system, and may vary with respect to both intrinsic (under the snake's control) and extrinsic (beyond the snake's control) circumstances (e.g., Hayes, 1992a, 1992b, 1993, 1995; Hayes et al., 1995). The optimal amount of venom to expend will depend upon its intended use.

In addition to the aforementioned families of venomous snakes, a number of colubrid snakes (the largest family of mostly nonvenomous taxa) also possess toxic secretions (McKinstry, 1983; Minton, 1990, 1996). These secretions are produced by the Duvernoy's gland that is always associated with enlarged rear maxillary teeth (McKinstry, 1983; Weinstein and Kardong, 1994). Because toxicity of the secretion is relatively weak in most species (Weinstein and Kardong, 1994) and the venom apparatus is poorly developed for delivery of venom (the

---

[1] Department of Natural Sciences, Loma Linda University, Loma Linda, California 92313, USA. E-mail (WKH): whayes@ns.llu.edu

[2] Department of Anatomy, University of Florida, Gainesville, Florida 32610, USA

teeth are not hollow and the gland is generally small, lacking a storage reservoir and extensive muscular attachment; Kochva, 1978; Kardong and Lavin-Murcio, 1993), the function of this secretion has been debated (Rodriguez-Robles, 1994; Kardong, 1996). Nevertheless, it clearly functions as a venom in some species for which it serves to subdue (or kill) prey or contributes to defense.

In this paper, we review the various factors that influence how viperid (and other snake species) allocate their venom supplies when biting. We begin by examining the biological roles, or functions, of venom, and how these relate to the need of snakes to have the ability to control the quantity of venom released during a bite. Although this ability has proved difficult to demonstrate, there are ample reasons why snakes should accrue selective benefits in having the capacity to "meter" their venom. We also briefly review the history of attempts to measure venom expenditure before we began to conduct our own studies. Following this, we focus on the primary factors that influence how snakes use their venom when biting. We first discuss a number of factors that influence venom delivery during predatory bites, and then we discuss those associated with defensive bites. Although we draw upon a number of studies, the majority of data available is based on our own work with North American crotalines. In some instances, we present new data that have not yet been published. Finally, we offer some general conclusions that emphasize the importance of envenomation strategies, not only to the snake, but to the human snakebite victim as well.

## The Functional Roles of Venom in Snakes

The primary roles of snake venom are for procuring food (predation) and for protection against attack (defense). Within each of these contexts, venom may function in several ways.

When acquiring food, venom serves three important functions: (1) rapid immobilization and killing of prey, (2) facilitation of prey relocation, and (3) acceleration of prey digestion. Most snakes swiftly strike, envenomate, and voluntarily release a larger prey item, which minimizes the risk of sustaining retaliatory injury (Kardong, 1986a). Prey that are released often travel several meters or more before dying, making it necessary for the snake to relocate its victim (Kuhn et al., 1991; Hayes, 1992a). The venom alters the scent of the prey such that the snake is able to relocate its meal by following the odoriferous trail deposited by the envenomated animal (reviewed by Chiszar et al., 1992, 1999; Lavin-Murcio et al., 1993). Thus, in addition to killing the prey, adequate venom delivery is important to minimize the distance the snake must travel to relocate its prey and to reduce the risk of losing its meal (Chiszar et al., 1983; de Cock Buning, 1983; Hayes et al., 1995). The proteolytic properties of venom also accelerate digestion, which may prevent putrefaction and regurgitation of larger, bulkier prey (Thomas and Pough, 1979; Rodriguez-Robles and Thomas, 1992). Moreover, selection may favor venom components that quickly immobilize prey but allow for longer survival to distribute the venom more effectively in the victim's tissues. Depending on local prey availability or other factors, selection may act on venom components for any of these functions independent of or in tandem with other functions (Chiszar et al., 1999; Aird, 2002). There is evidence to suggest that variation in venom composition within and between species can be attributed in part to dietary differences (e.g., Daltry et al., 1996; Jorge da Silva and Aird, 2001).

When confronted by predators (e.g., canids, raptors) or antagonists (e.g., ground squirrels, ungulates, humans), snakes also rely on venom for defense. It is important to distinguish between predators (which attack the snake to consume it) and antagonists (which harass or attack the snake but have no intention of eating it), because the snake's strategy for survival may vary with context of the attack. Snakes appear to benefit from defensive use of their venom in both proximate (current mechanisms) and ultimate (adaptations via natural selection) ways. Although envenomation may be fatal, venom injected into the tissues of an animal presumably causes a painful sensation, which, of itself, should be an effective deterrent to attack. Because a defensive bite is highly unlikely to cause death of the attacker before the snake itself dies, the proximate benefit to the snake is that a painful bite will often terminate an attack, allowing the snake to survive. In ultimate terms, the lethal bite confers protection against attack from predators that have been selected to avoid or reduce predation on snakes or to interact with them in a more cautious manner (e.g., Coss et al., 1993; O'Connell and Formanowicz, 1998). Given these considerations, the effectiveness of envenomation during defensive bites may vary with composition of venom or biochemical resistance of the target animal. Neurotoxic venoms, for example, do not elicit painful sensations as readily as hemorrhagic venoms (e.g., Minton, 1987). Thus, selection may favor particular venom components not only for their roles in procuring food but also for their effectiveness at defense.

For obtaining food, some authors have used the phrase "offensive strikes" rather than "predatory strikes." We prefer to use predatory strike, which is more descriptive of the context for the behavior. Moreover, throughout this paper we refer to bites that are generally associated with a rapid strike followed by release of the target. On occasion, more so for elapids and toxic colubrids, snakes will strike and then maintain a hold rather than release the target. Unless otherwise mentioned, our discussion of biting is restricted to the strike followed by an immediate release that is characteristic of many viperids.

## The Importance of Venom Metering

There are reasons why snakes should be judicious when deploying their venom reserves. Venom, no doubt, is a valuable commodity. Although we do not know how costly it is to produce or to store, we can assume there is some kind of metabolic expense to replacing venom that has been expended. Moreover, it may be disadvantageous for a snake to have a depleted supply of venom. A snake with insufficient venom may be unable to procure additional prey or defend itself against attack until its supply of venom has been at least partially restored (Hayes et al., 1995). The amount of time required to replenish venom is poorly understood. When the venom glands are completely emptied (e.g., by forceful venom extraction), up to two weeks may be required to refill the glands (Kochva, 1960; Schaeffer et al., 1972; Leinz and Janeiro-Cinquini, 1994). Presumably, less time is required after expenditure of smaller venom quantities, but this hypothesis has not been tested.

In addition to the need for conserving a valuable commodity, the optimal amount of venom to expend may vary with context of use. Prey that is large and/or more resistant to venom, for example, may be more effectively procured or digested when more venom is injected. Small prey, such as neonatal rodents, are often captured and consumed without any apparent use of venom (Klauber, 1972; Radcliffe et al., 1980). The amount of venom used in a defensive bite may vary depending on the identity of the attacker or the level of perceived threat. A snake that is physically grasped by an attacker, for example, is likely to inject more venom because the immediate risk of death is far greater than the risk of having depleted supplies subsequently (Hardy, 1991; Herbert, 1998).

Showing that snakes expend varying quantities of venom in different contexts does not necessarily imply intentional metering by the snake. There may be constraints to venom expenditure during a strike that cannot be controlled by the snake and must be taken into consideration by the investigator. For example, when a snake strikes a large, vertical surface (typical of defensive bites), as opposed to a small, horizontal surface (typical of predatory bites), the different alignment of jaw and fangs may impede venom flow (Kardong, 1986b). Brief duration of fang contact may also reduce delivery of venom, which may be deliberate on the part of the snake (hence, metering in a sense) or incidental to the reaction of the victim (Hayes, 1991a; Herbert, 1998). Failure to insert both fangs into the victim (Hokama, 1978; Kardong, 1986b), or the breakage of a fang during the bite, may likewise lead to reduced venom expenditure. The manner of prey handling can also influence venom delivery. For example, snakes probably inject more venom into prey that are struck and subsequently retained in their jaws than into prey that are released immediately after envenomation (Kardong, 1982, 1986a; Hayes, 1992b).

Until recently, the ability of snakes to meter their venom was the subject of intense speculation and debate (e.g., Gennaro et al., 1961; Klauber, 1972; Allon and Kochva, 1974; Russell, 1980a, 1984; Morrison et al., 1982, 1983a; Kardong, 1986a, b; Hardy, 1991). Several carefully designed experiments have now confirmed this ability in at least some taxa. The fact that rattlesnakes deliver more venom into larger prey without adjusting the kinematics of the bite (e.g., duration of fang contact) constitutes the strongest evidence to date that snakes indeed can meter their venom supplies (Hayes, 1995; Hayes et al., 1995). From the unpublished studies described in this paper, we present evidence that snakes can also meter their venom during defensive bites. Young et al. (2000) recently demonstrated that the venom gland musculature of rattlesnakes is functionally divided, which provides a mechanism for regulating venom flow during strikes.

Snakes are not unique in their ability to meter venom. Organisms as simple as anemones and jellyfish appear to regulate how much venom they expend. These cnidarians capture prey by use of their harpoon-like nematocysts, and the number of units recruited may correspond to the struggle of their prey. In addition to mechanical and vibrational cues, the supporting cells of anemone nematocysts can respond to chemical cues released by the prey that inhibit further discharge of nematocysts, thereby conserving venom (Watson and Hessinger, 1994; Thorington and Hessinger, 1998). Both spiders and tarantulas are similarly judicious in their use of venom. These arachnids release more venom if the prey struggles longer or more vigorously, and they inject more into larger prey or prey with greater ability to escape (Robinson, 1969; Perret, 1977; Pollard, 1990; Rein, 1993; Boeve, 1994; Boeve et al., 1995; Malli et al., 1998, 1999). Electric fish (eels and rays), which use an alternative weapon (electric shock) that is energetically costly to deploy, appear capable of metering the number and duration of electric organ discharges depending on prey responsiveness and the type of threat (e.g., Belbenoit, 1986; Lowe et al., 1994). They may also seek to stun but not kill their prey. We anticipate that eventually a wide diversity of venomous animals will be shown to have the capacity to meter their venom supplies. These non-reptilian organisms, however, generally deliver multiple doses of venom in contrast to single ones when subduing their prey, and their metering decisions are often made in response to the prey's reaction to the venom (e.g., Malli et al., 1999). Because many snakes inject their venom during a single brief bite, metering decisions may need to be made prior to the attack and without feedback from the prey's reaction to envenomation.

The fact that many venomous animals judiciously meter their venom confirms the biological importance of venom conservation and the need of conditional strategies for feeding and defense. The ability to meter venom has evolved independently in diverse groups of organisms, and it appears to have important adaptive value.

## Attempts to Measure Venom Expenditure

Interest in measuring venom expenditure during bites by snakes has inspired researchers to develop a variety of techniques. Early attempts to measure such quantities involved crude estimates based on forceful venom extractions (manual pressure exerted on the head and glands) that followed a voluntary bite of a membrane-covered beaker (e.g., Acton and Knowles, 1914a, b; Fairley and Splatt, 1929), or the weighing of a mouse before and after a bite (Kochva, 1960). More refined techniques were subsequently developed to measure venom expenditure directly.

The majority of studies on venom expenditure have focused on predatory bites. Gennaro et al. (1961), using injected radioiodide excreted in the saliva concomitantly with the venom as an indicator, concluded that *Agkistrodon piscivorus* delivered more venom into rats than into mice (but see below). In contrast, Allon and Kochva (1974), using snakes that produced $C_{14}$-labeled venom after consuming labeled food, found no difference in venom mass injected by *Vipera* (= *Daboia*; see Lenk et al., 2001) *palaestinae* into mice and rats. Kondo et al. (1972) developed a toxicity assay to show that *Trimeresurus* (= *Protobothrops*) *elegans* and *T.* (= *P.*) *flavoviridis* released less than 10% of their available venom in a single bite of mouse-sized (20 g) pieces of rabbit muscle. Morrison et al. (1982, 1983a,b, 1984) pioneered enzyme-linked immunosorbent assay (ELISA) to compare sequential strikes at mice among various Australian elapids. Although venom delivery patterns differed significantly between species, venom quantities injected generally declined through successive bites as strike coordination and efficiency deteriorated. Tun-Pe et al. (1991b) also used ELISA to show that *D. russelii* expended similar amounts of venom in each of four successive bites of mice. For many of these species, the envenomation efficiency (proportion of venom delivered into tissues rather than spilled on the surface) and percentage of available venom expended are summarized and compared by Hayes et al. (1992).

For defensive bites, Hokama (1978) used spectrophotometric measurements to quantify venom injected into saline bags by *P. flavoviridis*. There was no correlation between size of snake and amount of venom expended, but the snakes showed an apparent decline in venom delivery through successive bites (we cannot ascertain whether statistical tests supported their conclusion). Tun-Pe and Khin-Aung-Cho (1986) weighed pieces of plastic foam bitten by *D. russelii* and found that larger snakes delivered more venom than smaller snakes, and that the amount released declined through up to five successive bites. The mean amount of venom released during defensive bites by adult snakes (63 mg in first bite) was substantially more than that measured in predatory bites (1.2–2.4 mg in first bite) by adults of the same species in the aforementioned study of Tun-Pe et al. (1991b). Morrison et al. (1983a) used ELISA measurements to experimentally compare predatory versus defensive bites. They reported that the Australian Rough-scaled Snake (*Tropidechus carinatus*) injected less venom into an agar-filled glove (defensive stimulus) than into a mouse (predatory stimulus) in the first bite, but more in the second bite. Unfortunately, they did not cite statistical tests to support their conclusion.

**Table 1**. Comparisons of total venom expended during a single predatory bite of an adult mouse by various elapid, viperid, and colubrid snakes. The number of snakes used (N) and total number of bites measured are provided, as well as the length of the snakes (in some cases reported as snout-vent length, SVL).

| Species | N (bites) | Length (cm) | Venom expended (mg) | | Source |
|---|---|---|---|---|---|
| | | | Mean ± SE | Range | |
| **Elapidae** | | | | | |
| *Acanthophis antarcticus*[a] | 5(6) | < 100? | 42 ± 16 | 3–109 | Morrison et al., 1983[b] |
| *Notechis scutatus*[a] | 11(13) | ca. 100 | 14 ± 4 | 1–37 | Morrison et al., 1982 |
| *Oxyuranus microlepidotus* | 5(8) | 100–200 | 18 ± 5 | 1–46 | Morrison et al., 1984 |
| *O. scutellatus*[a] | 11(13) | 115–190 | 22 ± 7 | 1–74 | Morrison et al., 1982 |
| *Pseudonaja textilis*[a] | 10(12) | 90–150 | 5 ± 1 | 0–10 | Morrison et al., 1983[b] |
| *Tropidechus carinatus*[a] | 8(10) | 40–100 | 6 ± 2 | 0–23 | Morrison et al., 1983[b] |
| **Viperidae** | | | | | |
| *Agkistrodon piscivorus* | 10(35) | 152–175 | 14 ± 2 | 0–58 | Gennaro et al., 1961; this study |
| *Crotalus concolor* | 10(10) | 44–58 SVL | 6 ± 1 | 2–10 | W. Hayes, unpublished |
| *C. oreganus*[b] | 6(6) | 62–76 SVL | 15 ± 5 | 1–31 | Hayes et al., 1995 |
| *C. viridis* | 11(34) | 76–95 SVL | 15 ± 1 | 5–25 | Hayes, 1992[b] |
| *Daboia palaestinae*[c] | 7(21) | > 100? | 54 ± 52 | 1–188 | Allon and Kochva, 1974 |
| *D. russelii*[a] | 4(4) | 87–100 | 1.7 ± 0.5 | 1.2–2.4 | Pe et al., 1991 |
| *Protobothrops elegans*[d] | 21(21) | 102–132 | 11 | 2–31 | Kondo et al., 1972 |
| *P. flavoviridis*[d] | 17(17) | 123–165 | 13 | 3–33 | Kondo et al., 1972 |
| **Colubridae** | | | | | |
| *Boiga irregularis* | 9(9) | 122–184 SVL | 3.6 ± 0.3 | 2.5 | Hayes et al., 1993 |

[a] = studies that involved multiple bites in succession; only data from the first mouse bitten is included. [b] = range of venom expended not cited in paper, hence retrieved from notes. [c] = snakes were large (500–700 g) and assumed by us to be close to or in excess of 100 cm; each snake bit three mice and three rats in succession and in random order; we present values for all mice pooled from Table 2 of the original paper. [d] = mouse-sized (20 g) pieces of rabbit muscle were bitten; we calculated range of venom expended using their data.

In a series of recent studies, Hayes used the ELISA technique pioneered by Morrison et al. (1982) to quantify venom injected into various prey by rattlesnakes (genus *Crotalus*) under varying circumstances (Hayes, 1991a, b, 1992a, b, 1993, 1995; Hayes et al., 1992, 1995). In addition to venom measurements, video was used to carefully review the kinematics of individual strikes to better understand the proximate causes affecting venom delivery. More recently, we have begun to explore venom expenditure during defensive bites (Herbert, 1998; Rehling, 2002). In these studies, we are using a simple protein assay of venom injected into models of human limbs (warm, saline-filled gloves) or released during routine venom extraction. We will describe these studies in more detail in the following sections.

Finally, Young et al. (2001) recorded unilateral (one side only) venom flow through the venom duct of *Crotalus atrox* via surgically implanted transonic flow probes. Actual measurement of venom ejected from the fangs was not recorded. However, when flow traces were combined with high-speed digital videography, the mechanics of venom flow (and estimates of venom injection) were studied at an unprecedented resolution.

## FACTORS INFLUENCING VENOM EXPENDITURE

### Predatory Bites

Many factors potentially influence venom expenditure by snakes during predatory bites, and these are discussed below.

*Species of snake*.—The average amount of venom expended in a single bite of a mouse is summarized for various taxa in Table 1. Although venom delivery may vary substantially from species to species, the quantity injected may vary considerably from bite to bite within a single individual as well. Comparisons among closely related taxa, such as the two *Oxyuranus* species, the *Crotalus* species (when accounting for size differences), and the two aforementioned *Protobothrops* species, show similarity (Table 1). Whereas most investigators presumably used laboratory mice (*Mus musculus*) in their studies, W. Hayes (see Table 1) used wild mice (*Peromyscus maniculatus*) in most of his experiments. However, *Crotalus viridis* was found to inject similar quantities of venom into laboratory mice and wild mice (Hayes, 1991b).

One might predict that larger species of snakes inject more venom when feeding than smaller ones because size is an important determinant of venom expenditure (see below), but the comparisons in Table 1 offer little support for this assumption. The Australian Death Adder (*Acanthophis antarcticus*), for example, expended considerably more venom than other, larger snakes in the study of Morrison et al. (1983b). Although these authors did not disclose body size measurements of the specimens tested, *A. antarcticus* is relatively small and rarely attains a length in excess of 90 cm (Phelps, 1989). However, *A. antarcticus* is distinct from most other Australian elapids in having a stout, viper-like body form with a relatively large head and venom supply (Broad et al., 1979; Mirtschin and Davis, 1983). This morphological difference may account for the large amount of venom expended on mice. The apparent venom supply of *Pseudonaja textilis*, in contrast, is relatively small (mean extraction yield = 2 mg and maximum yield = 67 mg; Broad et al., 1979; see Whitaker et al., 2000). Despite the exceptionally large specimens of Cottonmouth (*A. piscivorus*) used by Gennaro et al. (1961), the mass of venom expended when feeding on mice was similar to that of rattlesnakes.

As reviewed by Hayes et al. (1992), the amount of venom injected during a single predatory bite is generally a small percentage of that which is available in the venom glands. The proportion varies from less than 12% in *D. russelii* (Tun-Pe et al., 1991b), *D. palaestinae* (Allon and Kochva, 1974), and two species of *Protobothrops* (Kondo et al., 1972), to up to 50% in the elapid snake, *A. antarcticus* (Morrison et al., 1983b). The amounts of venom delivered into mice by several species of *Crotalus* constitute roughly one-quarter to one-third of the venom available in the glands (Klauber, 1972; Glenn and Straight, 1982; Hayes et al., 1992), but these values are likely based on underestimates of the amount of venom available. There is a trend for elapids to release a proportionally greater amount of their venom than viperids when biting a mouse (Hayes et al., 1992). This trend may result from the tendency of elapids to hold on to prey for several seconds or more (Fairley and Splatt, 1929; Kardong, 1982; Radcliffe et al., 1986; Kardong et al., 1997b), which would allow for delivery of additional venom (unfortunately, the duration of fang contact was not reported in the studies of Australian elapids by Morrison et al., 1982, 1983a,b. 1984). Generalizations must be made with caution, however, because the measurement of venom available in the glands (venom yield) is problematic (Glenn and Straight, 1982; Whitaker et al., 2000) and the exponential relationship between snake size and venom availability is obscured when the "species average" for venom yield is used in these calculations (Hayes et al., 1992). As a point of clarification, three methods are typically used to extract venom from snakes (Glenn and Straight, 1982): *voluntary* (in which the snake bites once or repeatedly a membrane-covered vessel), *manual* (application of finger pressure to forcibly express venom from glands, which may be injurious to the snake), and *electrical* (shock delivered to stimulate contraction of glands). In our own studies, we conduct only voluntary extractions, which are less reliable for determining yield but are least injurious to the snake and more closely resemble a defensive bite.

The percentage of venom that is successfully injected into prey tissues, as opposed to being spilled harmlessly on the skin, varies from 89% in *C. viridis* to 97% in *A. antarcticus* (see review in Hayes et al., 1992). One might expect viperid snakes, with their longer fangs, to be more efficient at delivering venom into prey tissues, but viperids and elapids exhibit comparable envenomation efficiency (Hayes et al., 1992).

Although venom expenditure has not been evaluated for atractaspids, it has been quantified for one species of colubrid, the Brown Tree Snake (*Boiga irregularis*; Hayes et al., 1993; Table 1). As is true for most toxic colubrids (Hill and Mackessy, 1997), the amount of venom available in the glands is limited for this species, yet it expends on average 54% of its secretion (3.6 mg of 6.7 mg available) when consuming a mouse (Hayes et al., 1993). The amount delivered seems surprising, but is still less than that expended by elapid and viperid snakes of smaller sizes (Table 1). However, *B. irregularis* holds on to mice that are killed by constriction (Rochelle and Kardong, 1993), and venom flow is presumably continuous during the several minutes required for the prey to die. Moreover, much of the venom (45%) remains within the integument rather than penetrating into the viscera (Hayes et al., 1993; see Rodriguez-Robles and Leal, 1993). Thus, colubrid snakes differ from elapid and viperid snakes in several key aspects of venom delivery. Due to their poorly developed venom apparatus, they cannot deliver large quantities of venom quickly (Kardong and Lavin-Murcio, 1993), the reservoir of venom available is small

(Weinstein and Kardong, 1994; Hill and Mackessy, 1997), and the quantity delivered into prey viscera is limited (Hayes et al., 1993). Accordingly, Duvernoy's secretion in some species may function more in a digestive rather than killing capacity by opening holes in the integument of prey to facility entry of digestive enzymes from the snake's gut into the prey's viscera (Hayes et al., 1993). Other functions of the secretion, however, have been proposed (Rodriguez-Robles, 1994; Kardong, 1996).

Phylogenetic constraints on morphology (e.g., relative head, venom gland, and fang size), venom composition, and diet undoubtedly influence the quantities of venom expended by different species of snake. Other factors, such as body size, are better understood determinants of the amount of venom used when feeding (see below).

*Size of snake and venom availability.*—There are reasons to believe that the envenomation behavior of snakes may vary with ontogeny. For example, the fangs that introduce venom into prey are proportionally longer in juvenile rattlesnakes than in adults (Klauber, 1972). The composition and properties of venom vary with age (often from more toxic to more proteolytic), and the supply of venom increases exponentially with growth (e.g., Mackessy, 1985, 1988; Kardong, 1986a; Chippaux et al., 1991). Young snakes of some species (e.g., *Demansia psamnophis*, an Australian elapid) use alternative killing methods, such as constriction, because they cannot effectively dispatch prey with their venom (Shine and Schwaner, 1985). Diet also shifts with age, as adults take on larger, more potentially dangerous prey, and these are usually handled with much more caution (e.g., Kardong, 1986a; Mackessy, 1988). Of these, the exponential relationship between snake size and venom availability is probably the most relevant for questions of venom expenditure.

Hayes (1991a) examined the ontogeny of venom use in *C. viridis*. Snakes of three size classes (31–95 cm SVL) were offered mice of corresponding size (i.e., small snakes bit small mice, large snakes bit large mice). The mass of venom expended increased exponentially as a function of the length of the snake. Hayes also noted that larger snakes more quickly extricated their fangs from prey, which was probably a learned behavior in response to being bitten during prior feeding experiences (Kardong, 1986a). Nevertheless, despite the briefer period of fang contact, ample venom was delivered. Clearly, snake size is an important determinant of how much venom is used during a predatory bite.

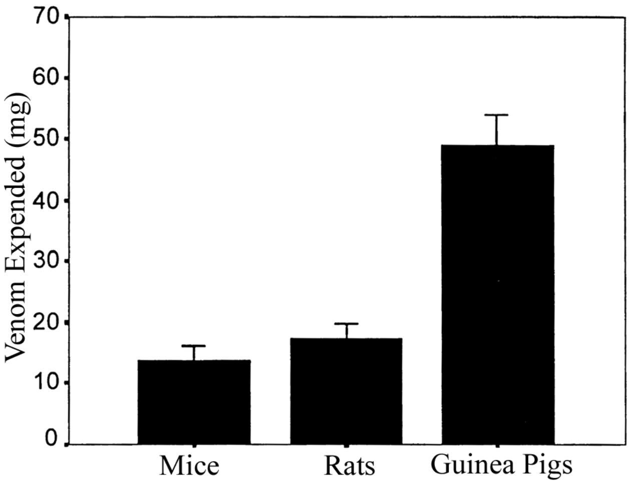

**Fig. 1.** Quantities of venom expended ($\overline{x}$ ± SE) during predatory strikes at prey of different sizes (mouse < rat < guinea pig) by exceptionally large Cottonmouths (*Agkistrodon piscivorus*) in the heretofore-unpublished study of Gennaro et al. (1961). For each mean, N = 35.

*Hunger level.*—Because the potential value of a meal corresponds to the risk of starvation, animals should place higher value on a prey item when they are hungry. Most venomous snakes typically release larger, more dangerous prey to avoid retaliatory injury. This strategy, although accompanied by a remarkable ability to relocate prey that are released and die some distance from the snake (Diller, 1990; Chiszar et al., 1992), nevertheless leaves the snake at risk of losing its meal.

Hayes (1993) hypothesized that hungry rattlesnakes (*C. viridis*) should reduce the risk of losing envenomated prey by holding on to them more often following the bite and/or by injecting more venom to produce more rapid immobilization. He found, however, that hungry snakes (food deprived for 28 days) actually injected less venom when feeding than well-fed snakes (food deprived for 7 days), and they showed no substantive changes in associated feeding behaviors. The reason for the difference in venom expenditure is unclear. Although the discrepancy could be the result of compositional changes in venom, we have subsequently learned that the protein content of venom is similar in *C. concolor* and *C. oreganus* after 7 and 28 days of food deprivation (Hayes et al., unpublished). It is conceivable that hungry snakes are attempting to conserve their venom, or in their eagerness to feed they are less efficient when biting, but more study is needed to clarify this issue. Copperheads (*Agkistrodon contortrix*), in contrast to the rattlesnakes studied by Hayes, do appear to hold on to mice more frequently when hungry

(G. Schuett, pers. comm.). There may also be a difference in venom use immediately following hibernation, when the snakes may have energy deficits and dehydration can result in higher protein concentrations within the venom.

*Size of prey*.—There are several reasons why it may be adaptive for snakes to meter more venom into larger prey (Hayes et al., 1995). First, larger animals are less affected by a given amount of venom than smaller ones. If insufficient venom is injected into prey released after the strike, large prey may flee beyond recovery range before dying. Second, because of the unfavorable (lower) surface-to-volume ratio of larger animals, snakes may obtain digestive benefits by injecting greater quantities of their proteolytic venom (Thomas and Pough, 1979; Kardong, 1986a; Mackessy, 1988; Rodriguez-Robles and Thomas, 1992) into larger prey. Third, injection of too much venom into smaller prey could be metabolically wasteful and temporarily deplete the snake's venom reserves. Fourth, larger prey may require injection of more venom to alter their chemistry so that snakes can more reliably distinguish between odor trails deposited by prey before and after being bitten (Chiszar et al., 1999).

Gennaro et al. (1961), in a widely cited abstract (see Gennaro, 1963), reported that the Cottonmouth (*A. piscivorus*) injects more venom into larger prey, but complete details of their study were never published (see below). Allon and Kochva (1974), in contrast, found no difference between venom quantities injected into mice and rats by *D. palaestinae*. Russell (1980a) cited unpublished data of his own to support the findings of Gennaro et al. (1961). Hayes (1995) compared naive (never exposed to large prey) and "experienced" juvenile Prairie Rattlesnakes (*C. viridis*) feeding on mice of three size classes. With experience, the snakes injected significantly more venom into larger prey. Because no other behavioral aspects of striking varied among prey sizes or changed between the two trials (naive, experienced) for each snake, venom expenditure was concluded to be under intrinsic control of the central nervous system (i.e. a decision was made by the snake) rather than subject to extrinsic aspects of striking, such as duration of fang contact. Hayes et al. (1995) similarly found that medium and large Northern Pacific Rattlesnakes (*C. oreganus*) delivered more venom when feeding on large compared to small mice. Again, no extrinsic factors of striking varied between the prey sizes, which further supports the notion that snakes have intrinsic control of venom expenditure and make decisions regarding how much venom to release when biting.

Data from the original study of Gennaro et al. (1961) are presented in Fig. 1. Ten exceptionally large *A. piscivorus* (152–175 cm, 5.5–6.6 kg) were given repeated opportunities to bite small prey (mice, 10–20 g), medium prey (rats, 80–100 g) and large prey (guinea pigs, 350–400 g). The snakes injected similar quantities of venom into mice and rats, but significantly more venom into guinea pigs (one-way ANOVA of rank-transformed data: $F_{2, 20} = 19.3$, $P < 0.001$; Scheffe post hoc tests). The feeding strikes were not videotaped for more detailed analyses of strike kinematics, but the results constitute evidence that crotaline snakes meter their venom when feeding on prey of different sizes. The results also suggest that a wide range in prey size may be required to detect venom metering in large snakes (Hayes, 1992a; Hayes et al., 1995).

*Species of prey*.—One might expect snakes to allocate different quantities of venom not only for prey of different sizes, but also for prey of different species. Many venomous snakes are opportunistic predators, feeding on a wide range of vertebrates and invertebrates (e.g., Klauber, 1972; Gloyd and Conant, 1990; Greene, 1997). Thus, snakes may utilize a variety of strategies for acquiring different types of prey that vary in ease of detection, escape tactics, and biochemical resistance to venom.

Several experimental studies suggest that rattlesnakes use more venom on some prey types than others. Whereas Prairie Rattlesnakes (*C. viridis*) typically strike and release mice, they usually strike and hold on to songbirds (sparrows similar in mass to mice; Hayes, 1992b). The snakes also appear to inject more venom into sparrows. These strategies may be adaptive because, if the bird is released after being bitten, it may be able to fly some distance and, therefore, not leave a chemical trail by which it could be relocated by the snake. By holding on to sparrows that are bitten, the snakes presumably trade off the risk of losing prey for an increased risk of being injured. The snakes appear to reduce the risk of injury by metering more venom into the birds and by aiming the strike at the head-neck region to immobilize the bird's most effective weapon—its beak. Hayes (unpublished) also found that Midget Faded Rattlesnakes (*C. concolor*) treat mice and lizards similarly by usually striking and releasing both and delivering the same quantities of venom (5.7 mg and 6.2 mg, respectively). However, because the mice were much larger than the lizards

(20 g vs 5 g, respectively), the snakes could be expected to deliver more venom into the mice. The fact that lizards received a similar quantity of venom during a period of similar fang contact time suggests that the snakes deliberately injected (or metered) more venom into lizards than into mice. This strategy also may be adaptive because lizards are less affected by venom than mice and survive much longer after envenomation.

Numerous anecdotal observations suggest that many venomous snakes swallow without envenomation a number of prey types that can be ingested with minimal risk or struggle (e.g., invertebrates, neonatal vertebrates, fish, amphibians; Klauber, 1972; Radcliffe et al., 1980; Savitzky, 1992). The West Indian colubrid snake, *Alsophis portoricensis*, has a toxic secretion from the Duvernoy's gland that appears to be effective in subduing prey. Rodriguez-Robles and Leal (1993) found that these snakes use their venom when capturing and swallowing lizard prey, but seldom use it when feeding on anuran prey. Jones (1988) reported that another colubrid snake with a toxic secretion, *Trimorphodon biscutatus*, uses its venom to paralyze lizards, but relies largely on constriction to kill mice. The ability of ectothermic prey to resist capture presumably varies with body temperature. Warmer ectotherms not only have more energy available for sustained activity, but also die more quickly after envenomation (W. Hayes, unpublished). Thus, although not studied yet, snakes may allocate different amounts of venom when feeding on ectotherms of varying body temperature.

From these studies, we conclude that many venomous species not only distinguish specific prey species but utilize different envenomation strategies when feeding on them. The strategies employed appear to be adaptive and reflect the need to conserve their venom.

*Multiple bites*.—When snakes strike repeatedly at a single prey item or at multiple prey within a relatively short period, the amounts of venom expended can be expected to decline eventually. Snakes possess a finite quantity of venom that requires several days (presumably) or several weeks to replenish (Kochva, 1960; Schaeffer et al., 1972; Leinz and Janeiro-Cinquini, 1994). Thus, the amount of venom remaining after biting once or several times may influence how much is released in subsequent bites. Numerous anecdotal reports, at least for rattlesnakes, suggest that multiple predatory bites within a short time frame are routine in nature (e.g., Klauber, 1972).

A number of studies have explored venom expenditure by viperid snakes when biting multiple prey offered in sequence. Kochva (1960) observed that some individuals of *D. palaestinae* could deliver up to 10 potent bites of mice before experiencing a decline in venom delivery. Kondo et al. (1972) measured venom delivered in three successive bites of mouse-sized pieces of meat by two specimens of *P. elegans*. Although variability was substantial, the total amount expended was approximately three times that released in a single bite. Allon and Kochva (1974) compared venom expenditure by *D. palaestinae* striking at 5–6 mice in succession (two snakes) and at mice and rats (seven snakes) offered in semi-random order within a series of 4–6 presentations in succession. Due to the nature of their study design, data on multiple bites and prey size were confounded in the larger study. Nevertheless, there was substantial variation in venom delivery regardless of bite order or prey size, and the snakes appeared to use similar quantities of venom in each of up to six bites. Tun-Pe et al. (1991b) similarly found no differences among four mice bitten in sequence by *D. russelii*. Kardong (1982) reported that up to four mice bitten in sequence by *A. piscivorus* died in a similar amount of time, though mice bitten later in the sequence were more likely retained in the jaws rather than released. Rehling (2002) measured a decline in the mean amounts of venom expended by *A. piscivorus* (N = 8) and *C. helleri* (N = 13) that struck three agar/condom models of mice, but also suggested that the snakes might have treated the models somewhat different than live mice. Clearly, viperid snakes are adequately equipped to procure a sizeable number of prey before their venom reserves are depleted.

Morrison et al. (1982, 1983a, b) studied multiple bites (up to five in succession) by various species of Australian elapid snakes. In most taxa, the quantities of venom expended declined consistently after the first bite as strike coordination and envenomation efficiency (measured as proportion of venom spilled harmlessly on skin) appeared to wane. The Taipan (*Oxyuranus scutellatus*), however, was an exception in that there was no decline in venom delivery through the third bite. This elapid species has relatively long fangs, a large supply of venom, is aggressive, and has a tendency to strike repeatedly (Morrison et al., 1982; Mirtschin and Davis, 1983; Phelps, 1989). Because the Tiger Snake (*Notechis scutatus*) showed a dramatic reduction in venom expenditure after the first bite and the Taipan was consistent (or injected more venom) in subsequent bites, the authors concluded that the

Taipan can probably control, or meter, its venom release. Kardong (1982) did not measure actual venom expended, but showed that when Egyptian Cobras (*Naja haje*) were presented up to four mice in close sequence, time to death was similar for each.

When compared to viperid snakes, it appears that elapids may be less capable of maintaining effective delivery of venom when striking multiple prey items. This may be because elapids seem to expend a higher proportion of their venom in the first bite (Hayes et al., 1992), but the difference could also result from dissimilar methods used by investigators. Snakes apparently exhaust quickly after several or more strikes, which could be expected to lead to reduced coordination and efficiency in biting. It is important that investigators carefully describe all details of strike trials, including the interval between each bite and presentation of the next prey, as well as the number of missed strikes, which can also contribute to exhaustion of a snake.

Do snakes inject more venom by striking a single prey item more than once? In contrast to elapid snakes, which tend to hold on to their prey (until bitten in retaliation) or to bite prey items more than once (Kardong, 1982; Radcliffe et al., 1986; Kardong et al., 1997b), many crotaline snakes are inhibited from striking prey more than once (Chiszar and Scudder, 1980; Kardong, 1986a; Hayes, 1992a). Kardong (1986a) observed adult *C. oreganus* strike the same mouse more than once (2–3 times each) in only 6% of 727 feeding episodes. Mice that were bitten multiply took significantly longer to die than those bitten once. In the study by Hayes (1992a), four of 38 deer mice (*P. maniculatus*) bitten by adult rattlesnakes (*C. viridis*) were struck multiply (2–3 times each) within a 5 min period during which the rodents were always within view of the snake due to the confines of the open-field arena. The amount of venom expended was not doubled or tripled in these rodents, which received a mean of 21 mg venom compared to 15 mg venom injected into mice struck only once. Thus, rattlesnakes appear capable of treating multiple prey items spaced apart in time differently than a single prey item that remains in close enough vicinity to be bitten several times.

*Environment, physiology, and composition of venom.*—Snakes that occupy different habitats may be under different selective pressures for use of their venom when feeding. We know, for example, that geographical variation in venom composition occurs (reviewed in Chippaux et al., 1991), and this may reflect adaptation to local prey (Daltry et al., 1996) or predators. It is tempting to speculate that more toxic snakes require less venom to kill their prey, but they may also need to inject more to compensate for the reduced proteolytic (digestive) capacity of their venom. The physiological status of a snake may also affect the amount of venom expended during feeding. In addition to hunger, as discussed previously, other factors that remain to be considered include level of hydration, body temperature, time of day, time of year, and pregnancy. We suspect that body size and other variables discussed in this section are likely to be more important determinants of venom expenditure, but future studies may reveal important influences associated with local environment, physiological status, and/or venom composition. It would be interesting to determine whether any such variation reflects constraints on venom delivery or adaptive strategies of venom usage.

*Labeling of prey for relocation after envenomation.*—The amount of venom injected into prey released after being bitten may facilitate relocation of the dispatched meal by chemosensory searching. Several studies have shown that the action of venom on prey tissues actually alters the odor of a bitten mouse, and this distinctive odor is then incorporated into the trail of the fleeing rodent (e.g., Chiszar et al., 1992; Smith et al., 2000; Stiles et al., this volume). Chiszar et al. (1999) explored the possibility that snakes need to inject a minimum or threshold amount of venom to be able to discriminate efficiently between the two odoriferous trails left behind by a rodent before and after being bitten. Their estimate of the minimal perceptible dose for discrimination by *C. atrox* was 6–7 mg of venom. Laboratory studies suggest that rattlesnakes have some capacity to discriminate trails of mice that are punctured by fangs without injection of venom, but the delivery of venom into tissues substantially improves trailing ability (Lavin-Murcio et al., 1993; Smith et al., 2000). Thus, the remarkable ability of snakes to distinguish and respond to venom-altered prey odors suggests that they are under selective pressure to inject a threshold amount of venom to more efficiently relocate their dispatched prey.

*Kinematics of biting.*—There are many proximate factors associated with the act of biting that might influence the amount of venom delivered. The successful injection of adequate venom during a bite is dependent upon a kinematically complex set of behaviors that typically not only involve the head, jaws, and placement of fangs, but also a substantial portion of the trunk (Kardong and Bels, 1998).

**Table 2.** Comparisons of total venom expended during a single defensive bite of a model human limb (or comparable target) by various elapid and viperid snakes.

| Species | N (bites) | Length (cm) | Venom expended (mg) | | Source |
|---|---|---|---|---|---|
| | | | Mean ± SE | Range | |
| **Elapidae** | | | | | |
| *Naja kaouthia*[a] | 20(20) | 105–172 SVL | 66 ± 8 | 0–125 | S. Herbert et al., unpublished |
| *Tropidechus carinatus*[b] | 6(6) | 66–91 | 4 | 0–16 | Morrison et al., 1983a |
| **Viperidae** | | | | | |
| *Agkistrodon contortrix*[c] | 4(4) | 58–60 SVL | 24 ± 13 | 3–61 | Rehling, 2002 |
| *A. piscivorus*[c] | 8(8) | 50–92 SVL | 23 ± 5 | 8–39 | Herbert, 1998 |
| | 8(8) | 52–97 SVL | 18 ± 11 | 0–83 | Rehling, 2001 |
| *Crotalus atrox*[d] | 28(28) | 45–140 SVL | 21± 5 | 1–103 | Herbert, 1998 |
| *C. helleri*[c] | 11(11) | 29–102 SVL | 72 ± 25 | 4–235 | Herbert, 1998 |
| | 7(7) | 58–105 SVL | 57 ± 19 | 0–145 | Rehling, 2002 |
| *C. lutosus*[c] | 2(2) | 59–71 SVL | 33 ± 17 | 16–50 | Herbert, 1998 |
| *C. oreganus*[c] | 3(3) | 46–78 SVL | 64 ± 15 | 41–93 | Herbert, 1998 |
| *Daboia russelii*[e] | 17(17) | 50–90 | 41 ± 8 | 3–138 | Tun-Pe and Khin-Aung-Cho, 1986 |
| | 31(31) | 91–123 | 63 ± 7 | 6–147 | Tun- Pe and Khin-Aung-Cho, 1986 |
| *Protobothrops flavoviridis*[f] | 38(38) | ~ 100–190 | 23 | 0–106 | Hokama, 1978 |

[a] = posterior of snake grasped by investigator during entire trial; struck at warm saline-filled gloves. [b] = struck at warm agar-filled gloves; mean is for first of up to three successive bites, and range is based on 13 bites from six snakes. [c] = unrestrained snakes in open field arena; struck at warm saline-filled gloves. [d] = unrestrained snakes on roads at night or in open field arena at rattlesnake round-up; struck at warm saline-filled gloves. [e] = snakes physically restrained by snake hook; struck at piece of plastic foam (L35 x W75 x H25 mm). [f] = snake length and range of venom expended based on 168 bites from 82 individuals; struck at warm 100 ml saline bags.

The recent use of video to examine proximate variables associated with venom expenditure has led to some interesting conclusions. Gennaro et al. (1961), for example, suggested that the force of the strike appeared to be correlated with the amount of venom expended by crotaline snakes. Although this variable has not been measured simultaneously with quantification of venom, the force of the strike is likely correlated with distance of the strike or duration of the launch (measured from initiation of strike to contact with prey). Hayes (1992a), however, found no correlation between these latter variables and amount of venom expended by Prairie Rattlesnakes feeding on mice. Although the site of fang penetration affects survival time of mice (mice bitten anteriorly succumb more rapidly; Kardong , 1982, 1986a; Hayes, 1992a), it does not appear to influence venom delivery (Hayes, 1992a). When mice are released immediately after being bitten, the duration of fang contact has no discernable effect on venom expenditure (Hayes, 1992a). However, mice that are held on to rather than released after envenomation die more quickly (Kardong, 1986a), presumably because more venom is injected (but see Kardong, 1982). Hayes (1993) suggested that by holding on to sparrows, rattlesnakes inject more venom into birds than mice. Elapid snakes frequently hold on to prey and probably deliver more venom in the process. Kardong (1982) showed that mice held on to by *N. haje* died more quickly than those that were released. Likewise, many colubrid snakes having Duvernoy's secretion hold on to their prey for a period of time before they begin to swallow it (e.g., McKinstry, 1978). It appears that by doing so, the prey may become weakened or immobilized by the Duvernoy's secretion, which makes ingestion easier.

Despite the remarkable guidance systems that assure well-coordinated strikes from crotaline snakes (eyes, facial pits, tactile receptors in the mouth and jaws), errant and ineffective strikes sometimes occur. Kardong (1986b) examined high-speed movies of ineffective bites by rattlesnakes that resulted in exceptionally long survival of mice, presumably from poor venom delivery. In some cases, only one fang penetrated the prey. Young and Zahn (2001) also reported unilateral (single fang) bites in which venom was not expelled from the fang that failed to penetrate. Thus, successful fang placement can be crucial to envenomation success. Some snakes are capable of reinserting fangs that missed during initial contact with prey (Kardong, 1975), which illustrates the importance of a bilateral bite. In Kardong's (1986b) study, poor envenomation also resulted from bites that were immediate follow-ups to missed strikes and from strikes that involved a collision with an obstacle (the side of the cage). Kardong concluded that flaws in the strike could disrupt normal jaw kinesis and neural control of venom delivery.

The timing of venom expulsion during the bite may influence venom delivery, particularly if prone to errors. The fact that venom is often spilled (or perhaps leaked from the wound) harmlessly on the skin of mice (Morrison et al., 1982, 1983a, b; Hayes et al., 1992) suggests that venom may be ejected just before or after fang contact during the bite. If such venom spillage occurs often and can be perceived by snakes during a given bite, they may compensate by delivering more venom during the bite. However, in high-speed video analyses of strikes by *C. atrox*, venom spillage (in ca. 5% of all strikes, including predatory and defensive) was observed only after withdrawal of the fang (B. Young, pers. comm.), at which time the snakes would be unable to compensate by delivering more venom. Young and Zahn (2001) showed that venom expulsion from the fang of *C. atrox* usually begins simultaneously with fang penetration or within 0.01 sec after penetration, but not prior to penetration.

Last, the reactions of prey to the incoming strike and during the bite itself may influence the site of fang penetration as well as the kinematics of fang penetration and disengagement of fangs from prey (Klauber, 1972; Hayes, 1992a). Unfortunately, we can only speculate on how such reactions may influence the success of venom delivery into prey tissues. When snakes hold on to larger prey, retaliatory bites often induce release by the snake (Kardong, 1982; 1986a), which could potentially result in another pulse of venom injection prior to release but certainly removes the possibility of further injection after release.

## Defensive Bites

Despite considerable interest in issues of human snakebite and treatment, the amount of venom expended by snakes has been studied in much less detail for defensive bites compared to predatory bites. Nevertheless, speculation has abounded. The purpose of a defensive bite is to thwart the attack of a predator or antagonist. The strike itself, with or without the physical contact of a bite, may elicit a startle response from the attacker sufficient to end a confrontation. Thus, it may be wasteful for the snake to expel substantial venom during attack. Even when venom is used in defense, the attacker is highly unlikely to become incapacitated before it can kill the snake (even very small prey often survive several minutes or more after being bitten). This reasoning, combined with the well-documented occurrence of dry bites in human snakebite victims (e.g., Hardy, 1991; Silveira and Nishioka, 1995; Rezende et al., 1998), led many researchers to suggest that snakes expend less venom when biting defensively compared to predatory bites (e.g., Morrison et al., 1983b; Kardong, 1986a, b; Minton, 1987; Hardy, 1991; Hayes, 1991b; but see Klauber, 1972; O'Connell et al., 1982). Speculation is no substitute for experimentally collected data, however, so we have turned our

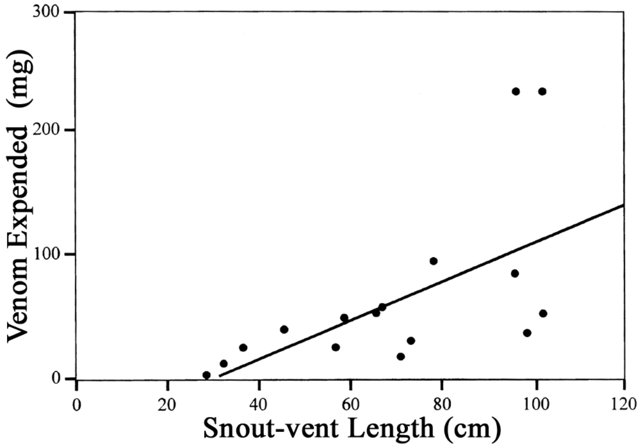

**Fig. 2.** The significant relationship between snake size (snout-vent length) and venom expenditure (mg) for defensive strikes at human limb models by 16 *Crotalus* spp., all formerly subspecies of *Crotalus viridis*. Data from Herbert (1998).

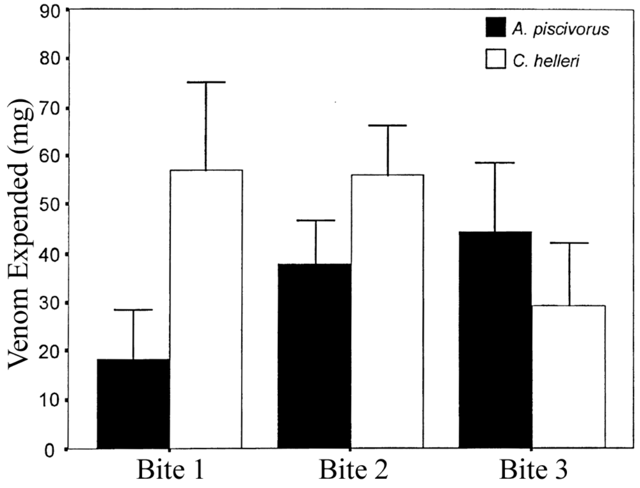

**Fig. 3.** Quantities of venom expended ($\bar{x}$ ± SE) during three successive defensive bites at human limb models by adult Cottonmouths (*Agkistrodon piscivorus*; N = 8) and adult Southern Pacific Rattlesnakes (*Crotalus helleri*; N = 7). Data from Rehling (2002).

**Table 3.** Duration of fang contact (mean ± 1 SE sec) during predatory (adult mouse) and defensive bites (adult rat, model of human limb) by elapid and viperid snakes, as determined from S-VHS videotape review (30 fields/sec). The brevity of strikes at rats is apparent when compared to mice and humans. The sample size (N) applies to each mean in the study.

| Species | N | SVL (cm) | Mouse | Rat | Human | Source |
|---|---|---|---|---|---|---|
| **Elapidae** | | | | | | |
| *Naja kaouthia* | 18 | 105–172 | — | — | 1.54 ± 0.38 | S. Herbert et al., unpublished |
| **Viperidae** | | | | | | |
| *Agkistrodon contortrix* | 5 | adult | 0.17 ± 0.04 | 0.07 ± 0.01 | — | W. Hayes, unpublished |
| | 4 | adult | — | — | 0.16 ± 0.06 | Rehling, 2002 |
| *A. piscivorus* | 7 | adult | 0.21 ± 0.05 | 0.09 ± 0.02 | — | W. Hayes, unpublished |
| | 8 | 50–92 | — | — | 0.20 ± 0.02 | Herbert, 1998 |
| | 8 | 52–97 | — | — | 0.12 ± 0.03 | Rehling, 2002 |
| *Crotalus atrox*[a] | $6^{+}$ | 78–112 | 0.32 ± 0.11 | 0.23 ± 0.02 | — | Young and Zahn, 2001 |
| *C. helleri* | 11 | 29–102 | — | — | 0.22 ± 0.06 | Herbert, 1998 |
| | 7 | 58–105 | — | — | 0.23 ± 0.10 | Rehling, 2002 |
| *C. lutosus* | 2 | 59–71 | — | — | 0.12 ± 0.05 | Herbert, 1998 |
| *C. oreganus* | 6 | adult | 0.18 ± 0.03 | 0.06 ± 0.01 | — | W. Hayes, unpublished |
| | 3 | 46–78 | — | — | 0.29 ± 0.13 | Herbert, 1998 |
| *C. viridis* | 10 | 76–93 | 0.16 ± 0.02 | 0.07 ± 0.01 | — | Hayes, 1991b |
| *Sistrurus* spp. | 7 | adult | 0.19 ± 0.04 | 0.08 ± 0.01 | — | W. Hayes, unpublished |

[a] = based on six predatory bites at mice and 10 defensive bites at rats by four snakes.

attention recently to experimental assessment of venom expenditure during defensive bites. In this section, we discuss what can be inferred about various factors that potentially affect venom usage during defensive bites. In doing so, we also present recent analyses of our own unpublished data.

*Species of snake*.—The amounts of venom expended by various snake species biting models of human limbs (saline-filled gloves) or comparable objects are summarized in Table 2. At present, too few elapid snakes have been studied to make valid comparisons between elapids and viperids. Whereas the Monacled Cobra (*Naja kaouthia*) appears to deliver a substantial quantity of venom when biting defensively, the small amount expended by the Australian Rough-scaled Snake (*T. carinatus*) reflects on the small supply of available venom (Broad et al., 1979), as was also apparent for predatory bites by this species (Table 1). The large amount expended by *N. kaouthia* may be attributed in part to the relatively long duration of its bites (often > 0.5 sec) compared to crotaline snakes (usually < 0.5 sec; Table 3). The several species of *Crotalus* studied by Herbert (1998) and Rehling (2002) show similarities to each other, but *A. piscivorus* appears to deliver less venom in an initial bite compared to rattlesnakes (Table 2; see comments below on multiple bites). Compared to other taxa in Table 2, *C. atrox* and *P. flavoviridis* expend somewhat less venom when striking defensively. Whether the apparent difference is an artifact of dissimilar testing procedures, or reflects distinctly different behaviors, is unclear at this time. Herbert (1998) believed that the *C. atrox* he tested, most of which were grouped together in large pens during a rattlesnake round-up, were disinclined to bite in part because it was their mating season. More studies of a wider range of taxa will be necessary before firm generalizations can be made.

How does venom expenditure for defensive strikes compare to that of predatory bites? Morrison et al. (1983a) experimentally compared venom expenditure by *T. carinatus* during predatory bites of mice and defensive bites of agar-filled gloves. In the first bite, the snakes delivered more venom into mice and less into gloves (means of 7.1 mg and 3.8 mg, respectively). In the second bite, however, the snakes injected less venom into mice (1.5 and 3.2 mg, respectively). In the third bite, equal amounts of venom were delivered into each target (1.0 mg). Unfortunately, measures of variance and statistical tests were not provided to evaluate these otherwise fascinating differences. *Daboia russelii* injected substantially more venom in defensive bites compared to predatory bites (means of 63 mg and 2 mg, respectively; see Tables 1–2). The enormous discrepancy between these values is difficult to understand, but the data were obtained in separate

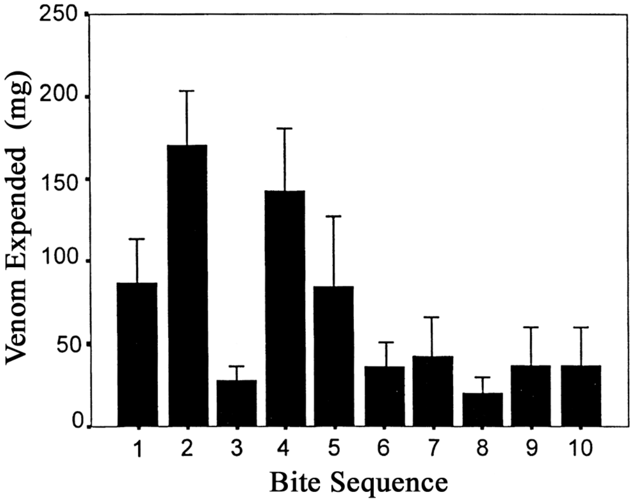

**Fig. 4**. Quantities of venom expended ($\bar{x}$ ± SE) during ten successive defensive strikes at large pieces of meat by four exceptionally large Cottonmouths (*Agkistrodon piscivorus*) in the heretofore-unpublished study of Gennaro et al. (1961). For each mean, N = 4.

studies rather than within a single experiment. For *A. piscivorus* and *Crotalus*, it appears that these snakes likewise expend more venom in defensive bites compared to predatory bites (see Tables 1–2). These greater amounts of venom in defensive bites are delivered in the same amount of time for fang contact as in predatory bites (Table 3). More impressive is the much greater range of venom expended during defensive bites by these crotaline snakes, with maximum amounts far exceeding those observed in predatory bites. Young and Zahn (2001), based on measures of venom flow through a single duct, concluded that duration of venom flow, maximum venom flow rate, and total venom volume expelled were all significantly greater in defensive compared to predatory bites at mice by *C. atrox*. Thus, the widely held opinion that snakes inject more venom in a predatory bite compared to a defensive bite can be summarily dismissed.

Unfortunately, no data have been reported for venom expenditure during defensive bites by colubrid or atractaspidid snakes. Based on human envenomation histories for colubrids (e.g., McKinstry, 1978; Hayes and Hayes, 1985; Fritts et al., 1990), however, most toxic colubrid species may have to bite and then hold on to the victim for an extended period of time before an effective dose of venom can be delivered. This is because the venom apparatus is poorly suited for rapid delivery of venom into deeper tissues (Hayes et al., 1993; Kardong and Lavin-Murcio, 1993). However, there are species for which brief bites are reportedly sufficient to induce alarming symptoms of envenomation, including immediate pain (e.g., Seib, 1980). Hence, in some toxic colubrids, Duvernoy's secretion can provide an effective defense. Atractaspidids have a well-developed venom apparatus, but their typical mode of biting—a unique, single-fanged, sidewise bite with the mouth closed (Golani and Kochva, 1988; Kurnik et al., 1999)—imposes a severe constraint on how much venom is delivered (only half as much as could be delivered with a more typical bite).

*Size of snake and venom availability.*—The relative size of a snake appears to be the most important factor that influences venom expenditure during defensive bites by snakes. Although Tun-Pe and Khin-Aung-Cho (1986) did not statistically compare venom expenditure by small and large *D. russelii*, a comparison of the means and variance (Table 2) is strongly suggestive of a size-related difference. Herbert (1998) found a highly significant effect of snake size in his study of *A. piscivorus* and *C.* spp. (primarily *C. helleri*) (Spearman's rho = 0.69, $P$ = 0.003, N = 16; Fig. 2) striking saline-filled gloves. Using a multifactorial model, snake size explained considerably more variance in venom expenditure than did species of snake or duration of fang contact. The positive correlation between snake size and severity of envenomation in human snakebite victims has been confirmed statistically in several clinical studies (Tun-Pe et al., 1991a; Thomas et al., 1998; see Kouyoumdjian and Polizelli, 1989, and Jorge et al., 1997, 1999).

*Size or species of predator/antagonist.*—One might expect snakes to inject more venom into larger, more dangerous predators or antagonists compared to smaller ones. However, while larger animals are generally less susceptible to the effects of venom, the size of the attacker may not correspond to the risk of attack or death of the snake. Thus, rather than a generalized strategy of injecting more venom into larger attackers, snakes may have evolved strategies for dealing with specific predators and antagonists. In the only experiment to address this issue in the context of venom usage, Young and Zahn (2001) showed that several measures of venom (mean and maximum flow rate, total volume) in *C. atrox* were similar for defensive bites of small and large targets (mice and rats, respectively). Nevertheless, differences in duration of fang contact between defensive bites of rats and humans suggest that predator-specific strategies exist. These differences will be discussed below in the section on kinematic influences.

*Assessment of threat level.*—Many animals appear to be capable of assessing different levels of threat, and snakes appear to be no exception (Duvall et al., 1985; Goode and Duvall, 1989; Rowe and Owings, 1990; Whitaker et al., 2000; Gibbons and Dorcas, 2002). With this capacity, it is tempting to speculate that snakes have evolved a strategy for injecting more venom when the risk of injury or threat to life is greater than in other circumstances. For example, a snake that is being harassed by an attacker at close proximity might escape serious harm without a bite or by delivering a brief bite with minimal venom release. The startle effect from the bite and the immediate painful sensation (from mechanical damage by the fangs and action of the venom) could be sufficient to ward off further attack. By releasing only a small portion of its venom with the first bite, more can be delivered if need be in subsequent bites. In contrast, a snake that is physically grasped by an attacker is faced with the immediate risk of death, and delivery of a substantial amount of venom may be an effort of last resort to terminate the attack (Hardy, 1991; Herbert, 1998). In this latter context, often called an "illegitimate bite" when the enemy is a human (Klauber, 1972), the risk of having a depleted venom reserve is irrelevant because immediate survival is most important.

Unfortunately, we presently lack experimental data to properly evaluate this hypothesis, but we do have some interesting findings that may shed light on the matter. In the study of Herbert (1998), rattlesnakes (*Crotalus* spp.) expended similar quantities of venom when striking model human limbs and during venom extractions (means of 65 and 70 mg, respectively). Because the snakes were physically grasped during extractions, one might expect them to release more venom during extractions, but such was not the case. *Agkistrodon piscivorus*, in contrast, expended much less venom when striking model limbs compared to venom extractions (23 and 73 mg, respectively). Rehling (2002) replicated the results of Herbert (1998) for rattlesnakes and the Cottonmouth, and showed further that the snakes expended more venom when physically contacted by the model limbs. The Monacled Cobra (*N. kaouthia*), like the Cottonmouth, also delivered less venom into model limbs than during venom extractions (66 and 145 mg, respectively; Herbert et al., unpublished). Although the two types of bites are kinematically different (a strike vs a simple bite while grasped), these data suggest that at least some species of snakes (e.g., *A. piscivorus*, *N. kaouthia*) are more willing to part with their venom in a context of higher threat. We intend to explore this hypothesis in a more rigid experimental design. In addition to the level of harassment, irritability of a snake may also influence assessment of threat and contribute to decisions on venom expenditure. Some individuals escalate their defensive behaviors more rapidly than others, which may be dependent not only on individual temperament but also proximity to cover, snake size, and body temperature (e.g., Duvall et al., 1985; Goode and Duvall, 1989; Whitaker and Shine, 1999; Whitaker et al., 2000).

*Multiple bites and depleted venom reserves.*—As mentioned previously for predatory bites, venom resources may become depleted during sequential bites. Hokama (1978) found decreasing venom expended in sequential bites by *P. flavoviridis*, as well as a correlation between number of fang marks (1–4 fang marks) and venom expended per bite. Presumably, the occurrence of more than two fang marks in their saline-filled bags was the result of multiple penetrations in a single biting event. Bites with four fang marks delivered almost four times as much venom as bites with two fang marks; this may be due to exceptionally long duration of fang contact (when summed for all fang penetrations), but these trials apparently were not videotaped for analyses of bite kinematics. Tun-Pe and Khin-Aung-Cho (1986) found that venom expenditure in *D. russelii* clearly declined over the course of five bites at plastic foam targets. In this species, the first bite accounted for 45% of available venom in adults (107 cm mean SVL) and 53% of available venom in juvenile snakes (87 cm mean SVL). In the only study of multiple defensive bites by an elapid, Morrison et al. (1983a) found that *T. carinatus* injected similar amounts of venom in the first two bites of agar-filled gloves, but substantially less in the third bite. The proportion of venom spilled harmlessly on the glove surface, however, increased with each bite, probably as a consequence of snake fatigue (Morrison et al., 1983b).

More recently, Rehling (2002) found that the Southern Pacific Rattlesnake (*C. helleri*) and the Cottonmouth (*A. piscivorus*) have venom resources to deliver at least three potent defensive bites, but the two species differed in how they allocated their supplies, as shown in Figure 3. When striking model human limbs, *C. helleri* injected more in the first two bites and less in the third bite (respective means of 57, 56, and 29 mg venom; N = 7 for each), whereas *A. piscivorous* expended less in the first bite and more in subsequent bites (respective means of 18, 38, and 45 mg venom; N = 8 for each). The significant interaction (two-way ANOVA, $F_{2,24} = 3.67$, $P = 0.041$) suggests that these two crotaline snakes use different strategies for multiple defensive bites. Gennaro et al. (1961) also show that *A. piscivorous* can inject less venom in the first bite compared to the second bite. Four of their large *A. piscivorous* were provoked to strike defensively at large pieces of meat (rat-sized) presented by forceps during harassment by the investigator. The previously unpublished results of their study are shown in Figure 4. Although there was much variation in venom expended, especially among the first three bites in sequence, there was a significant decline in venom delivery that became most evident after the fifth mouse was bitten (one-way repeated-measures ANOVA, $F_{9,27} = 3.99$, $P = 0.002$).

Clinical data show considerable variation in severity of envenomation amongst multiple bite victims. Russell (1978) described a case of envenomation of three people by a single *C. atrox* within 15 sec. The first patient showed fang marks but no venom injected, the second suffered substantial envenomation, and the third mild envenomation. Apparently, the level of envenomation was not affected by degree of harassment by the human agitator, as all three patients had grasped the rattlesnake by hand. Sean Bush (pers. comm.) at Loma Linda University Medical Center has a handful of case histories involving multiple bites from single rattlesnakes that demonstrate unpredictability in the severity of envenomation based on sequence of the bite.

Though these data reveal a great deal of variance in defensive venom expenditure by vipers, delivery of venom in multiple bites is still constrained by limited venom resources within the glands. Recent expenditure of venom will deplete the venom reservoir, but because snakes seldom expend all of their venom, they are generally capable of delivering potent bites regardless. Tun-Pe et al. (1991a) found no statistical difference in severity of envenomation among humans bitten by Russell's Vipers that had recently eaten (N = 67) vs those having empty stomachs (N = 34). Parrish and Thompson (1958) reported three cases of moderate to severe envenomation from rattlesnakes whose venom had been extracted within 24–48 h. Nevertheless, recently milked rattlesnakes exhibit reduced envenomation success since mice bitten by these snakes (in a predatory context) took longer to die than those struck by control snakes (Kardong, 1986a). Unfortunately, we do not know the length of time it takes for snakes to regenerate their venom after routine expenditure, but it is doubtful that the venom is replaced within minutes or hours of use. Venom extraction studies suggest that several days and up to two weeks may be required when a substantial portion of the venom is removed from the glands (Kochva, 1960; Schaeffer et al., 1972; Leinz and Janeiro-Cinquini, 1994). More study is needed on the duration and energetic cost of venom replacement following routine usage.

These above findings suggest that snakes (or at least viperids) have evolved envenomation mechanisms and strategies that allow them to deliver multiple potent bites without excessive risk of their venom supply becoming overly depleted. The fact that *A. piscivorous* expends less venom in the first strike and more in subsequent bites (Figs. 3–4) suggests that venom can also be metered during defensive bites.

*Environment and temperature*.—Although natural selection resulting from species-typical or local ecological circumstances (e.g., habitat, microclimate, body temperature, predators, prey availability) may influence how much venom is injected, we suspect that this variance is usually small relative to snake body size and other variables described here. Herbert (1998), for example, showed that despite the widely divergent habitats and climates occupied by *A. piscivorus* and *Crotalus* spp., venom expenditure by these two genera in a single defensive bite at a glove was statistically similar in an ANCOVA model that treated snake size as a covariate (but see Rehling, 2002). Furthermore, although extreme temperatures were not considered, *A. piscivorus* and *Crotalus* spp. yielded similar quantities of venom during routine extractions at 18°C and 28°C (Herbert, 1998). Rowe and Owings (1990) found no differences in the duration of fang contact during defensive bites by *C. oreganus* at temperatures of 10–35°C. Whitaker et al. (2000) reported that temperature (over the range of 18–36°C) had little effect on duration, distance, speed, and accuracy of defensive strikes by the elapid *Pseudonaja textilis*. As discussed below, duration of fang contact has a significant influence on the amount of venom injected during defensive bites. Further evaluation of environmental variables may yield important new insights.

*Dry bites*.—In contrast to predatory bites by viperid snakes, for which dry bites are seldom documented (e.g., none in 768 mice bitten by *C. oreganus* in Kardong, 1986a), dry bites are frequently observed in defensive bites delivered to humans. Estimates of the frequency of human dry bites vary from 20% (Russell, 1980b) to 25% (Minton, 1987) for rattlesnakes and in excess of 50% for venomous snakes worldwide (Reid and Theakston, 1983; Russell et al., 1997). Perhaps because of their generally shorter fangs, elapid snakes are believed to deliver dry bites with greater frequency than viperid snakes (e.g., Russell et al., 1997; Warrell, 1996). Likewise, toxic colubrids have a high incidence of dry bites, estimated at 80–90% in the Boomslang (*Dispholidus typus*) and > 99% in gartersnakes (genus *Thamnophis*; Minton, 1990). Unfortunately, none of these estimates appear to have been based on empirical data.

Recent clinical studies provide interesting data on dry bites for viperid, elapid, and even colubrid snakes. For viperids, Hardy (1991) indicated that 7% of 159 rattlesnake bites (from various species) admitted to Tucson, Arizona, hospitals were apparently dry. Silveira and Nishioka (1995) determined that 30.3% of 33 *Bothrops* bites and 42.9% of seven *C. durissus* bites in Brazil were dry. Jorge et al. (1997) stated that 8% of 97 *C. durissus* bites in Brazil were dry, with 11% for 18 snakes ≤ 40 cm and 8% for 79 snakes > 40 cm. Rezende et al. (1998) found that 12% of 41 *C. durissus* bites in Brazil were dry. Stahel et al. (1985) determined that 12% of 113 adder bites (*Vipera berus* and *V. aspis*) in Switzerland lacked symptoms of envenomation. For elapids, Tibballs (1992) stated that 53% of 19 conclusive bites and 81% of 46 confirmed or suspected bites by Australian species were dry. Tibballs (1992) cites three additional studies that report 80–83% of confirmed or suspected bites by Australian elapids to be dry. For colubrids, 37% of 43 bites by *Philodryas olfersii* showed no evidence of envenomation (Ribeiro et al., 1999). The empirical approach of these investigators is to be applauded, though there may be a bias in victims consenting to treatment (hence asymptomatic bites may go unreported), and the interpretation of a dry bite is based on clinical criteria (e.g., no local swelling) without actual measurement of venom.

Measurements of venom injected into models of human limbs suggest that approximately 10–20% of the bites by rattlesnakes and cottonmouths are dry (Herbert, 1998; Rehling, 2002). We recognize that our experimental conditions are much more restrictive than those occurring under natural conditions in the field, where most human snakebites are incurred, but our data are consistent with clinical studies.

We suggest two major hypotheses for the occurrence of dry defensive bites (after Hayes, 1991b). Dry bites may result from a deliberate decision by the snake to withhold venom (the venom metering hypothesis), or they may result from kinematic constraints during striking and/or biting (the kinematic constraints hypothesis). Recent evidence supports the venom metering hypothesis. Young and Zahn (2001) documented “dry” bites by *C. atrox* in which kinematically normal bites resulted in no venom flow through the one duct containing a flow probe. They concluded that the lack of venom expulsion resulted from control of venom injection. However, it is difficult to establish that all dry bites result from a deliberate decision on the part of the snake to withhold its venom. Two kinematic constraints have been proposed to explain dry bites. Kardong (1986b) suggested that when snakes strike a large object, particularly a vertical one (such as an upright human leg), the different positioning of jaw and fangs relative to predatory bites may disrupt venom delivery. Hayes (1991b) suggested that very brief bites, which may occur more often for defensive bites (directed toward rats, at least) than predatory bites, may also constrain venom flow through the fangs, especially if the timing of venom expulsion is prone to errors. The fairly strong correlation between duration of fang contact and amount of venom expended during defensive bites, as discussed below, supports the latter suggestion by Hayes.

*Kinematics of biting*.—The duration of fang contact may be the most important kinematic variable to explain the quantity of venom expended during defensive bites. Rowe and Owings (1990) first proposed the relationship when they noted that larger specimens of *C. oreganus* tend to maintain longer fang contact than smaller snakes during defensive strikes at squirrel puppets. Recent data from Herbert (1998) and Rehling (2002) show a significant relationship between duration of fang contact and amount of venom injected by *C. helleri* into human limb models. This finding is in contrast to what we have seen in predatory bites, wherein no such relationship has been observed (Hayes, 1992a).

The duration of fang contact during a strike, however, may vary between defensive targets, as shown in Table 3. Whereas snakes hold on to mice and human limb models for a similar duration of time (averaging roughly 0.20 sec), most crotaline snakes examined treat adult rats very differently, consistently holding on for a much briefer period of time (averaging 0.07 sec in species we have examined, but somewhat longer in *C. atrox* tested by Young and Zahn, 2001). The difference between predatory strikes at mice and defensive strikes at rats is apparent from representatives of all three North American genera of crotaline snakes (*Agkistrodon*, *Crotalus*, and *Sistrurus*; Table 3). Although we are uncertain as to what may be occurring, we are confident that the strikes elicited from the snakes by adult rats were not predatory bites, as judged by criteria set forth in Hayes and Duvall (1991). (We should add that while larger snakes clearly will strike rats for predatory purposes, few if any of the snakes we tested were of sufficient size to consume an adult rat.) We suspect that the brief strikes at rats may represent a generalized defensive strategy against squirrel-sized antagonists. Because squirrels are capable of inflicting serious or fatal wounds to rattlesnakes (Rowe and Owings, 1990), the snakes may attempt to minimize the risk of retaliatory injury by reducing the amount of time when they are vulnerable—when their necks are extended and their fangs engaged (Hayes, 1991b). Our experiments with saline-filled gloves suggest that humans are treated differently than rats and may on average receive greater levels of envenomation due to the longer duration of fang contact.

Although crotaline snakes, when defensive, typically exhibit the same strike-and-release strategy they use when feeding on larger prey, elapid snakes are more prone to hold on after biting or to bite for a longer duration of time (e.g., Pearn et al., 2000; Table 3). During videotaped venom extractions, the Monacled Cobras (*N. kaouthia*) studied by Herbert et al. (unpublished; Tables 2–3) delivered multiple pulses of venom from each fang that were associated with obvious “chewing” motions by the jaws. Similar chewing actions were seen during defensive bites of model human limbs. Thus, we infer that by holding on for a longer period of time, and especially by chewing, elapids effectively deliver much more venom than could be accomplished during a brief defensive bite involving immediate release of the victim. Crotaline snakes are also capable of delivering multiple pulses of venom, as commonly observed during routine venom extraction. However, the videotape analyses of Herbert (1998) suggest that fang contact during defensive bites by rattlesnakes and cottonmouths normally allows for only a single pulse of venom from each fang, though multiple pulses are sometimes observed (Young and Zahn, 2001). Although rarely exhibited, viperid snakes are capable of holding on during a defensive bite (Klauber, 1972). Sean Bush (pers. comm.) has treated or consulted on more than 100 snakebite cases from southern California, and the two worst cases resulted from adult *C. helleri* that actually bit and held on to the victims until pried loose.

There are additional kinematic factors that may influence venom expenditure. In rattlesnakes, the gape of the jaw upon fang contact is inversely proportional to the pressure exerted on the venom glands (Mitchell, 1860). Thus, more venom may be expelled when snakes bite smaller objects (Mitchell, 1860; Kardong, 1986b), but clinical data on snakebite severity do not support this view (Moss et al., 1997; Hayes et al., unpublished). For rattlesnakes and presumably other viperids, the fangs must be erected approximately 60 degrees relative to the roof of the mouth before venom begins to flow freely (Young et al., 2001). Once the fangs are erect, pressure gradients within the venom duct, venom chamber (space between duct and fang surrounded by the sheath), and fang may on occasion direct venom between the chamber and fang rather than through the fang (Young et al., 2001), thereby reducing venom delivery into the target. Venom may also flow through a replacement fang in addition to the main fang (i.e., venom flows through three or more fangs), but the total venom delivered should be the same or possibly less (Herbert, 1998; Young et al., 2001). Fangs also may be broken off during a bite, particularly when the target animal physically reacts to the bite, and this could impair envenomation success. Thus, depth and trajectories of fang penetration, as well as accompanying shearing forces on the fangs, may influence the success of venom delivery, particularly into deeper tissues (Ruben and Geddes, 1983). The timing of venom expulsion may also be subject to errors. When filming strikes by *C. atrox* in the laboratory, Bruce Young (pers. comm.) observed venom leakage from the fangs during fang withdrawal from the target in a small percentage of bites (5%, including both predatory and defensive bites; see Young and Zahn, 2001). Usually the leakage was seen from the fang tip. Curiously, when only one fang penetrated during a bite, venom did not flow from the free fang (N = 5, Young and Zahn, 2001). We anticipate that the functional relationship between venom expenditure and these and other kinematic variables will receive additional attention, particularly in the laboratory of Bruce Young.

*Spitting of venom*.—Spitting cobras comprise a diverse group of elapids having the unique capability of spraying their venom through the air for defensive purposes. They have a remarkable ability to aim their venom at the eyes of a predator or antagonist. If successfully sprayed in the eyes, the venom exerts toxic effects, including an immediate burning sensation and potential blindness (Greene, 1997). The lumen of fangs from most species (and a few non-spitting cobras) is uniquely channeled to facilitate venom expulsion (Greene, 1997). Several investigators have noted the ability of these snakes to spit over 50 times in an interval of several minutes without exhausting their supply of venom (Rasmussen et al., 1995; Greene, 1997). Cascardi et al. (1999) determined that adult Red Spitting Cobras (*Naja pallida*) (147–182 cm SVL) consistently expelled approximately 1.7% of their venom supply with each spit induced by movements of the investigators. There was no significant difference in quantity between the first few spits ($\bar{x}$ = 3.9 mg, range = 1.9–6.3 mg) and the 20th spit ($\bar{x}$ = 4.0 mg, range = 2.1–5.9 mg), but there was a significant decline in venom expulsion between the 20th and 40th spits ($\bar{x}$ = 1.8 mg, range = 0.9–3.6 mg). Curiously, in every specimen (N = 6) the first few spits were chemically unique from all subsequent spits in having a distinct protein never observed beyond the seventh spit. Freyvogel and Honegger (1965) obtained similar results for venom expenditure by Black-necked Spitting Cobras (*N. nigricollis*), which expelled an average of 3.7 mg with each spit induced by electrical stimulation. This average amount represented 3.3% of the total venom yielded by electrostimulation milking.

We assume these species, like other cobras (e.g., *N. kaouthia*; Herbert et al., unpublished; Table 2), are also capable of delivering large quantities of venom when biting defensively. If so, then spitting cobras appear to have a more sophisticated delivery system than other snakes that allows them to expel not only large quantities of venom when biting, but also to repeatedly eject small fractions of venom via spitting. The functional control of venom gland compression is probably distinct for biting and spitting. It would be interesting to learn of interspecific variation in spitting strategies or of variation dependent on size or species of target. If the snakes meter their venom when spitting, they most likely do so by the number of spits delivered rather than by the quantity per spit.

*Nuchal gland secretions*.—Some natricine (colubrid) snakes in Asia possess toxic secretions that are stored in a series of paired glands embedded under the skin of the neck region (Mori et al., 1996). The glands have no duct, but the skin covering the glands is relatively thin and easily broken under pressure to release the secretion (Mori et al., 1996). Studies of *Rhabdophis tigrinus* suggest that certain characteristic behaviors, such as neck-flattening, neck-arching, and dorsal-facing posture (directing the dorsal neck toward the threatening stimulus), serve a predator deterrent function of the glands (Mori et al., 1996; Mori and Burghardt, 2000). Deployment of this venom appears to be passive, such as by the pressure exerted during a bite by a predator. Despite the highly toxic substances secreted by this snake's Duvernoy's glands (Minton, 1990), the snake appears to rely more on nuchal gland secretions rather than its teeth and oral secretions for defense (Mori et al., 1996).

## CONCLUSIONS AND FUTURE DIRECTIONS

Snakes are remarkably designed to deploy their venom effectively, and appear capable of metering their venom based on decisions made in advance of the strike. Because venom is a limited commodity and serves a vital function for acquiring food and facilitating defense, the optimal amount of venom to use should be acted upon by natural selection. Selection on venom composition and deployment strategies, however, may be subject to the multiple needs that venom serves to immobilize and kill prey, to facilitate chemosensory relocation of released prey, to enhance prey digestion, and to defend against attack by potential predators and antagonists. Recognition of the multiple roles of venom is essential before we begin to make predictions or conclusions about optimal venom expenditure. For example, while the amount of venom delivered by *C. viridis* into mice appears to represent "overkill" (i.e., more than necessary to kill effectively; Hayes et al., 1992; Hayes, 1992a; Chiszar et al., 1999; S. Herbert and W. Hayes, unpublished), the additional volume delivered may be important to facilitate digestion or relocation of the prey.

Snakes are not unique in their ability to meter venom, as numerous invertebrates have independently evolved mechanisms for metering and conserving their venom supplies. It is tempting to suggest that, because snakes often use a rapid strike followed by immediate release, they are the only organisms with the ability to make metering decisions prior to attack and without feedback from the envenomated animal. However, nature has an amazing knack for reinventing the wheel, so to speak, and similar strategies may one day be documented in other venomous animals. Because snakes are so prominent among venomous animals and so fascinating to those who study them, investigators need to resist the temptation of an overly narrow ophidian focus if we are to better understand the functional roles of venoms and the adaptive value of venom allocation strategies.

Of the many factors that influence venom delivery, the relative size of the snake, and its corresponding supply of venom, appears to be the most important determinant of how much venom is used by an individual when biting. This appears to be true regardless of whether the strike is made within a predatory or defensive context. Because viperids generally have stouter bodies and larger heads than elapids, for a given size they are often capable of delivering more venom in a bite. There are exceptions, however, including the stout-bodied Australian elapid *A. antarcticus*, which can deliver substantial amounts of venom when biting. Colubrid snakes, in contrast, have a poorly developed apparatus for delivery of venom. Thus, when compared to elapids and viperids of similar size, most (but perhaps not all) toxic colubrids deliver substantially less venom, though they may compensate—as many elapids are also inclined to do—by maintaining a hold on the victim subsequent to biting. There is a widespread view among the general public (in the United States) that smaller snakes are more dangerous than larger ones because the smaller snakes have poor control over the venom. However, substantial data are now available to entirely debunk this myth. Young rattlesnakes typically produce more toxic venom than adults, but the supply of available venom is considerably less (e.g., Mackessy, 1985, 1988; Kardong, 1986a; Chippaux et al., 1991) and they inject much less venom during both predatory (Hayes, 1995) and defensive bites (Herbert, 1998; Rehling, 2002). The study of Hayes (1995) suggests that young snakes can indeed control venom release.

There are notable differences in venom expenditure between the two primary contexts of striking. For predatory bites, studies of rattlesnakes suggest there is relative consistency in the amount of venom expended (e.g., Hayes, 1992a). Although errant and ineffective bites occur, dry predatory bites are quite rare (e.g., Kardong, 1986a, b; Hayes, 1992a; but see Rehling, 2002, who reported a number of dry predatory bites by cottonmouths striking models of mice). In contrast, the amount of venom expended by rattlesnakes during defensive bites at model human limbs is highly variable, and dry bites occur with much greater frequency (Herbert, 1998; Rehling, 2002). Further, the maximum amounts of venom injected far exceed those reported previously for predatory bites. Obviously, it is time to dispel the supposition that snakes usually expend less venom in a defensive strike than in a predatory bite. Rattlesnakes appear capable of delivering these higher quantities of venom during approximately the same duration of fang contact as for predatory strikes at mice (averaging 0.20 sec; Table 3). This latter fact suggests that snakes can control or meter their venom when biting defensively. More compelling, however, are the data of Gennaro et al. (1961) and Rehling (2002), in which *A. piscivorus* delivers less venom in the first of several bites in succession and more in subsequent bites (Figs. 3–4). This pattern of venom appropriation is different from that seen in rattlesnakes (Rehling, 2002). Dry bites by rattlesnakes may also result from bites kinematically similar to envenomating bites, which suggest control of venom delivery (Young and Zahn, 2001). Thus, there is sufficient evidence to conclude that at least some venomous snakes are capable of metering their venom during both predatory and defensive strikes. Some snakes, particularly the viperids, are capable of delivering multiple potent bites and, therefore, are seldom at risk of having dangerously depleted venom reserves. Any venomous snake should be treated as dangerous regardless of its prior feeding or biting history. Nevertheless, we urgently need an improved understanding of how routine venom use relates to the time and energy investment necessary for venom replenishment.

There are a number of kinematic factors that can influence envenomation success, and more work is needed to fully tease these apart. The duration of fang contact, however, is clearly an important determinant of envenomation success for defensive bites. Surprisingly, data suggest that duration of fang contact does not influence the amount of venom delivered during a predatory strike (unless the snake simply holds on to the prey after venom injection). Duration of fang contact may play into decisions made by snakes when dealing with potential predators and enemies, and represents another option for venom metering. When striking is deemed necessary in a defensive context, there may be trade-offs between reducing vulnerability to counterattack (by minimizing duration of fang contact) and the need to inject a large dose of venom (by increasing fang contact time). The best decision may well depend on the snake's assessment of the severity of threat. Accordingly, the correlation between fang contact and venom expended may be an important consideration for concerns about human snakebite. For example, snakes that are trod upon or picked up by a human may perceive a greater threat and therefore inject more venom. Moreover, any article of clothing that might interfere with biting (but still allow the fangs to penetrate) could reduce the time in which fangs are embedded in the victim and, therefore, reduce the severity of envenomation. We are presently testing these hypotheses, as the results should be of interest to those who venture regularly into the habitats of snakes.

The capacity to meter venom is likely best developed among snakes that have evolved the strike-release strategy typically used by viperids. Through rapid delivery of venom and a quick retreat, these snakes can deploy their toxins with much reduced risk of retaliatory injury. Decisions on how much venom to deliver are best made in advance of the strike, and selection can act to optimize the amount of venom expended depending on the context and target. The evidence presented in this paper suggests that some snakes have evolved the capacity to meter their venom in this manner. For snakes that retain the presumed ancestral strike-hold strategy of envenomation (many elapids and colubrids), the venom can still be metered on an as-needed basis. When feeding, the snake can deliver additional pulses of venom until the prey ceases its struggles, and selection can act to optimize this amount. Metering for these snakes is largely dependent on the prey's reaction and renders the snake vulnerable to prey retaliation. More study is needed to confirm whether snakes that exhibit strike-hold strategies do indeed meter their venom, as suggested by data on time to prey death (Kardong, 1982).

With regard to defensive bites, many of our conclusions are drawn from measurements of venom injected into human limb models. We have some concerns about the validity of these model limbs, especially since venom is delivered into a fairly viscous medium (saline). When snakes inject venom into animal tissues, peripheral resistance to venom flow may provide some kind of feedback to which the snakes respond, or it may constrain how much venom can be delivered. We have recently compared the resistance to venom flow in various artificial media (air, water, several densities of agar) and in animal tissues (supermarket meats). We will use these data to evaluate the influence of flow resistance to venom delivery by snakes. The use of models, of course, is essential to better understand the factors that influence severity of envenomation in human snakebites, but the models, ideally, should be representative of real bite conditions. Fortunately, some of our conclusions derived from experiments are supported by clinical data. Nevertheless, additional prospective or retrospective studies of snakebite cases would be useful in validating more of our experimental findings.

*Acknowledgments*.—We wish to thank David Chiszar and Bruce Young for their thoughtful comments on an earlier draft. Gordon Schuett, Matt Rowe, and Wayne VanDevender loaned us several snakes used in our analyses. Jim Harrison, Scott Delay, and Jason Strack assisted in data collection for one or more experiments. David Duvall contributed to the conceptual design of Hayes' earlier studies, for which Ivan Kaiser and Corrine Seebart assisted with development of the ELISA methods. Support was provided by Southern Adventist University, Loma Linda University, and two grants from the National Science Foundation.

## LITERATURE CITED

ACTON, H. W., AND R. KNOWLES. 1914a. The dose of venom given in nature by a cobra at a single bite. Indian J. Med. Res. 1:388–413.

———, AND ———. 1914b. The dose of venom given in nature by the *Echis carinata* at a single bite. Indian J. Med. Res. 1:414–422.

AIRD, S. D. 2002. Ophidian envenomation strategies and the role of purines. Toxicon 40:335–393.

ALLON, N., AND E. KOCHVA. 1974. The quantities of venom injected into prey of different size by *Vipera palaestinae* in a single bite. J. Exp. Zool. 188:71–76.

BELBENOIT, P. 1986. Fine analysis of predatory and defensive motor events in *Torpedo marmorata* (Pisces). J. Exp. Biol. 121:197–226.

BOEVE, J.–L. 1994. Injection of venom into an insect prey by the free hunting spider *Cupiennius salei* (Araneae, Ctenidae). J. Zool., Lond. 234:165–175.

———, L. KUHN-NENTWIG, S. KELLER, AND W. NENTWIG. 1995. Quantity and quality of venom released by a spider (*Cupiennius salei*, Ctenidae). Toxicon 33:1347–1357.

BROAD, A. J., S. K. SUTHERLAND, AND A. R. COULTER. 1979. The lethality in mice of dangerous Australian and other snake venoms. Toxicon 17:661–664.

CASCARDI, J., F. A. YOUNG, H. D. HUSIC, AND J. SHERMA. 1999. Protein variation in the venom spat by the red spitting cobra, *Naja pallida* (Reptilia: Serpentes). Toxicon 37:1271–1279.

CHIPPAUX, J. P., V. WILLIAMS, AND J. WHITE. 1991. Snake venom variability: methods of study, results and interpretation. Toxicon 29:1279–1303.

CHISZAR, D., C. W. RADCLIFFE, K. M. SCUDDER, AND D. DUVALL. 1983. Strike-induced chemosensory searching by rattlesnakes: the role of envenomation related chemical cues in the post-strike environment. Pp. 25–44 *In* D. Müller-Schwarze and R. M. Silverstein (Eds.), Chemical Signals in Vertebrates III. Plenum Press, New York.

———, C. W. Radcliffe, and H. M. Smith 1992. Searching behaviors by rattlesnakes following predatory strikes. Pp. 369–382 *In* J. A. Campbell and E. D. Brodie, Jr. (Eds.), Biology of the Pitvipers. Selva , Tyler, Texas.

———, and K. M. Scudder. 1980. Chemosensory searching by rattlesnakes during predatory episodes. Pp. 125–139 *In* D. Müller-Schwarze and R. M. Silverstein (Eds.), Chemical Signals: Vertebrates and Aquatic Invertebrates. Plenum Press, New York.

———, A. Walters, J. Urbaniak, H. M. Smith, and S. P. Mackessy. 1999. Discrimination between envenomated and nonenvenomated prey by western diamondback rattlesnakes (*Crotalus atrox*): chemosensory consequences of venom. Copeia 1999:640–648.

Coss, R. G., K. L. Guse, N. S. Poran, and D. G. Smith. 1993. Development of antisnake defenses in California ground squirrels (*Spermophilus beecheyi*): II. Microevolutionary effects of relaxed selection from rattlesnakes. Behaviour 124:137–164.

Daltry, J. C., W. Wuster, and R. S. Thorpe. 1996. Diet and snake venom evolution. Nature 379:537–540.

de Cock Buning, T. 1983. Thermal sensitivity as a specialization for prey capture and feeding in snakes. Amer. Zool. 23:363–375.

Diller, L. V. 1990. A field observation on the feeding behavior of *Crotalus viridis lutosus*. J. Herpetol. 24:95–97.

Duvall, D., M. B. King, and K. J. Gutzwiller. 1985. Behavioral ecology and ethology of the prairie rattlesnake. Nat. Geogr. Res. 6:457–469.

Elliott, W. B. 1978. Chemistry and immunology of reptilian venoms. Pp. 163–436 *In* C. Gans (Ed.), Biology of the Reptilia, Vol. 8. Academic Press, London.

Fairley, N. H., and B. Splatt. 1929. Venom yields in Australian poisonous snakes. Med. J. Aust. 1:336.

Freyvogel, T., and C. Honegger. 1965. Der ‘Speiakt’ von *Naja nigricollis*. Acta Tropica 22:289–302.

Fritts, T. H., M. J. McCoid, and R. L. Haddock. 1990. Risks to infants on Guam from bites of the brown tree snake (*Boiga irregularis*). Am. J. Trop. Med. Hyg. 42:607–611.

Gans, C. 1961. The feeding mechanism of snakes and its possible evolution. Am. Zool. 1:217–227.

———. 1978. Reptilian venoms: some evolutionary considerations. Pp. 1–16 *In* C. Gans (Ed.), Biology of the Reptilia, Vol. 8, Academic Press, New York.

Gennaro, J. F. 1963. Observations on the treatment of snakebite in North America. Pp. 427–449 *In* Proceedings of the 10th Pacific Science Conference. H. Keegan and W. MacFarlane (Eds.), The Macmillan Company, New York.

———, R. S. Leopold, and T. W. Merriam. 1961. Observations on the actual quantity of venom introduced by several species of crotalid snakes in their bite. Anat. Rec. 139:303.

Gibbons, J. W., and M. E. Dorcas. 2002. Defensive bite behavior of cottonmouths (*Agkistrodon piscivorus*) toward humans. Copeia 2002:195–198.

Glenn, J. L., and R. C. Straight. 1982. The rattlesnakes and their venom yield and lethal toxicity. Pp. 3–119 *In* A. T. Tu (Ed.), Rattlesnake Venoms: Their Actions and Treatment. Marcel Dekker, New York.

Gloyd, H. K., and R. Conant. 1990. Snakes of the *Agkistrodon* Complex: A Monographic Review. Society for the Study of Amphibians and Reptiles, Contributions to Herpetology 6. Oxford, Ohio.

Golani, I., and E. Kochva. 1988. Biting behaviour of *Atractaspis*. Copeia 1988:792–797.

Goode, M. J., and D. Duvall. 1989. Body temperature and defensive behavior of free-ranging prairie rattlesnakes, *Crotalus viridis viridis*. Anim. Behav. 38:360–362.

Greene, H. 1997. Snakes: The Evolution of Mystery in Nature. University of California Press, Berkeley and Los Angeles.

Haas, G. 1973. Muscles of the jaw and associated structures in the Rhyncocephalia and Squamata. Pp. 285–490 *In* C. Gans and T. Parsons (Eds.), Biology of the Reptilia, Vol. 4. Academic Press, New York.

Hardy, D. L. 1991. Epidemiology and medical management of rattlesnake bite in southern Arizona. Sonoran Herpetol. 4:153–158.

Hayes, W. K. 1991a. Ontogeny of striking, prey-handling and envenomation behavior of prairie rattlesnakes (*Crotalus v. viridis*). Toxicon 29:867–875.

———. 1991b. Envenomation strategies of prairie rattlesnakes. Unpublished Ph.D. dissertation, University of Wyoming, Laramie.

———. 1992a. Factors associated with the mass of venom expended by prairie rattlesnakes (*Crotalus v. viridis*) feeding on mice. Toxicon 30:449–460.

———. 1992b. Prey-handling and envenomation strategies of prairie rattlesnakes (*Crotalus v. viridis*) feeding on mice and sparrows. J. Herpetol. 26:496–499.

———. 1993. Effects of hunger on striking, prey-handling, and venom expenditure of prairie rattlesnakes (*Crotalus v. viridis*). Herpetologica 49:305–310.

———. 1995. Venom metering by juvenile prairie rattlesnakes, *Crotalus v. viridis*: effects of prey size and experience. Anim. Behav. 50:33–40.

———, AND D. DUVALL. 1991. A field study of prairie rattlesnake predatory strikes. Herpetologica 47:78–81.

———, AND F. E. HAYES. 1985. Human envenomation from the bite of the eastern garter snake, *Thamnophis s. sirtalis* (Serpentes: Colubridae). Toxicon 23:719–721.

———, I. I. KAISER, AND D. DUVALL. 1992. The mass of venom expended by prairie rattlesnakes when feeding on rodent prey. Pp. 383–388 *In* J. A. Campbell and E. D. Brodie, Jr. (Eds.), Biology of the Pitvipers. Selva, Tyler, Texas.

———, W. K., P. LAVIN-MURCIO, AND K. V. KARDONG. 1993. Delivery of Duvernoy's secretion into prey by the brown tree snake, *Boiga irregularis* (Serpentes: Colubridae). Toxicon 31:881–887.

———, P. A. LAVIN-MURCIO, AND K. V. KARDONG. 1995. Northern Pacific rattlesnakes (*Crotalus viridis oreganus*) meter venom when feeding on prey of different sizes. Copeia 1995:337–343.

HERBERT, S. S. 1998. Factors influencing venom expenditure during defensive bites by cottonmouths (*Agkistrodon piscivorus*) and rattlesnakes (*Crotalus viridis, Crotalus atrox*). Unpublished M.S. thesis, Loma Linda University, Loma Linda, California.

HILL, R. E., AND S. P. MACKESSY. 1997. Venom yields from several species of colubrid snakes and differential effects of ketamine. Toxicon 35:671–678.

HOKAMA, Z. 1978. Study on experimental envenomation by the Habu (*Trimeresurus flavoviridis*). The Snake 10:107–113.

JONES, K. B. 1988. Influence of prey on the feeding behavior of *Trimorphodon biscutatus lambda* (Colubridae). Southwest. Nat. 33:488–490.

JORGE, M. T., L. A. RIBEIRO, AND S. A. NISHIOKA. 1997. A comparison of clinical and epidemiological aspects of bites by small and large South American rattlesnakes. Trop. Doct. 27:106–107.

———, ———, AND J. L. O'CONNELL. 1999. Prognostic factors for amputation in the case of envenoming by snakes of the *Bothrops* genus (Viperidae). Ann. Trop. Med. Parasitol. 93:401–408.

JORGE DA SILVA, JR., N., AND S. D. AIRD. 2001. Prey specificity, comparative lethality and compositional differences of coral snake venoms. Comp. Biochem. Physiol. 128C:425–456.

KARDONG, K. V. 1975. Prey capture in the cottonmouth snake (*Agkistrodon piscivorus*). J. Herpetol. 9:169–175.

———. 1982. The evolution of the venom apparatus in snakes from colubrids to viperids and elapids. Mem. Inst. Butantan 46:105–118.

———. 1982. Comparative study of changes in prey capture behavior of the cottonmouth (*Agkistrodon piscivorus*) and Egyptian cobra (*Naja haje*). Copeia 1982:337–343.

———. 1986a. The strike behavior of the rattlesnake, *Crotalus viridis oreganus*. J. Comp. Psychol. 100:304–314.

———. 1986b. The predatory strike of the rattlesnake: when things go amiss. Copeia 1986:816–820.

———. 1996. Snake toxins and venoms: an evolutionary perspective. Herpetologica 52:36–46.

———, AND V. L. BELS. 1998. Rattlesnake strike behavior: kinematics. J. Exp. Biol. 201:837–850.

———, T. KIENE, AND V. BELS. 1997a. Evolution of trophic systems in squamates. Neth. J. Zool. 47:1–17.

———, ———, AND E. K. JOHNSON. 1997b. Proximate factors affecting the predatory behavior of the red spitting cobra, *Naja mossambic pallida*. J. Herpetol. 31:66–71.

———, AND P. A. LAVIN-MURCIO. 1993. Venom delivery of snakes as high-pressure and low-pressure systems. Copeia 1993:644–650.

KLAUBER, L. M. 1972. Rattlesnakes: Their Habits, Life Histories and Influence on Mankind, 2 Vols., 2nd ed. University of California Press, Berkeley and Los Angeles.

KOCHVA, E. 1960. A quantitative study of venom secretion by *Vipera palaestinae*. Am. J. Trop. Med. Hyg. 9:381–390.

———. 1978. Oral glands of the Reptilia. Pp. 43–161 *In* C. Gans and K. A. Gans (Eds.), Biology of the Reptilia, Vol. 8. Academic Press, New York.

———. 1987. The origin of snakes and evolution of the venom apparatus. Toxicon 25:65–106.

Kondo, H., S. Kondo, S. Sadahiro, K. Yamauchi, A. Ohsaka, and R. Murata. 1972. Estimation by a new method of the amount of venom ejected by a single bite of *Trimeresurus* species. Japan. J. Med. Sci. Biol. 25:123–131.

Kouyoumdjian, J. A., and C. Polizelli. 1989. Snake bites by *Bothrops moojeni*: correlation of the clinical picture with the snake size. Rev. Inst. Med. Trop. Sao Paulo 31:84–90.

Kuhn, B. F., M. J. Rochelle, and K. V. Kardong. 1991. Effects of rattlesnake (*Crotalus viridis oreganus*) envenomation upon the mobility and death rate of laboratory mice (*Mus musculus*) and wild mice (*Peromyscus maniculatus*). Bull. Maryland Herpetol. Soc. 27:189–194.

Kurnik, D., Y. Haviv, and E. Kochva. 1999. A snake bite by the burrowing asp, *Atractaspis engaddensis*. Toxicon 37:223–227.

Lavin-Murcio, P., B. G. Robinson, and K. V. Kardong. 1993. Cues involved in relocation of struck prey by rattlesnakes, *Crotalus viridis oreganus*. Herpetologica 49:463–469.

Leinz, E. F., and T. R. F. Janeiro-Cinquini.1994. Influence of the extraction interval on the venom yield from snakes *Bothrops jararaca*. The Snake 26:67–70.

Lenk, P., S. Kalyabina, M. Wink, and U. Joger. 2001. Evolutionary relationships among the true vipers (Reptilia: Viperidae) inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 19:94–104.

Lowe, C. G., R. N. Bray, and D. R. Nelson. 1994. Feeding and associated electrical behavior of the Pacific electric ray *Torpedo californica* in the field. Mar. Biol. 120:161–169.

Mackessy, S. P. 1985. Fractionation of red diamond rattlesnake (*Crotalus ruber ruber*) venom: protease, phosphodiesterase and L-amino acid oxidase activities. Toxicon 23:337–340.

———. 1988. Venom ontogeny in the Pacific rattlesnakes *Crotalus viridis helleri* and *C. v. oreganus*. Copeia 1988:92–101.

———. 1991. Morphology and ultrastructure of the venom glands of the northern Pacific rattlesnake, *Crotalus viridis oreganus*. J. Morphol. 208:109–128.

Malli, H., H. Imboden, and L. Kuhn-Nentwig. 1998. Quantifying the venom dose of the spider *Cupiennius salei* using monoclonal antibodies. Toxicon 36:1959–1969.

———, L. Kuhn-Nentwig, H. Imboden, and W. Nentwig. 1999. Effects of size, motility and paralysation time of prey on the quantity of venom injected by the hunting spider *Cupiennius salei*. J. Exp. Biol. 202:2083–2089.

McKinstry, D. M. 1978. Evidence of toxic saliva in some colubrid snakes of the United States. Toxicon 16:523–534.

———. 1983. Morphologic evidence of toxic saliva in colubrid snakes: a checklist of world genera. Herpetol. Rev. 14:12–15.

Minton, S. A. 1987. Poisonous snakes and snakebite in the U.S.: a brief review. Northwest Sci. 61:130–137.

———. 1990. Venomous bites by nonvenomous snakes: an annotated bibliography of colubrid envenomation. J. Wildl. Med. 1:119–127.

———. 1996. Are there any nonvenomous snakes? An update on colubrid envenoming. Pp. 127–134 *In* Advances in Herpetoculture, P. D. Strimple (Ed.), Spec. Publ. Internatl. Herpetol. Symp., Inc., Des Moines, Iowa.

Mirtschin, P., and R. Davis. 1983. Dangerous Snakes of Australia. Rigby Publishers, Adelaide.

Mitchell, S. W. 1860. Researches upon the venom of the rattlesnake. Smithson. Contrib. Knowledge 1860:1–139.

Mori, A., and G. M. Burghardt. 2000. Does prey matter? Geographic variation in antipredator responses of hatchlings of a Japanese natricine snake (*Rhabdophis tigrinus*). J. Comp. Psychol. 114:408–413.

———, D. Layne, and G. M. Burghardt. 1996. Descriptions and preliminary analysis of antipredator behavior of *Rhabdophis tigrinus tigrinus*, a colubrid snake with nuchal glands. Japanese J. Herpetol. 16:94–107.

Morrison, J. J., J. H. Pearn, and A. R. Coulter. 1982. The mass of venom injected by two Elapidae: the taipan (*Oxyuranus scutellatus*) and the tiger snake (*Notechus scutatus*). Toxicon 20:739–745.

———, N. T. Charles, and J. H. Pearn. 1983a. The use of experimental models to study the biting habits of Australian snakes in both “defensive” and “hunting” bites. Toxicon Suppl. 3:305–308.

———, J. H. Pearn, N. T. Charles, and A. R. Coulter. 1983b. Further studies on the mass of venom injected by Elapid snakes. Toxicon 21:279–284.

———, ———, J. Covacevich, C. Tanner, and A. Coulter. 1984. Studies on the venom of *Oxyuranus microlepidotus*. Clin. Toxicol. 21:373–385.

Moss, S. T., G. Bogdan, R. C. Dart, S. P. Nordt, S. R. Williams, and R. F. Clark. 1997. Association of rattlesnake bite location with severity of clinical manifestations. Ann. Emerg. Med. 30:58–61.

O'Connell, D. J., and D. R. Formanowicz, Jr. 1998. Differential handling of dangerous and non-dangerous prey by naive and experienced Texas spotted whiptail lizards, *Cnemidophorus gularis*. J. Herpetol. 32:75–79.

O'Connell, B., T. Poole, P. Nelson, H. M. Smith, and D. Chiszar 1982. Strike-induced chemosensory searching by prairie rattlesnakes (*Crotalus v. viridis*) after predatory and defensive strikes which made contact with mice (*Mus musculus*). Bull. Maryland Herpetol. Soc. 18:152–160.

Parrish, H. M., and R. E. Thompson. 1958. Human envenomation from bites of recently milked rattlesnakes:a report of three cases. Copeia 1958:83–86.

Pearn, J., B. McGuire, L. McGuire, and P. Richardson. 2000. The envenomation syndrome caused by the Australian red-bellied black snake *Pseudechis porphyriacus*. Toxicon 38:1715–1729.

Perret, B. A. 1977. Determination of the amount of venom released by striking tarantulas. J. Georgia Ent. Soc. 12:329–333.

Phelps, T. 1989. Poisonous Snakes. Blanford Press, London.

Pollard, S. D. 1990. The feeding strategy of a crab spider, *Diaea* sp. indet. (Araneae: Thomisidae): post-capture decision rules. J. Zool. Lond. 222:601–615.

Radcliffe, C. W., D. Chiszar, and B. O'Connell. 1980. Effects of prey size on poststrike behavior in rattlesnakes (*Crotalus durissus*, *C. enyo* and *C. viridis*). Bull. Psychon. Soc. 16:449–450.

———, K. Estep, T. Boyer, and D. Chiszar. 1986. Stimulus control of predatory behaviour in red spitting cobras (*Naja mossambica pallida*) and prairie rattlesnakes (*Crotalus v. viridis*). Anim. Behav. 34:804–814.

Rassmussen, S., B. Young, and H. Krimm. 1995. On the 'spitting' behavior in cobras (Serpentes: Elapidae). J. Zool. Lond. 237:27–35.

Rehling, G. C. 2002. Venom expenditure in multiple bites by rattlesnakes and cottonmouths. Unpublished M.S. thesis, Loma Linda University, Loma Linda, California.

Reid, H. A., and R. D. G. Theakston. 1983. The management of snake bite. Bull. World Health Organiz. 61:885–895.

Rein, J. O. 1993. Sting use in two species of *Parabuthus* scorpions (Buthidae). J. Arachnol. 21:60–63.

Rezende, N. A., F. M. Torres, M. B. Dias, D. Campolina, C. Chavez-Olortegui, and C. F. Amaral. 1998. South American rattlesnake bite (*Crotalus durissus* sp) without envenoming: insights on diagnosis and treatment. Toxicon 36:2029–2032.

Ribeiro, L. A., G. Puorto, and M. T. Jorge. 1999. Bites by the colubrid snake *Philodryas olfersii*: a clinical and epidemiological study of 43 cases. Toxicon 37:943–948.

Robinson, M. H. 1969. Predatory behavior of *Argiope argentata* (Fabricius). Amer. Zool. 9:161–173.

Rochelle, M J., and K. V. Kardong. 1993. Constriction versus envenomation in prey capture by the brown tree snake, *Boiga irregularis* (Squamata: Colubridae). Herpetologica 49:301–304.

Rodriguez-Robles, J. A. 1994. Are the Duvernoy's gland secretions of colubrid snakes venoms? J. Herpetol. 28:388–390.

———, and M. Leal. 1993. Effects of prey type on the feeding behavior of *Alsophis portoricensis* (Serpentes: Colubridae). J. Herpetol. 27:163–168.

———, and R. Thomas. 1992. Venom function in the Puerto Rican racer, *Alsophis portoricensis* (Serpentes: Colubridae). Copeia 1992:62–68.

Rosenberg, H. 1967. Histology, histochemistry and emptying mechanisms of the venom gland of some elapid snakes. J. Morphol. 122:133–156.

Rowe, M. P., and D. H. Owings. 1990. Probing, assessment, and management during interactions between ground squirrels and rattlesnakes. Part 1: Risks related to rattlesnake size and body temperature. Ethology 86:237–249.

Ruben, J. A., and C. Geddes. 1983. Some morphological correlates of striking in snakes. Copeia 1983:221–225.

Russell, F. E. 1978. Consecutive bites on three persons by a single rattlesnake. Toxicon 16:79–80.

———. 1980a. Snake Venom Poisoning. J. B. Lippincott Co., Philadelphia.

———. 1980b. Snake venom poisoning in the United States. Ann. Rev. Med. 31:247–259.

———. 1984. Snake venoms. Symp. Zool. Soc. Lond. 52:469–480.

———, F. G. Walter, T. A. Bey, and M. C. Fernandez. 1997. Snakes and snakebite in Central America. Toxicon 35:1469–1522.

Savitzky, B. A. C. 1992. Laboratory studies on piscivory in an opportunistic pitviper, the cottonmouth, *Agkistrodon piscivorus*. Pp. 347–368 *In* J. A. Campbell and E. D. Brodie, Jr.(Eds.), Biology of the Pitvipers. Selva, Tyler, Texas.

Schaeffer, R. C., Jr., S. Bernick, T. H. Rosenquist, and F. E. Russell. 1972. The histochemistry of the venom glands of the rattlesnake *Crotalus viridis helleri*: II. Monoamine oxidase, acid and alkaline phosphatase. Toxicon 10:295–297.

Seib, R. L. 1980. Human envenomation from the bite of an aglyphous false coral snake, *Pliocercus elapoides* (Serpentes: Colubridae). Toxicon 18:399–401.

Shine, R., and T. Schwaner. 1985. Prey constriction by venomous snakes: a review, and new data on Australian species. Copeia 1985:1067–1071.

Silveira, P. V. P., and S. A. Nishioka. 1995. Venomous snake bite without clinical envenoming ('dry-bite'): a neglected problem in Brazil. Trop. Geog. Med. 47:82–85.

Smith, T. L., K. V. Kardong, and P. A. Lavin-Murcio. 2000. Persistence of trailing behavior: cues involved in poststrike behavior by the rattlesnake (*Crotalus viridis oreganus*). Behaviour 137:691–703.

Stahel, E., R. Wellauer, and T. A. Freyvogel. 1985. Poisoning by domestic vipers (*Vipera berus* and *Vipera aspis*). A retrospective study of 113 patients. Schweiz. Med. Wochenschr. 115:890–896.

Thomas, R. G., and F. H. Pough. 1979. The effect of rattlesnake venom on digestion of prey. Toxicon 17:221–228.

Thomas, L., B. Tyburn, J. Ketterle, T. Biao, H. Mehdaoui, V. Moravie, C. Rouvel, Y. Plumelle, B. Bucher, D. Canonge, C. A. Marie-Nelly, and J. Lang. 1998. Prognostic significance of clinical grading of patients envenomated by *Bothrops lanceolatus* in Martinique. Trans. R. Soc. Trop. Med. Hyg. 92:542–545.

Thorington, G. U., and D. A. Hessinger. 1998. Efferent mechanisms of discharging cnidae: II. A nematocyst release response in the sea anemone tentacle. Biol. Bull. 195:145–155.

Tibballs, J. 1992. Diagnosis and treatment of confirmed and suspected snakebite: implications from analysis of 46 paediatric cases. Med. J. Australia 156:270–274.

Tu, A. T. (Ed.). 1977. Venoms: Chemistry and Molecular Biology. John Wiley & Sons, New York.

———. 1982. Rattlesnake Venoms: Their Actions and Treatment. Marcel Dekker, New York.

———. 1991. Handbook of Natural Toxins. Vol. 5. Reptile Venoms and Toxins. Marcel Dekker, New York.

Tun-Pe, Ba-Aye, Aye-Aye-Myint, Tin-Nu-Swe, and D. A. Warrell. 1991a. Bites by Russell's vipers (*Daboia russelii siamensis*) in Myanmar: effect of the snake's length and recent feeding on venom antigenaemia and severity of envenoming. Trans. Royal Soc. Trop. Med. Hyg. 85:804–808.

———, and Khin-Aung-Cho. 1986. Amount of venom injected by Russell's viper (*Vipera russelli*). Toxicon 24:730–733.

———, ———, and Aye-Aye-Myint. 1991b. Amount of venom injected by Russell's viper (*Vipera russelli*) in killing prey. The Snake 23:71–74.

Warrell, D. 1996. Clinical features of envenoming from snake bite. Pp. 64–76 *In* C. Bon and M. Goyffon (Eds.), Envenomings and their Treatment. Foundation Marcel Merieux, Lyons.

Watson, G. M., and D. A. Hessinger. 1994. Antagonistic frequency tuning of hair bundles by different chemoreceptors regulates nematocyst discharge. J. Exp. Biol. 187:57–73.

Weinstein, S. A., and K. V. Kardong. 1994. Properties of Duvernoy's secretions from opisthoglyphous and aglyphous colubrid snakes: a critical review. Toxicon 32:1161–1185.

Whitaker, P. B., K. Ellis, and R. Shine. 2000. The defensive strike of the eastern brownsnake, *Pseudonaja textilis* (Elapidae). Funct. Ecol. 14:25–31.

Whitaker, P. B., and R. Shine. 1999. Responses of free-ranging brownsnakes (*Pseudonaja textilis*, Elapidae) to encounters with humans. Wildlife Res. 26:689–704.

Young, B. A., M. Blair, K. Zahn, and J. Marvin. 2001. Mechanics of venom expulsion in *Crotalus*, with special reference to the role of the fang sheath. Anat. Rec. 264:415–426.

———, and K. Zahn. 2001. Venom flow in rattlesnakes: mechanics and venom metering. J. Exp. Biol. 204:4345–4351.

———, ———, M. Blair, and J. Lalor. 2000. Functional subdivision of the venom gland musculature and the regulation of venom expulsion in snakes. J. Morphol. 246:249–259.