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Buccal Expansion during Hissing in the Puff Adder, *Bitis arietans*

BRUCE YOUNG, JADE JAGGERS, NANCY NEJMAN, AND NATHAN J. KLEY

Videoradiographic analyses were made of hissing in puff adders (*Bitis arietans*). During the hiss the larynx remains relatively stationary immediately adjacent to the internal nares. The exhalatory portion of the hiss is characterized by a distinct expansion of the caudal buccal cavity, including a depression of the hyoid and divergence of the cornua. This buccal expansion is hypothesized to be an epiphenomenon of intraoral pressure generated by the exhalatory airstream of the hiss.

EXPANSION of the body, particularly the anterior portion, is a common feature of the defensive repertoire of snakes (for reviews see Mertens, 1946; Carpenter and Ferguson, 1977; Greene, 1988). These localized body expansions may be horizontal (such as ''hooding'' in *Naja*; Young and Kardong, 1989) or vertical (such as throat expansion in *Ptyas mucosus*; Young et al., 1999a); the plane of expansion generally correlates with the main visual axis of the potential predator (Greene, 1979; Senter, 1999). Although many snakes use respiratory mechanisms for generalized body expansion (e.g., Kinney et al., 1998; Young et al., 1999b), the specialized defensive displays appear to result from differential contributions of the cranial ribs and the anterior portion of the respiratory tract (Young et al., 2000).

The specializations of the anterior respiratory tract, which produce defensive visual displays, may also result in unusual acoustic properties (Young, 1991b; Young et al., 1999a). Most defensive sounds produced by snakes have a simple acoustic structure characterized by very low levels of frequency and amplitude modulation and little or no temporal patterning (Young, 1997, 1998b). Many snakes such as *Heterodon platyrhinos* (Young and Lalor, 1998) and *Daboia russelii* (Young, 1998a) are obligate nasal hissers in which the exhalent airstream is always passed through the internal nares. Experimental analyses of hissing in another obligate nasal hisser, the puff adder (*Bitis arietans*), revealed that the larynx plays a passive role during sound production, remaining patent throughout the sound-producing portions of the hissing cycle (Young et al., 1999b).

The larynx of snakes is structurally simple (Kardong, 1972a,b; Young, 2000) and with the exception of *Pituophis melanoleucus* (Young et al., 1995) shows few anatomical specializations. Changes in the relative position of the larynx within the oral cavity during the hiss and/or changes in the dimensions of the buccal cavity itself could result in a resonance effect and a visible distension of the buccal cavity similar to what has been described as a defensive display in *Psammophis* (Werner, 1985). Recent studies using cineradiography and videoradiography on squamates have provided documentation of intrabuccal processes relating to chemosensory searching (Young, 1990, 1991a), defensive displays (Bels et al., 1995), prey transport and swallowing (Janoo and Gasc, 1992; Cundall, 1995; Kley and Brainerd, 1996), drinking (Bels and Kardong, 1995), and ventilation (Owerkowicz et al., 1999). In this study, we used videoradiography to study intrabuccal processes associated with defensive displays in the puff adder, *B. arietans*.

MATERIALS AND METHODS

The snakes used for this study were long-term captives maintained in the venomous snake room at Lafayette College at a temperature of 29–32 C, with a 12:12 L:D photoperiod, and a diet of prekilled mice. All animal maintenance and experimental procedures comply with existing guidelines for both live reptiles and venomous snakes. We recorded x-ray video data from five adult puff adders (*B. arietans*) with snout–vent lengths (SVLs) ranging from 74.5–128 cm.

The specimens were individually anesthetized by exposure to ice until the cessation of independent movement. Once immobile, the specimen was placed on a bed of ice and the mouth held open with a surgical retractor. A soft applicator stick was used to repeatedly smear powdered barium sulfate ($BaSO_4$) onto the epithelial lining of the larynx. Additional barium sulfate was placed into the caudal portion of one of the nasal passageways. Any stray barium sulfate was rinsed and wiped from the oral cavity prior to returning the specimen to normal temperature for recovery from the anesthesia. One day after the application of the barium marker to the larynx, the water bowl was removed from

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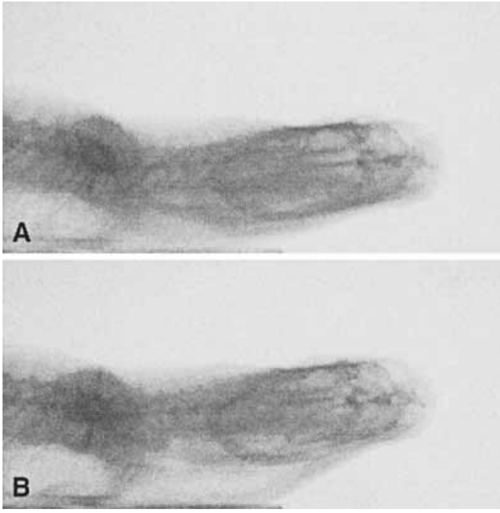

Fig. 1. Lateral images of a 128 cm SVL *Bitis arietans* during the onset (A) and termination (B) of the exhalatory phase of the defensive hiss. Approximately two seconds separates these two images. Note the distinct expansion of the buccal floor in (B).

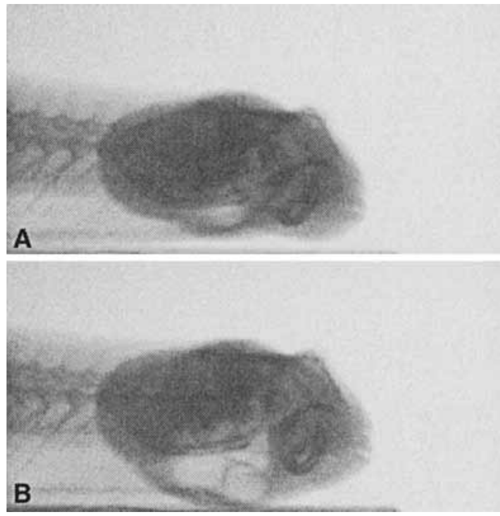

Fig. 2. Frontal images of a 128 cm SVL *Bitis arietans* during the onset (A) and during (B) the exhalatory phase of the defensive hiss. Note the expansion of the buccal floor, and the depression and divergence of the hyoid (arrow).

the snake's cage, and no further water was provided for one week prior to data acquisition.

The snakes were transported to the radiographic facility at the Museum of Comparative Zoology, Harvard University for videoradiography. During transport and data collection, the snakes were kept at temperatures from 25–30 C. During data collection, the specimens were placed individually into 52.5 × 40 × 22.5 cm plastic containers with secure lids. One end of the plastic container opened into a detachable filming chamber. Two filming chambers were made to accommodate animals of different sizes; both were 30 cm long and 11 cm high, but one was 5 cm wide and the other 10 cm wide. Both filming chambers were made of Plexiglas®, but each had a partial wooden floor. Defensive behaviors were evoked either by motion of one of the researchers or through direct stimulation of the caudal end of the animal. Many of the specimens were subsequently removed and placed in appropriately sized clear Plexiglas® tubes for additional data collection. Following videoradiography, each snake was placed in a tank of warm water to ensure hydration and was subsequently given water ad libitum.

Videofluorscopy was performed using a Siemens radiographic unit equipped with a Sirecon image intensifier. X-ray videos were recorded at 30 fps on a Sony DCR VX1000 digital video camera. The x-ray source and image intensifier were aligned in a lateral position, and the distal portion of the filming chamber or Plexiglas® tube was positioned against the image intensifier. Although most of the videographic data were recorded in lateral projection, the filming chambers were large enough that the snake could fully turn its head, affording us a frontal perspective. Video sequences were downloaded directly from the Sony digital camera to a PowerMac G3 computer via a Radius MotoDV Firewire card.

Results

Multiple hisses were obtained from four of the *B. arietans*; a total of 48 hisses were examined. During each hiss, the larynx appeared to remain stationary in a protracted position near the level of the internal nares. Although our marker system did not allow us to quantify the contact between the larynx and the internal nares, there was no evidence for intrabuccal movement of the larynx during sound production. During the terminal portion of each exhalatory hiss, there was a marked depression of the caudal portion of the buccal floor (Fig. 1). In the larger specimens, this buccal expansion lasted approximately two seconds. This buccal expansion was extensive enough that the hyoid extended ventral to the lower jaws and the cornua diverged laterally (Fig. 2). Although not quantified, the extent of buccal expansion appeared to be related to the volume of air exhaled with each hiss and, thus, probably with air pressure as well. Although some buccal expansion was observed in each hissing *B. arietans*, it was most pronounced in the largest specimen.

Discussion

The combined use of videoradiography and radiopaque barium markers permitted clear visualization of the larynx and other features of the buccal cavity, thereby documenting the relative spatial stability of the larynx during sound production in *B. arietans*. This technique also provided clear evidence of buccal expansion associated with sound production (Figs. 1–2). This expansion may represent an unusual form of buccal pump in which exhalatory air is pulled into the buccal cavity then redirected either externally or back into the lung. Although buccal pumps are known in reptiles (Owerkowicz et al., 1999) and other terrestrial vertebrates, they normally function to pump in fresh inhalatory air not to expel exhalatory air (Brainerd, 1994). Although we consider it unlikely that this is a buccal pump, further experimental data are necessary to test this hypothesis.

Rather than an active buccal pump, this buccal expansion may represent the passive result of increased air pressure in the buccal cavity. Puff adders move considerable volumes of air during defensive hisses, particularly during the exhalatory phase (Young et al., 1999b). If the larynx does not form an airtight seal with the internal nares, the resistance created by the narrow diameter of the nasal passageways (relative to the larynx) could force some of the exhalent airstream into the oral cavity. Because *B. arietans* keep the mouth closed during hissing, this pressurized air could cause expansion of the caudal portion of the oral cavity, which is distended similarly during prey transport. This hypothesis would explain why the buccal expansion is seen only during exhalation and why the extent of buccal expansion appeared correlated with the volume of exhaled air associated with each defensive hiss. Furthermore, the duration of the observed buccal expansions (approximately 2 sec) is in close agreement with the duration of the exhalatory phase of the hiss of *B. arietans* (mean 1.54 sec, range = 1.2–2.2 sec; Young et al., 1999b). A similar pattern of buccal expansion has been described in *Psammophis aegyptius* (Werner, 1985).

The use of exhalatory air pressure for body expansion, such as we hypothesize, occurs in the buccal region of *B. arietans*, is rather common in snakes (Noble, 1921; Carpenter and Ferguson, 1977; Greene, 1988), and may be active as well as passive (Young et al., 2000). The buccal expansion in *B. arietans* is functionally isolated from the respiratory-based expansion of the puff adder's body, which also occurs during defensive displays. This general body expansion is produced through movements of the ribs (Young et al., 1999b), and deflation of the body is synchronous with expansion of the buccal region. Viewed without the assistance of videoradiography, the buccal expansion of *B. arietans* is not dramatic; indeed, it does not appear to have been previously described. In part, this may be explained by the defensive posture adopted by *B. arietans* in which the snout is angled down toward the ground, completely obscuring the buccal region.

As a defensive display, this buccal expansion in *B. arietans* is quite unusual. Body expansion is generally interpreted as a way of making the animal appear more intimidating by increasing the visible surface of the snake (Greene, 1979; Senter, 1999). Terrestrial snakes that increase the dimensions of the head during defensive displays typically do so in the horizontal plane (e.g., Young et al., 1999c). Given the modest external evidence of buccal expansion, and the defensive posture that obscures the buccal region, we consider it more likely that the buccal expansion in *B. arietans* is an epiphenomenon of the dramatic hisses produced by this species rather than a true defensive behavior in which the body is inflated for intimidation.

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(BAY, JJ, NN) DEPARTMENT OF BIOLOGY, LAFAYETTE COLLEGE, EASTON, PENNSYLVANIA 18042; AND (NJK) ORGANISMIC AND EVOLUTIONARY BIOLOGY PROGRAM, UNIVERSITY OF MASSACHUSETTS, AMHERST, MASSACHUSETTS 01003. E-mail: (BAY) youngab@lafayette.edu. Send reprint requests to BAY. Submitted: 20 March 2000. Accepted: 24 July 2000. Section editor: R. E. Gatten Jr.