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Venom flow in rattlesnakes: mechanics and metering

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Summary

The functional morphology of venom injection in *Crotalus atrox* was explored using high-speed digital videography combined with direct recording of venom flow using perivascular flow probes. Although venom flow was variable, in most strikes the onset of venom flow was coincidental with fang penetration, and retrograde flow (venom suction) was observed prior to fang withdrawal. The duration of venom flow was consistently less than the duration of fang penetration. The occurrence of retrograde flow, 'dry bites' (which accounted for 35 % of the strikes) and unilateral strikes all support a hypothesis for venom pooling in the distal portion of the venom-delivery system. No significant difference in temporal or volumetric aspects of venom flow were found between defensive strikes directed at small and large rodents. With the species and size of target held constant, the duration of venom flow, maximum venom flow rate and total venom volume were all significantly lower in predatory than in defensive strikes.

Key words: feeding, prey capture, Reptilia, squamata, functional morphology, kinematics, rattlesnake, *Crotalus atrox.*

Introduction

The prey-capture system of venomous snakes is one of the most specialized feeding mechanisms among vertebrates. The biomechanics and kinematics of prey ingestion have been documented from several snake taxa, although the full diversity of ingestive mechanics has only recently been appreciated (Cundall and Greene, 2000). Less attention has been paid to prey capture and, in particular, the mechanics of venom injection (Savitzky, 1980; Kochva, 1987). Venom is expelled when the skeletal musculature surrounding the venom gland contracts, causing an increase in pressure within the venom gland and forcing venom out (Rosenberg, 1967). This explanation, commonly referred to as the intraglandular pressure hypothesis, has been supported, directly and indirectly, by a number of investigations (Freyvogel and Honegger, 1965; Kardong and Lavin-Murcio, 1993; Young et al., 2000). To date, no study has directly recorded the flow of venom or directly linked venom flow with the mechanics and kinematics of the strike.

In recent years, the behavioral ecology of snake venoms, particularly rattlesnake (*Crotalus* spp.) venom, has received considerable attention. Using techniques such as enzyme-linked immunosorbent assay and protein assay, researchers have quantified the amount of venom injected into, or onto, a target and compared these results with theoretical arguments for venom use based on optimization and energy conservation (for a review, see Hayes et al., 2001). Although causal evidence is lacking, these studies frequently attribute differential venom flow to differences in the kinematics of the strike or to the mechanics of venom injection (Gennaro et al., 1961; Kardong, 1986a,b; Rowe and Owings, 1990; Hayes, 1991, 1992).

The present study reports on a new technique for the study of venom mechanics. Transonic flow probes, surgically implanted onto the venom duct, use ultrasound to directly quantify venom flow through the venom-delivery system. The output of these flow probes is synchronized with a high-speed digital video camera, which permits both venom flow and strike kinematics to be quantified with a temporal resolution of 2 ms. The primary purpose of this study was to explore the mechanics of venom expulsion in western diamondback rattlesnakes, *Crotalus atrox*. Venom flow was recorded during strikes directed at different-sized biological targets and under different behavioral contexts, thus enabling a direct quantitative test for the regulation of venom flow.

Materials and methods

Four wild-caught adult (snout–vent length 78–112 cm) western diamondback rattlesnakes, *Crotalus atrox* (Baird and Girard, 1853), were used for this study. These animals were maintained in individual terraria within a specially designed venomous snake room at 26–31 °C on a 12 h:12 h light:dark cycle and were provided with water *ad libitum* and a diet of pre-killed mice. The specimens were sedated by oral exposure to isoflurane, then anesthetized with an intramuscular injection of 65 mg kg^{-1} Ketamine hydrochloride:Acepromazine (9:1 v/v) and maintained on 0.5 % isoflurane in oxygen using a low-flow respirator (Anesco). Using sterile surgical techniques, a

perivascular flow probe (Transonic Systems, Inc.) was unilaterally implanted onto the primary venom duct and surrounded by Sylastic sheeting (Dow Corning). The cable from the flow probe coursed subcutaneously along the dorsolateral margin of the head and neck, then extended 5 cm through the scalation on the dorsal midline of the neck. Surgical incisions were closed with a combination of sutures and surgical adhesive (Vetbond).

Following a minimum recovery period of 14 days, the specimens were connected, through 2 m of cable, to a T106 flowmeter (Transonic Systems, Inc.) and placed in a 125 cm×47 cm×48 cm high filming cage. A partition gradually narrowed the available area in the cage, reducing one end of the cage to only 16 cm×10 cm. While in the filming cage, the snakes were induced to strike at live rodents. Previous work has shown that the physical interaction between the soft tissue surrounding the fang, the fang sheath, and the target can influence venom flow (Young et al., 2001a). This finding, combined with our need for a strong behavioral reaction, necessitated our use of live biological targets. For defensive strikes, the proximity of the researchers was used to induce a suite of defensive behaviors in the rattlesnakes (including sustained elevated body coiling, slow tongue flicks and continuous rattling) prior to introduction of the targets. Once these behaviors were sustained, we presented the snake with either a rat (mean mass 173.6 g, range 159.2–185.3 g) or a mouse (mean mass 25.6 g, range 20.4–28.8 g) that was held by the tail using 60 cm forceps and rapidly advanced at the snake in a consistent manner. Every defensive encounter took place in the largest portion of the filming cage, and all resulted in a strike.

For predatory strikes, the snake was coupled to the flowmeter and placed in the filming cage, as described above. The filming cage was surrounded by a floor-to-ceiling screen that visually isolated the snake. The snake was given at least 3 h to accommodate to the cage. A clear Plexiglas sliding door extending the height of the cage prevented the snake from entering the 10 cm×16 cm end of the cage before the presentation of the live mouse, but allowed visual stimulation when the mouse was in the chamber. The door had 5 mm holes drilled near its bottom edge, allowing the snake thermal and olfactory stimulation. A sealable portal hole in the wall of the cage allowed a mouse (mean mass 24.6 g) to be introduced without inducing defensive behavior in the snake. Once the snake showed a suite of predatory behaviors (including absence of rattling, rapid tongue flicks, orientation of body and head towards the prey), the Plexiglas door was retracted using a pulley system, allowing the snake to strike the mouse.

Strikes were filmed using a 1000S high-speed digital video camera (Redlake Instruments) at 500 frames s^{-1} with a shutter speed of 1/20 000 s. Output from the video camera was streamed to a PowerMac 8500 (Apple) and captured using Premiere 4.0 (Adobe) prior to frame-by-frame analysis with Image1.6.2 (N.I.H.). Output from the T106 flowmeter was transferred to a G4 computer (Apple) using the Instrunet data-acquisition system (G.W. Instruments) and quantified using SuperScope (G.W. Instruments). The kinematic and venom flow recordings were synchronized using a S88 dual-channel stimulator (Grass) which sent a simultaneous signal to the video camera and the data-acquisition computer.

Mean and maximum flow rates were measured directly from the venom traces (using SuperScope) following both internal (using the built-in function of the T106 flowmeter) and external (manual extraction using light pressure) calibrations. Total venom volume was defined as the area under the flow curve above the baseline; as such, venom volume was not corrected for retrograde flow. The quantitative data were imported into SYSTAT 5.12 and compared using the two-tailed *t*-test (with unequal variance). Venom mass was not measured directly, but was calculated from volume using a conversion of 1 ml venom=200 mg dry mass (S. Mackessy, personal communication). This conversion was performed largely to allow comparison with values in the literature, where venom quantity is normally reported as dry mass, not volume.

To ensure venom regeneration between strikes, the snakes were given a minimum of 7 days between trials (or post-feeding), with the average interval being 11 days. Only a single strike was recorded from each trial. Sixty strikes were recorded, roughly evenly divided among the four specimens. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Lafayette College.

Results

The correct size of Transonic flow probes was of critical importance; probes that were too small could occlude the venom duct, while larger probes produced excessive stretching of the scalation and soft tissue. These flow probes are designed for chronic implantation and work better following connective tissue encapsulation. Two of these implants were left in place for almost a year. Our initial experimental design was to combine the Transonic flow probes with patch electromygraphic (EMG) electrodes on the extrinsic venom gland musculature; however, the presence of the flow probe cable coursing over the patch electrode introduced too much artifact into the EMG signals. None of the venom tracings recorded during these early experiments is included in the present report. By comparison with a previous quantitative analysis (Young et al., 2001b), we found that the presence of the Transonic flow probe had no apparent influence on the kinematic features of the strike of *Crotalus atrox*.

Mechanics of venom expulsion

Integrating the kinematic and flow probe recordings for all of the strikes (defensive and predatory) yielded fairly consistent venom flow profiles (Fig. 1). No venom flow was recorded during the launch of the strike or the early portions of fang erection; however, the terminal portions of fang erection were associated with a modest, and variable, amount of retrograde venom flow. The onset of venom expulsion was marked by the reversal of this retrograde flow (Fig. 1) and was closely linked to fang penetration. The mean time from fang

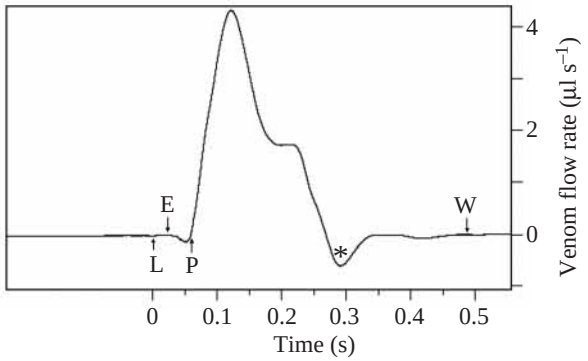

Fig. 1. A 'typical' venom flow profile. The key kinematic episodes of the strike are indicated: L, launch; E, fang erection; P, fang penetration; W, fang withdrawal. Note the low level of retrograde flow immediately prior to fang penetration, the temporal coincidence between venom expulsion and fang penetration and the prominent venom suction (*) following venom expulsion.

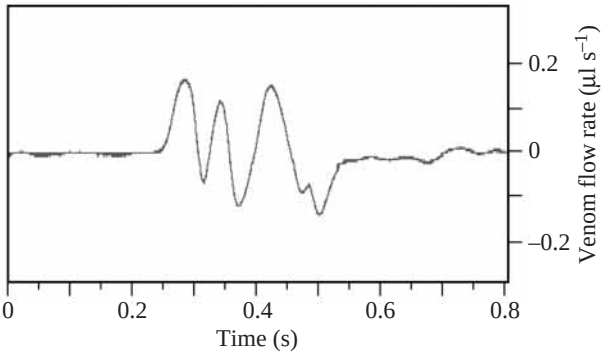

Fig. 2. Venom flow profile illustrating multiple pulses of venom expulsion associated with asymmetric movements of the fangs. Analysis of the synchronized video recording revealed that the three pulses correspond to penetration of the contralateral fang, penetration of the ipsilateral fang and repositioning of the contralateral fang.

penetration to venom flow was 16 ms, but this value is skewed by one interval that lasted almost 100 ms; in over half the strikes, the onset of venom flow was coincidental with fang penetration. The rate and total duration of venom expulsion, as well as the total volume of venom expelled, were highly variable. The termination of venom expulsion was marked by a second larger, and more consistent, episode of retrograde venom flow (Fig. 1). A second, much smaller, pulse of venom expulsion was occasionally observed during fang withdrawal. The duration of fang penetration showed little correlation to the duration of venom flow (Pearson's correlation coefficient=0.294). Nevertheless, there was a clear pattern between these two variables in that the duration of fang penetration was longer than the duration of venom flow (Table 1) in all but two strikes; in these two strikes, venom was expelled from the fangs for approximately 10 ms after fang withdrawal.

Variations from this typical venom flow profile provide insight into the mechanics of venom expulsion. In eight (13 %) of the strikes, we recorded multiple pulses of venom flow (Fig. 2), although each pulse typically ejected less venom than in a 'typical' strike. Kinematic analysis revealed that each pulse corresponded to an asymmetric penetration of the fangs (i.e. the left fang penetrated before the right or the right fang was unilaterally repositioned after the initial penetration). The Transonic flow probe was only implanted on the right venom duct; however, penetration of either the left or right fang was coincidental with equivalent venom flow through the right venom duct (Fig. 2). Venom flow following penetration of the contralateral fang was particularly evident in the five (8 % of total strikes) unilateral strikes we observed. In these strikes, only the left fang penetrated the target during the initial strike, and the exposed right fang was visible on the video recording. Penetration of the contralateral fang was associated with venom flow through the right venom duct (Fig. 3); however, no venom could be seen leaving the exit orifice or fang sheath of the right fang. In each of these strikes, the snake pivoted such that the formerly free right fang penetrated the prey, resulting in a second pulse of venom being recorded from the venom duct (Fig. 3).

Strikes in which fang penetration was not accompanied by venom expulsion were also recorded. Four conservative criteria for the identification of these strikes were adopted: (i)

Table 1. *Quantitative comparison of venom expulsion by target size and behavioral context*

	Defensive, rat	*t*	*P*	Defensive, mouse	*t*	*P*	Predatory, mouse
Duration of fang penetration (ms)	233.4±24.7	1.57	0.14	305.9±39.1	0.14	0.89	322±111.8
Duration of venom flow (ms)	205.8±25.0	0.63	0.54	228.5±25.8	3.82	0.001	113.2±15.6
Time from penetration to venom flow (ms)	4.8±2.7	1.62	0.13	22.7±10.7	0.14	0.89	25.3±15.9
Mean venom flow rate (µl s^{-1})	529.4±149	0.34	0.73	625.5±225	2.17	0.057	133.6±28.0
Maximum venom flow rate (µl s^{-1})	1161.5±262	0.71	0.49	1582.6±534	2.61	0.028	185.3±42.1
Total venom volume rate (µl s^{-1})	130.7±54.1	0.14	0.93	137.4±47.1	2.69	0.025	10.4±1.8
Venom mass (mg)	26.1±10.8			27.5±9.4			2.1±0.4

Values are means ± S.D.

Data are from defensive strikes at rats and mice (10 strikes each) and predatory strikes at mice (six strikes).

Results of *t*-tests are given between the two comparison groups. Note that there were no significant differences in venom expulsion between defensive strikes directed at rats and mice, but three significant differences between strikes directed at equivalent-sized mice under defensive and predatory behavioral contexts.

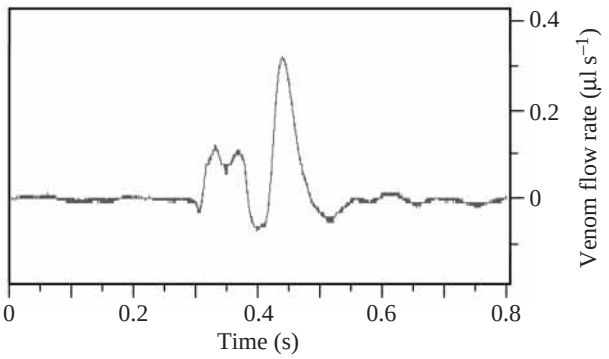

Fig. 3. Venom flow profile from a unilateral strike. In the initial strike, only the contralateral fang penetrated the target, which corresponded to the initial broad pulse of venom flow. Subsequently, the snake pivoted so that its ipsilateral fang could penetrate, which resulted in the second, narrower pulse of venom flow. During the initial pulse of venom flow, no venom was ejected from the free fang tip.

a 'typical' strike with venom injection had to be observed both before and after the strike without venom expulsion; (ii) a test and calibration of the flow probe were performed before each strike (these are internal functions of the T106 flowmeter), and only strikes with normal calibrations and 'good' test results were considered; (iii) the fang penetration had to be clear on the kinematic recording and the kinematic aspects of the strike had to agree with results of the previous study; and (iv) no discernible venom expulsion could be evident in the venom flow profile (Fig. 4). With these conservative criteria in place, approximately 35 % of the strikes recorded lacked venom expulsion.

Differential venom flow

The three behavioral contexts used in this study (defensive strikes at large targets, defensive strikes at small targets and predatory strikes) resulted in similar distributions of 'typical' and 'atypical' strikes. Our experimental design was compromised by a tendency for the flow probe cable to disrupt the predatory strikes. The necessity of hard-wiring the snake to the flowmeter frequently resulted in a cessation of predatory activity; the snake maintained its defensive behavior, unplugged itself from the cable by strenuous activity or simply failed to strike at the predatory target when it was presented. Omitting the strikes that lacked venom flow and those marked by multiple pulses of venom, 26 typical strikes were compared (10 defensive strikes at rats, 10 defensive strikes at mice and six predatory strikes).

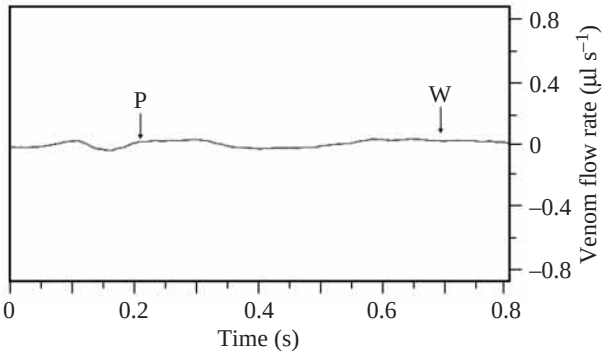

Fig. 4. Venom flow profile from a strike in which the fangs penetrated the target but no venom was ejected. A small retrograde pulse of venom flow was observed in the terminal portions of fang erection, but no other venom flow was recorded. P, fang penetration; W, fang withdrawal.

The two defensive targets, live mice (mean mass 25.6 g) and live rats (mean mass 173.6 g), were presented to the snake in a similar fashion only after the snakes exhibited a sustained suite of behavioral features. The three temporal variables quantified (duration of fang penetration, duration of venom flow and time from fang penetration to the onset of venom flow) were all reduced in strikes directed at rats, but none of these differences was significant (Table 1). Similarly, the three venom variables quantified (mean and maximum flow rate and total volume) were all reduced in strikes directed at the larger target (Table 1). As with the temporal variables, none of these differences proved significant using a cut-off of $P<0.05$. Linear regression was performed on target mass against venom volume, and the resulting coefficient (–0.345) was not significantly different from zero (t=0.767, P=0.45, N=20, r^2=0.032). Three outliers were identified, two from strikes at mice and one from a rat strike, but eliminating these points produced a similar (non-significant) regression equation.

Similar-sized targets were presented to the snakes within a defensive (target mean mass 25.6 g) and predatory (target mean mass 24.6 g) context, with context judged by a suite of behavioral and kinematic features. Two of the temporal variables (duration of fang penetration and time from fang penetration to the onset of venom flow) were greater in predatory strikes (Table 1); however, these differences were not significant, perhaps reflecting the substantial variation measured in the predatory strikes. The third temporal variable, duration of venom flow, differed by a factor of nearly 2 with relatively low variation; the predatory strikes had significantly shorter durations of venom flow (Table 1). The three quantitative features of venom flow were all reduced in predatory strikes (Table 1); the mean venom flow rate and venom volume were both significantly reduced in predatory strikes.

Discussion

The methodology employed in this study provides the first direct record of venom flow from a conscious unrestrained snake and is the only analysis available that combines venom flow data with high-speed kinematics. Previous functional analyses of the venom-delivery system (performed on anesthetized rattlesnakes) suggested that venom delivery could be regulated by fine control over the extrinsic venom gland musculature (Young et al., 2000) and that there were passive barriers to venom flow within the fang sheath that were influenced both by fang erection and by the displacement of

the fang sheath during fang penetration (Young et al., 2001a). The results of the present study indicate that venom expulsion is more complex than was previously appreciated.

Mechanics of venom expulsion

The first evidence of venom flow was a pulse of retrograde flow (suction) that occurred during the later portions of fang erection (Fig. 1); this retrograde flow was present in only 57 % (15 out of 26) of the typical strikes, and was always of small magnitude (mean volume 9.35 µl). We hypothesize that this retrograde venom flow is produced through straightening and elongation of the normally tortuous primary venom duct (Mitchell, 1860; Young et al., 2001a) combined with a reduction in the size of the venom chamber (a space located between the end of the venom duct and the entrance orifice of the fang, surrounded and defined by the epithelium and connective tissue of the fang sheath) brought about by fang erection (Young et al., 2001a). The presence of retrograde flow in the venom-delivery system, prior to the main expulsion of venom, may have important implications for the accessory venom gland. This gland is not surrounded by skeletal muscle and appears to contain differing amounts of myoepithelial cells (Kochva and Gans, 1965; Kochva, 1975); early retrograde venom flow could function as a means of mixing the glandular secretions of the accessory and main venom glands (Gans and Elliot, 1968).

The termination of this early retrograde flow marked the onset of venom expulsion and was typically coincidental with fang penetration (Fig. 1). The temporal relationship between fang penetration and venom flow could be caused by the displacement of passive barriers within the fang sheath (Young et al., 2001a). Reflex actions involving sense organs on the fang sheath and/or oral epithelium and the extrinsic venom gland musculature may also play a role, although reflexes alone are too slow to explain the coincidental timing. The variable rate and duration of venom expulsion recorded (see below) presumably reflect a complex interaction between differential activation of portions of the extrinsic venom gland musculature, variation in venom volume within the venom gland and differential resistance to venom flow within the distal venom-delivery system and the target tissue.

The termination of venom expulsion was typically (96 % or 25 out of 26 of the typical strikes) associated with a distinct episode of retrograde venom flow (Fig. 1) which was over twice the volume (mean 21.3 µl) of the initial retrograde pulse. This suction of venom is presumably caused by a recoiling of the venom gland following contractile termination of the extrinsic venom gland musculature. Since this venom suction occurs prior to fang withdrawal, the suction pressure could draw fluid from the accessory venom gland, from the venom chamber or even from the tissue of the target. Such post-expulsion suction may function to keep excess venom out of the venom canal of the fang, where it could crystallize and impede venom injection. The smaller secondary episode of venom expulsion occasionally observed during fang withdrawal is presumably caused by the increased skeletal displacement and muscular activity required to extract the fangs from the target (Cundall, 2001).

The pattern of venom flow recorded during several strikes suggests that there is bilateral venom flow with each fang penetration. This was most evident in strikes in which the fangs were not implanted synchronously or in which the fangs were repositioned after the initial penetration of the target. Venom flow was recorded irrespective of whether the fang ipsilateral or contralateral to the flow probe penetrated the prey (Fig. 2), suggesting that the activity of the extrinsic venom gland muscles is normally synchronized during venom injection. Synchronized activity within the compressor glandulae has been described in the few previous EMG studies of striking in viperids (Kardong et al., 1986).

The significance of fang penetration without venom injection (a phenomenon commonly referred to as a ‘dry bite’) has long been recognized (e.g. Warrell, 1996); however, understanding the incidence and functional basis of this phenomenon has been impeded by the multiple criteria used to determine the absence of venom injection.

The synchronized recording of high-speed kinematics and direct measurement of venom flow presented in this study provides the first direct evidence of fang penetration without venom injection (Fig. 4). Given the conservative recognition criteria employed, this study also provides strong evidence that the lack of venom expulsion results from a control of venom injection rather than from kinematic variation or methodological ambiguity. Previous estimates of the relative incidence of strikes without venom expulsion vary considerably (e.g. Russell, 1980; Reid and Theakston, 1983; Tibbals, 1992); the value of 35 % determined in the present study is slightly lower than was reported in most previous studies.

Five unilateral strikes, in which the fang ipsilateral to the flow probe failed, at least initially, to penetrate the target (Fig. 4), were recorded during this study. In each of these strikes, venom flowed through the primary venom duct, although no venom discharge was observed from the fang tip, as was clearly evident on the video recording. Venom flow through the primary venom duct without discharge from the fang indicates that the venom can pool in the distal portion of the venom-delivery system, presumably in the venom chamber (Young et al., 2001a), a conclusion also supported by the presence of retrograde flow in the initial part of the flow profile. There is no experimental evidence to suggest that venom flow out of the venom chamber is constant; rather, the morphology of the region suggests that venom flow would be determined by a combination of venom fluid pressure, flow resistance from the prey tissue, venom pressure within the venom chamber and the relative displacement of the passive barriers to venom flow associated with the venom chamber.

The mechanics of venom flow revealed in the present study appears to be consistent with the prevailing intraglandular pressure hypothesis for venom flow (Rosenberg, 1967), which was also supported by an earlier functional analyses (Young et al., 2000). The venom flow profiles recorded in this study

suggest that the complexity of venom flow cannot be explained solely by differential pressures on the venom gland. The present study supports the earlier hypothesis (Young et al., 2001a) that the fang sheath and enclosed venom chamber play a key role in influencing venom expulsion.

Differential venom flow

Previous studies of the behavioral ecology of snake venom injection have quantified, using a variety of techniques, the amount of venom injected into biological and non-biological targets (for a review, see Hayes et al., 2001). Driving most of these studies is the hypothesis of 'venom metering', which argues that snakes will regulate the amount of venom injected depending on prey size and/or the behavioral context of the strike. The results of these studies do not offer a clear picture of venom metering: Hayes et al. (1995) report venom metering from *Crotalus v. oreganus* when presented with mice differing by approximately 20 g in mass; Genarro [reported in Hayes et al. (1995)] found no difference in the amount of venom expelled by *Agkistrodon piscivorus* when striking rodents that differed in mass by 70 g, but did find differential venom output when the targets differed by over 250 g; Allon and Kochva (1974) found no difference in venom expenditure when *Vipera palestinae* struck at rodents that differed in mass by approximately 175 g. Some of this discrepancy may be because both total venom mass and venom mass per gram of target have been used as the basis of comparisons (Hayes et al., 2001), resulting in the unfortunate situation that virtually any result can be interpreted as venom metering under one of the two definitions. Similar complications arise from the use of non-biological and biological targets and from variation in target presentation.

Our direct measurements of venom flow in *Crotalus atrox* during defensive encounters with targets varying by approximately 150 g revealed no significant difference in the temporal or volumetric characteristics of the venom profile (Table 1). Although these data are suggestive of a trend towards lower venom expulsion (in terms of both time and volume) into the larger targets, this experiment yielded no evidence of venom metering. Previous workers (e.g. Rowe and Owings, 1990; Hayes, 1992) have suggested that aspects of the kinematics of the strike, particularly strike duration, could influence venom metering. The 2 ms temporal resolution of the present study revealed that the duration of venom flow was consistently less than, and poorly correlated with, the duration of fang penetration (Fig. 1) (Table 1), which suggests that, for all but aberrantly short strikes, the duration of fang penetration does not influence venom flow.

The present study demonstrates that venom injection by *Crotalus atrox* is influenced by behavioral context; when the same target was presented in a predatory and defensive context, significant differences were recorded in both temporal and volumetric features of venom injection (Table 1). Although previous studies had compared venom expenditure during predatory and defensive strikes (Hayes et al., 2001), they used targets of different size and often combined biological and non-biological targets. Although many authors have argued that snakes should inject more venom during predatory than during defensive strikes, the few previous studies agree with the present results in showing a far greater amount of venom expulsion during defensive strikes (for a review, see Hayes et al., 2001).

To minimize the potential influences of surgical implantation and hard-wiring the snakes, we recorded venom flow rates and volumes from the right primary venom duct only. The right and left venom-delivery systems of rattlesnakes can function independently, and observations suggest that both the temporal patterns and venom quantities may vary between the contralateral venom-delivery systems (B. A. Young). As such, the incidence of 'dry bites' we recorded from the right venom duct (35 %) may be higher than the actual incidence of dry bites experienced by the targets. The potential independence of the left venom-delivery system would not influence the venom mechanics or differential venom flow detailed here unless the rattlesnakes preferentially regulate the left venom-delivery system to counterbalance the right. It is difficult to see the functional utility of such a system, and there is no experimental evidence to support this type of preferential regulation.

Although the calculation of dry mass from venom volume is a source of potential error, the final values for venom mass presented here are in general agreement with values in the literature. All the mice envenomated during the predatory encounters exhibited classic symptoms of snake bite, including partial paralysis and seizures, and died shortly after being struck. The mean venom mass recorded from a single fang during the predatory encounters was 2.1 mg (Table 1). Since the LD_{50} for *Crotalus atrox* is generally given as just over 2.0 mg kg^{-1} (Russell, 1980), even without an equivalent contribution from the contralateral fang, these snakes were injecting enough venom to kill the prey. The amount of venom recorded during defensive bites in the present study is similar in range to that reported by Herbert [data from Hayes et al. (2001)] in bites directed at model limbs. Furthermore, the volumes we report are lower than those reported by Klauber (1972) from milking *C. atrox* and much lower than the dry mass yields given by Russell (1980).

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