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COMPARATIVE STUDY OF TONGUE PROTRUSION IN THREE IGUANIAN LIZARDS, *SCELOPORUS UNDULATUS*, *PSEUDOTRAPELUS SINAITUS* AND *CHAMAELEO JACKSONII*

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Summary

The goal of this study was to investigate the function of the hyolingual muscles used during tongue protraction in iguanian lizards. High-speed videography and nerve-transection techniques were used to study prey capture in the iguanid *Sceloporus undulatus*, the agamid *Pseudoptrapelus sinaitus* and the chameleonid *Chamaeleo jacksonii*. Denervation of the mandibulohyoideus muscle slips had an effect only on *P. sinaitus* and *C. jacksonii*, in which tongue protrusion or projection distance was reduced. In *C. jacksonii*, denervation of the M. mandibulohyoideus completely prevented little hyoid protraction. Denervation of the M. verticalis had no effect on *S. undulatus*, but reduced tongue protrusion distance in *P. sinaitus*. Denervation of the accelerator muscle in *C. jacksonii* inhibited tongue projection completely. The function of the M. mandibulohyoideus and M. verticalis has become increasingly specialized in *P. sinaitus* and especially in *C. jacksonii* to allow greater tongue protrusion. The combined results of these treatments suggest that these three groups represent transitional forms, both morphologically and functionally, in the development of a projectile tongue.

Key words: reptile, lizard, Iguanidae, Agamidae, Chamaeleonidae, *Sceloporus undulatus*, *Pseudotrapelus sinaitus*, *Chamaeleo jacksonii*, tongue protrusion, prey capture, kinematics, nerve transection.

Introduction

The form of the tongue has long been considered an important character in squamate classification (Camp, 1923; Schwenk, 1988). Early studies noted the similarities in internal tongue form that seemed to separate iguanian lizards (Agamidae, Chamaeleonidae and Iguanidae) from all other lizard families (Scleroglossa). Whereas all iguanian lizards have a large fleshy tongue, the trend in other lizards is towards an increasingly narrow and bifid tongue. The use of the tongue as a prehensile organ for capturing prey is generally restricted to iguanian lizards (Schwenk and Throckmorton, 1989). Although there are a few exceptions (Gans et al., 1985; Urbani and Bels, 1995; Smith et al., 1999), all other lizards use the jaws to capture prey, and the tongue has become specialized for chemoreception.

Although all iguanians use the tongue to capture prey, there are striking differences in the distance the tongue is protruded or projected in the different groups of iguanians. Tongue protrusion occurs in all groups and is defined as the distance the tongue moves beyond the mandible tip. Tongue projection occurs only in chamaeleonid lizards and refers to the ballistic movement of the tongue as it is propelled off the entoglossal process of the hyobranchium. In most iguanids, the tongue can be protruded out of the mouth by only 30 % of mandible length, whereas some members of the Agamidae can protrude the whole tongue out of the mouth, often by more than 50 % of the mandible length (Schwenk and Throckmorton, 1989; J. J. Meyers, personal observations). In chameleons, the sister taxon to the Agamidae (Estes et al., 1988; Schwenk, 1988; Lee, 1998), the tongue is protruded out of the mouth on the entoglossal process and then projected off the entoglossal process to a distance greater than the snout–vent length (Houston, 1828; Zoond, 1933; Wainwright et al., 1991).

Prey capture in chameleons is uniquely different from that in other iguanian lizards. Unlike other iguanians, chameleons project the body of the tongue off the entoglossal process at the prey item. This is made possible through modifications of the tongue musculature and the hyolingual apparatus. Although several studies have begun to address the diversity in intrinsic tongue form and function within the Iguania (Smith, 1984; Schwenk, 1988; Bell, 1989; Wainwright and Bennett, 1992a,b; Delheusy et al., 1994; Herrel et al., 1995), it was not until recently that hypotheses of the evolution of this unique mechanism were proposed (Smith, 1988; Schwenk and Bell, 1988; Schwenk and Throckmorton, 1989).

Smith (1984) suggested three possible mechanisms of tongue protrusion in lizards on the basis of anatomy, cine-radiographic and electromyographic studies: (i) the tongue may be drawn anteriorly out of the mouth by the Mm. genioglossus; (ii) the tongue may undergo hydrostatic elongation, which requires a decrease in its height, width or diameter that causes a corresponding increase in tongue length; (iii) contraction of the M. verticalis surrounding the entoglossal process may exert a centrally directed force on the entoglossal process and cause the tongue to slide forward. In addition to these mechanisms, Schwenk and Throckmorton (1989) proposed that the M. mandibulohyoideus (=M. geniohyoideus) also plays an important role in protruding the tongue.

The first mechanism of tongue protrusion requires the activation of the genioglossus muscles. In most lizards, the M. genioglossus is composed of two large bands of muscles, the M. genioglossus medialis and lateralis, which originate near the mandibular symphysis and insert on the M. hyoglossus (Secoy, 1971; Smith, 1984). Activity of the M. genioglossus medialis during forward movement of the tongue was noted by Smith (1984) in the iguanid *Ctenosaura similis* and in the agamid *Agama stellio* (Herrel et al., 1995). In chameleons, the M. genioglossus is reduced (Bell, 1989), and anterior tongue movement is thought to result from a combination of the three mechanisms described below.

Hydrostatic elongation could play a role in tongue protrusion, but this depends on the action of the intrinsic muscles and the structure of the surrounding musculature and connective tissues. If the intrinsic muscles work to flatten and/or decrease the diameter of the tongue, and the other tissues restrict lateral expansion, the tongue will increase in length (Kier and Smith, 1985). Hydrostatic mechanisms have been proposed for some scleroglossans (Smith, 1984, 1986); however, there is no experimental evidence that this mechanism is used by any iguanian lizards.

The third mechanism suggested by Smith (1984) requires action of the intrinsic muscle on the entoglossal process of the hyobranchium. The importance of this mechanism in iguanids is probably minimal because the M. verticalis borders the entoglossal process, instead of surrounding it as in chameleons (Smith, 1984). However, in most agamids, the M. verticalis forms a ring of fibers that surrounds the entoglossal process (Smith, 1988). No functional data exist on the M. verticalis in iguanids, but Herrel et al. (1995) found that the M. verticalis is active during tongue protrusion in *Agama stellio*. This muscle is most developed in chameleons, where it forms a dense ring of radially arranged fibers surrounding the entoglossal process (van Leeuwen, 1997). In both agamids and chamaeleonids, the structure of the entoglossal process is important in determining the extent of muscle sliding. The agamid entoglossal process is highly tapered (Smith, 1988), whereas in chameleons only the tip of the process is tapered, putatively allowing the contracting muscle to build up force before tongue projection (Wainwright and Bennett, 1992b). Several studies have addressed the function of the accelerator muscle in chameleons. Accelerator muscle activity was measured during tongue protrusion and projection in *Chamaeleo jacksonii* (Wainwright and Bennett, 1992a), and Zoond (1933) found that denervation of the M. accelerator prevented tongue projection completely.

The last muscle that may be used in tongue protrusion is the M. mandibulohyoideus complex (Schwenk and Throckmorton, 1989). There are three distinct slips of the mandibulohyoideus (MHI, II, III) in iguanians. In iguanids and agamids, these muscles originate medially (MHII) and laterally (MHI, III) on the mandible and then run posteriorly to insert on the ceratobranchials (Secoy, 1971; Tanner and Avery, 1982; Smith, 1984, 1988). In chameleons, both these muscles originate near the mandibular symphysis and run posteriorly to insert onto the basihyoid and ceratobranchials (Bell, 1989). Cine-radiography of markers implanted on the hyobranchium and in the tongue musculature in *Ctenosaura similis* shows anterior movement of the hyobranchium during transport, but data were not presented for tongue protrusion (Smith, 1984). Herrel et al. (1995, 1997) demonstrated activity of the mandibulohyoideus I and II during capture and processing cycles in *Agama stellio*. Given their position, these muscles are likely to move the hyobranchium forward in the mouth during tongue protrusion in iguanids and agamids. Wainwright and Bennett (1992a) documented the activity of the M. mandibulohyoideus during protraction of the hyobranchium and projection of the tongue in *Chamaeleo jacksonii*. By cutting the M. mandibulohyoideus in *Lophosaura pumila* (now *Bradypodion pumilum*), Zoond (1933) demonstrated that the hyobranchium was no longer protruded out of the mouth and that tongue projection was reduced.

Although Zoond (1933) demonstrated the role of the M. mandibulohyoideus, he was not able to discern interactions between this muscle and the hyobranchium. However, using high-speed videography, Wainwright et al. (1991) described fast hyobranchial movement that occurs simultaneously with tongue projection. Using nerve-transection techniques, we were able look at the role of the M. mandibulohyoideus and the hyobranchium during tongue projection.

The goals of the present study were to test the hypotheses of tongue protrusion put forth by Smith (1984) and Schwenk and Throckmorton (1989). Using nerve-transection experiments and high-speed videography, we will assess the relative importance of specific hyolingual and intrinsic muscles used during tongue protrusion and projection. The results suggest that the relative importance of each of these muscle groups differs for each species. The M. accelerator and M. mandibulhyoideus become increasingly important in *Pseudotrapelus sinaitus* and *Chamaeleo jacksonii*. For *C. jacksonii*, these muscles are critical for normal hyobranchium protrusion and tongue projection.

Materials and methods

The *Sceloporus undulatus* (Latreille, 1801) (snout–vent length 5.3–6.3 cm) in this study were collected in Coconino Co., Arizona, USA, between 1996 and 1998 (Arizona Game and Fish permit no. SP839083). *Pseudotrapelus sinaitus* (Heyden, 1827) and *Chamaeleo jacksonii* (Boulenger, 1896) were purchased from commercial animal dealers. These animals were of a similar size to the *S. undulatus*: the *P. sinaitus* ranged from 6.7 to 8.6 cm snout–vent length and the *C. jacksonii* from 6.9 to 8.0 cm snout–vent length. The *S. undulatus* and the *P. sinaitus* were housed in groups of 8–10 in 400 l terraria and kept on a 12 h:12 h L:D photoperiod. Lighting was provided by Vita-lite full-spectrum lights, and the terraria were heated using 50 W bulbs. The *Chamaeleo jacksonii* were provided with the same light. However, they were housed in groups of six in 12 cm×50 cm×38 cm screened cages with small *Ficus benjamina* for cover. Initially, lizards were fed crickets and waxworms in their cages. Once the experiments began, the animals were fed only while being filmed.

Anatomy

At least two preserved individuals of each species were dissected to examine the arrangement of the tongue and hyobranchial muscles. In addition to gross dissection, histological sections of the lower jaw and tongue were made of each species in three different planes (transverse, sagittal and frontal). Specimens were embedded in paraffin, serially sectioned at 10 µm and stained using Milligan's Trichrome stain (Humason, 1979). Another two individuals of each species were cleared using trypsin, and their nerves were stained with Sudan Black B (Nishikawa, 1987) to visualize the arrangement of nerves innervating the tongue and hyobranchial muscles. To determine innervation points for each muscle, preserved specimens were dissected and compared with the innervation patterns of the cleared and stained specimens. Drawings of the muscles and nerves were made *via* a *camera lucida* on a Zeiss dissecting microscope. Basic descriptions of muscle and neuroanatomy are given in this paper, but a more detailed account is in preparation.

Kinematic analysis

Prey capture was quantified using a Display Integration Technologies model DIT 660 high-speed, multi-frame video camera. The feeding sequences were filmed at 120 fields s^{-1} at room temperature (23–26 °C). The *Sceloporus undulatus* and *Pseudotrapelus sinaitus* were filmed in a 38 cm×6.5 cm×13 cm acrylic container, and the crickets were presented to the animal through a hole in the floor of the container. For *Chamaeleo jacksonii*, a wooden dowel was provided as a perch, and prey were presented at the same vertical height as the animal's head. A 1 cm grid background was used as a backdrop during all feeding bouts.

Feeding sequences were analyzed using Peak 2D motion-analysis software on an IBM-compatible computer. For each field of the feeding sequence, the *x,y* coordinates of a reference point, six points (seven for chameleons) on the animal and one on the prey item were digitized (Fig. 1). For each species and each treatment, three sequences were digitized for three individuals before and after nerve transection, giving a total of six digitized sequences per individual. Only lateral sequences were used for kinematic analysis and were defined as those in which the camera was arranged perpendicular to the lizard's body and in which the lizard did not turn or bend its head towards or away from the camera. Lateral sequences were most easily obtained if the long axis of the cricket was presented perpendicular to the body of the lizard. A description of the kinematic variables measured from the digitized sequences is given in Table 1.

Muscle denervation

Two different nerve-transection experiments were performed on each of the three species. The first treatment consisted of bilaterally transecting hypoglossal nerve branches innervating the M. mandibulohyoideus slips I, II and III. In the second treatment, the hypoglossal nerve branch innervating the muscle surrounding the entoglossal process of the hyobranchium was transected bilaterally (Fig. 2). This muscle has been referred to as the M. verticalis in *S. undulatus* and *P. sinaitus* and the M. accelerator in *C. jacksonii*. In this experiment, no sham surgery was performed to examine the effects of the surgical procedure. However, in anurans, sham surgery has revealed no effect of these procedures (Deban and Nishikawa, 1992; Ritter and Nishikawa, 1995). In addition, the two treatments performed here serve as controls for each other (Nishikawa and Roth, 1991).

Animals were anesthetized by flowing 5 % isoflurane

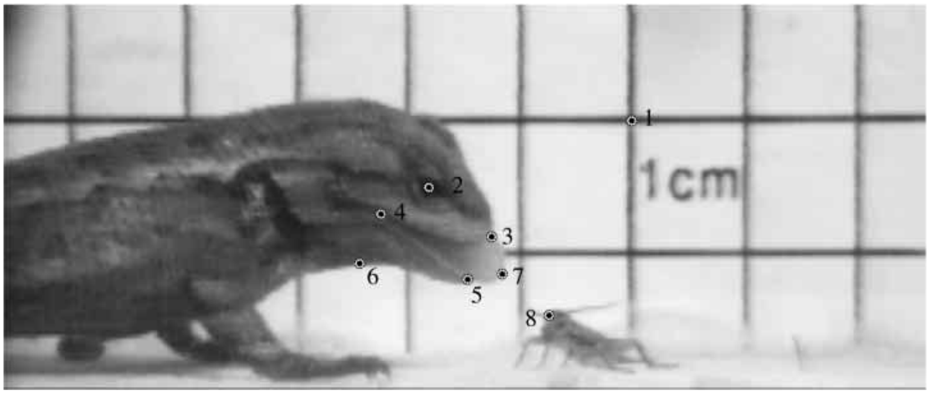

Fig. 1. Points used to calculate kinematic variables: 1, external reference; 2, middle of eye; 3, upper jaw tip; 4, jaw vertex; 5, lower jaw tip; 6, throat surface below jaw vertex; 7, tongue tip; 8, prey. In *Chamaeleo jacksonii*, there was also a point for the tip of the hyobranchium, which was placed on the tongue tip until the hyobranchium became visible after tongue projection.

Table 1. *Kinematic variables measured for all three species*

Variable	Measured as
Onset of mouth opening	Time at which the animal first opens the mouth
Onset of tongue protraction	Frame in which the tongue is first seen moving forward in the mouth
Onset of lunge	Time at which the animal begins to rotate over the forelimbs towards the prey item
Time of prey contact	Time of first contact of the tongue or jaw with the prey
Onset of tongue retraction	Time at which the tongue begins to move back into the mouth
Time of maximum gape	Time at which the mouth is opened widest
Time to completion tongue retraction	Time at which the tongue stops posterior movement in the mouth
Time to maximum horizontal displacement of the upper jaw tip	Time at which the upper jaw tip travels the farthest in the *x* coordinate
Onset of mouth closing	Time at which the distance between the upper and lower jaw begins to decrease, usually the first frame after maximum gape
Duration of approach	Time from the onset of mouth opening to the onset of the lunge
Duration of mouth opening	Time from the onset of mouth opening until the time of maximum gape
Duration of tongue protrusion	Time from the onset of tongue protrusion until the time of maximum protrusion (this is solely hyoid movement in chameleons)
Duration of tongue projection	Time from the onset of projection until the time of maximum tongue projection (this is variable is only relevant for chameleons)
Duration of lunge	Time from the onset of lunge until prey contact
Duration of tongue retraction	Time from the onset of tongue retraction until the time of completion of tongue retraction
Duration of mouth closing	Time from the onset of mouth closing until the time of completion of mouth closing
Duration of recovery	Time from maximum horizontal displacement of the upper jaw tip until the completion of mouth closing
Duration of feeding sequence	Time from onset of mouth opening until the jaw closes over the prey
Maximum gape angle	Angle between the upper jaw tip, jaw vertex and the lower jaw tip (points 3, 4 and 5, Fig. 1)
Head angle at onset of lunge	Angle calculated using a horizontal reference point, the jaw vertex and the upper jaw at the onset of lunge
Head angle at prey contact	Angle calculated using a horizontal reference point, the jaw vertex and the upper jaw at the time of prey contact
Gape distance	Distance between the upper and lower jaw tips (points 3 and 5, Fig. 1)
Distance to prey	Distance from the lower jaw tip to the prey at the beginning of the lunge for *Sceloporus undulatus* and *Pseudoptrapelus sinaitus*, and at the onset of tongue projection in *Chaemaeleo jacksonii*
Maximum tongue protrusion	Distance from the lower jaw tip to the anterior tip of the tongue

From digitized sequences we calculated the 18 timing events (ms), three angles (degrees) and three distance (cm) variables for each prey-capture sequence. All timing variables were calculated relative to the onset of mouth opening, which is set as time zero in this study.

(AErrane) through a 500 ml flask containing the animal. Once the animals had been sedated, they were placed on their backs on the stage of a Zeiss dissecting microscope. A small cone placed over the nose continued to deliver 5 % isoflurane at approximately 300 ml min^{-1}. When the anesthetic was removed, the animals came out of surgical anesthesia within a few minutes. To determine whether the animal was under surgical anesthesia, the forelimbs were squeezed with forceps. If no reaction was elicited, the animal was usually sufficiently anesthetized to perform the surgery. A small incision was made between the mandibles in the skin that covered the muscle and nerve of interest. The superficial musculature was teased apart parallel to the orientation of the fibers to expose the deep muscle layers. Once the nerve had been isolated close to the innervation point of the muscle, it was dissected away from the surrounding tissue, and a 1–2 mm section of the nerve was removed. The incision in the skin was closed using Nexaband surgical adhesive. Animals were then placed in separate containers and allowed to recover from anesthesia. Post-surgery feeding behavior was recorded as soon after recovery as possible. Filming of the animals was usually terminated within 3 weeks to avoid recording the behavior of reinnervated animals. After at least three feeding sequences had been recorded, the animals were killed with an overdose of 5 % isoflurane, and the nerve transections were confirmed by dissection.

Statistical analyses

Kinematic variables were analyzed using Statview statistical software on a Macintosh Power PC. A two-way analysis of variance (ANOVA) was performed to compare differences in the means of the kinematic variables before and after nerve transection. Of the 24 variables, only the durations, angles and distances were used in the analysis. These tests were performed for the following treatments: (i) before and after bilateral transection of the M. mandibulohyoideus complex; and (ii) before and after bilateral transection of the verticalis or accelerator muscle.

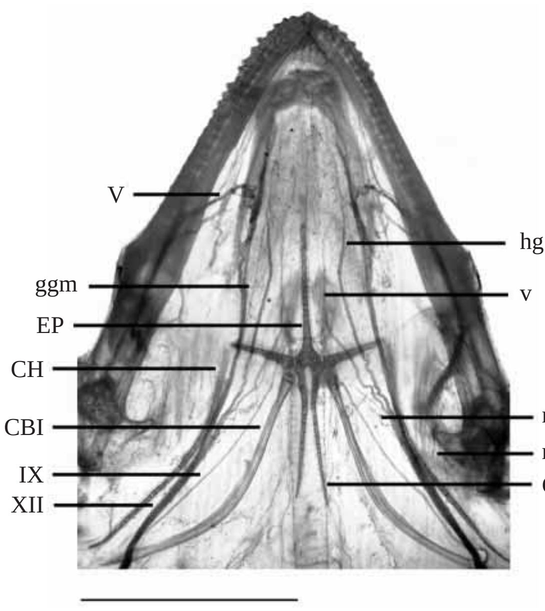

Fig. 2. Ventral view of a cleared and stained lower jaw of *Sceloporus undulatus*. CBI, ceratobranchial I; CBII, ceratobranchial II; CH, ceratohyal; EP, entoglossal process; IX, glossopharyngeal nerve; XII, hypoglossal nerve; ggm, ramus genioglossus medialis; hg, ramus hyoglossus; v, ramus verticalis; mhI, ramus mandibulohyoideus I; mhII, ramus mandibulohyoideus II; V, trigeminal nerve. Scale bar, 5 mm.

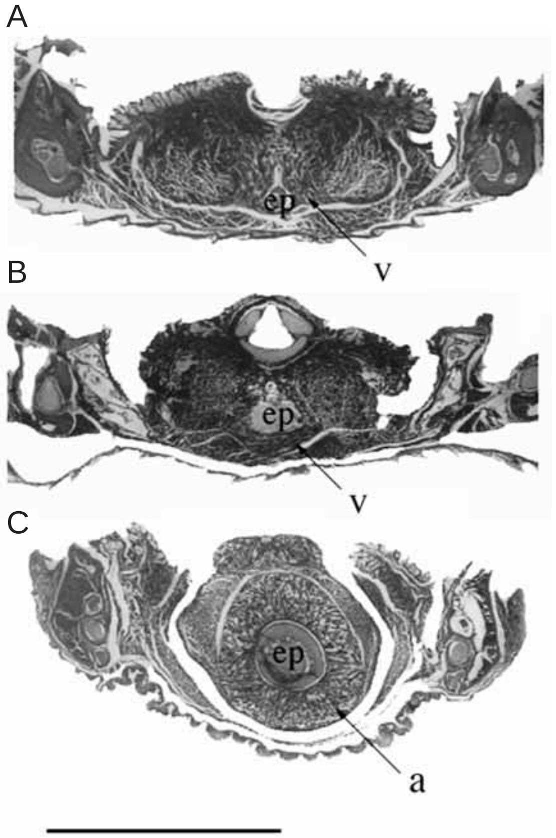

Fig. 3. Cross section through the lower jaw and tongue of each species. The selected sections represent those in which the verticalis or accelerator muscle was the most developed. (A) In *Sceloporus undulatus*, the M. verticalis does not form a complete ring of muscle around the entoglossal process. (B) In *Pseudoptrapelus sinaitus*, the addition of a ventral layer of muscle fibers creates a complete ‘ring’ around the process. (C) In *Chamaeleo jacksonii*, a dense group of radially arranged fibers can be seen surrounding the entoglossal process. ep, entoglossal process of the hyobranchium; v, M. verticalis; a, M. accelerator. Scale bar, 5 mm.

Results

Muscle anatomy

The tongue and hyobranchial musculature of iguanid (Oelrich, 1956; Secoy, 1971; Tanner and Avery, 1982; Smith, 1984; Schwenk, 1988; Delheusy et al., 1994), agamid (Sanders, 1872; Gnanamuthu, 1937; Tanner and Avery, 1982; Schwenk, 1988; Smith, 1988; Herrel et al., 1998) and chamaeleonid (Houston, 1828; Gnanamuthu, 1930, 1937; Tanner and Avery, 1982; Bell, 1989; Wainwright and Bennett, 1992a) lizards has been described in some detail. Here, we describe only those muscles thought to be important in protruding and projecting the tongue out of the mouth (Gnanamuthu, 1937; Smith, 1984, 1988; Schwenk and Bell, 1988; Schwenk and Throckmorton, 1989; Herrel et al., 1997). Although descriptions of the tongue and hyolingual apparatus are available for all three of these groups, there is none specifically for *S. undulatus*, *P. sinaitus* or *C. jacksonii*. Therefore, we provide a preliminary description for these three species.

The M. mandibulohyoideus (MH) complex lies dorsal to the M. intermandibularis and is made up of three distinct slips, including the M. mandibulohyoideus slips I, II and III. These slips are present in all three species, but only M. mandibulohyoideus slips I and II are well developed. Each of these muscle slips originates on the mandible and inserts ventrally onto the ceratobranchials in both *S. undulatus* and *P. sinaitus*. In *C. jacksonii*, slip I of the M. mandibulohyoideus inserts postero-ventrally on the basihyoid. Slip II of the M. mandibulohyoideus inserts onto the distal tip of the certobranchial II, but is also connected to the basihyoid by a band of fascia.

The M. genioglossus medialis and lateralis are present in both *S. undulatus* and *P. sinaitus* but have been reduced in *C. jacksonii*. The M. genioglossus medialis originates near the jaw symphysis dorsal to M. mandibulohyoideus slip II and runs posteriorly to insert on the M. hyoglossus at the level of the basihyoid. The M. genioglossus lateralis also originates at the jaw symphysis. It runs posteriorly and then dorsally to insert laterally on the M. hyoglossus.

The intrinsic muscle, M. verticalis or M. accelerator, is the last muscle that may be used to protrude the tongue. This muscle is present in all three species. In *S. undulatus*, the muscle fibers of the M. verticalis are arranged vertically and only border the entoglossal process of the hyobranchium (Fig. 3A). In *P. sinaitus*, the M. verticalis forms a ring of muscle that becomes increasingly developed in the posterior portion of the tongue (Fig. 3B). The accelerator muscle in *C. jacksonii* is the most developed, forming a dense ring of radially arranged fibers around the entoglossal process (Bell, 1989; van Leeuwen, 1997).

Neuroanatomy

The muscles of the tongue and hyobranchium of lizards are innervated by four cranial nerves, the facial (VII), trigeminal (V), glossopharyngeal (IX) and hypoglossal (XII), and the first spinal nerve (Willard, 1915; Oelrich, 1956; Sondhi, 1958). The pattern of innervation in *S. undulatus* and *P. sinaitus* is relatively conserved between the two taxa. However, in *C. jacksonii*, because the arrangement of the muscles is highly modified and the body is laterally compressed, it is difficult to compare the innervation patterns.

The hypoglossal nerve provides most of the motor input to the tongue and hyobranchial muscles. This nerve emerges posterior to the dentary and runs anteriorly on the ventral surface of the M. hyoglossus. As it proceeds anteriorly, small branches of the nerve diverge into the M. hyoglossus and M. mandibulohyoideus complex. Midway up the M. hyoglossus, near the insertion of the M. genioglossus medialis onto the M. hyoglossus, the hypoglossal nerve trifurcates into three main branches. The medial branch innervates the M. verticalis, the middle branch runs deep into the M. hyoglossus and the lateral branch runs forward to innervate the M. genioglossus internus, lateralis and medialis (Fig. 2). Although some of the smaller branches may vary in the number, size and position of the branches, gross innervation patterns are conserved among the taxa examined.

Normal feeding kinematics

Because the focus of this study was to look at the function of specific components of the feeding apparatus, a detailed analysis of movement kinematics is only presented with respect to treatment effects. A general description of normal feeding movements will be given for each species. Both *S. undulatus* and *P. sinaitus* exhibit similar feeding behaviors that

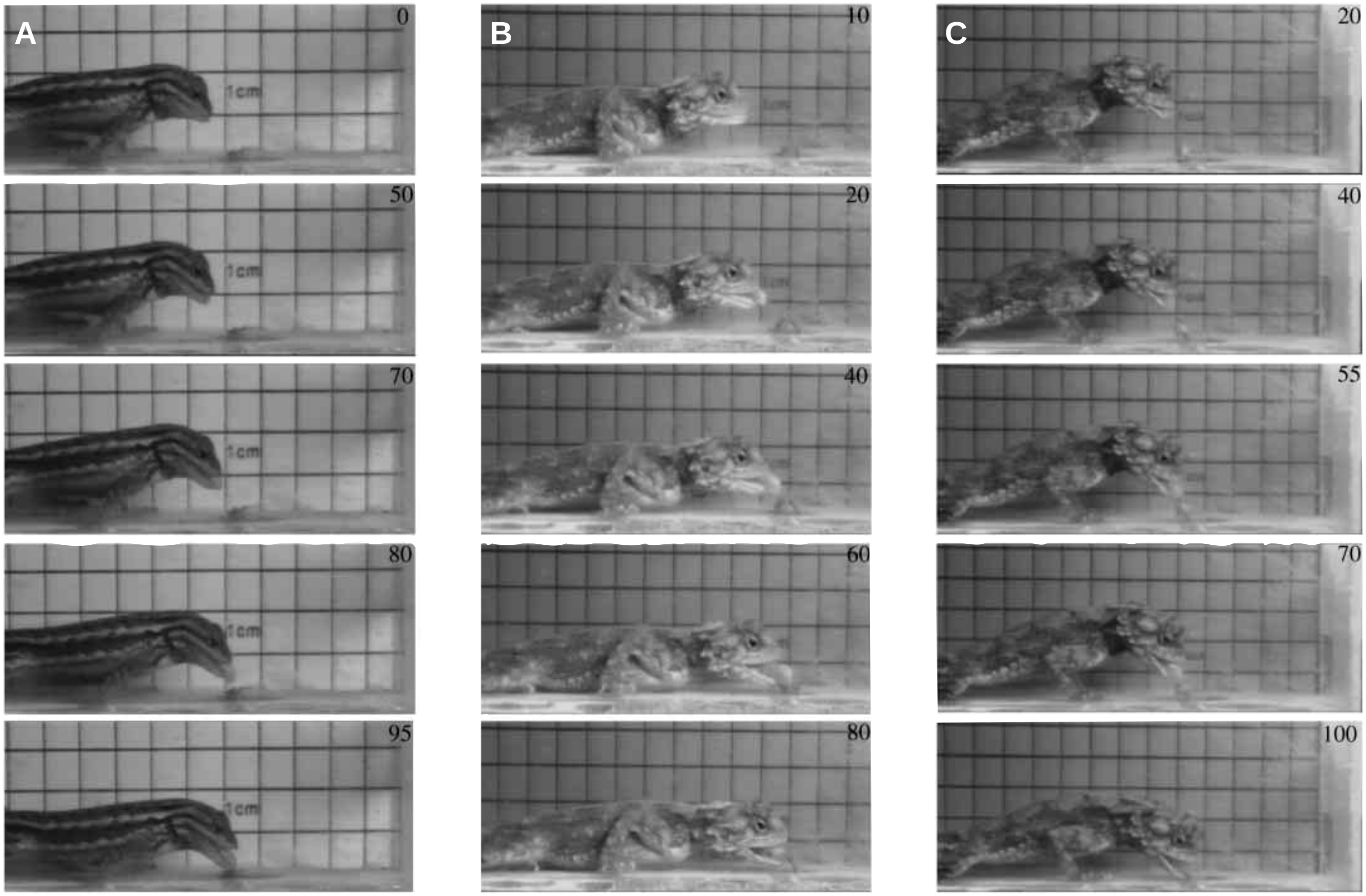

Fig. 4. Selected frames from feeding sequences for *Sceloporus undulatus* and *Pseudoptrapelus sinaitus*. The prey was presented through a hole in the floor of the container. Time (in milliseconds) is given in the upper right-hand corner. (A) Normal feeding sequence for *S. undulatus*. Post-surgery sequences are not presented because there were no differences in the ability to protract the tongue for either treatment. (B) Normal feeding sequence for *P. sinaitus*. Note the greater tongue extension compared with that of *S. undulatus*. (C) Feeding sequence in *P. sinaitus* after transection of the nerves innervating the M. mandibulohyoideus complex. There was a significant decrease in the maximum tongue protrusion distance after denervation of the M. mandibulohyoideus but not the M. verticalis. Both treatments had approximately the same effect (reducing maximum tongue protrusion distance), so only the effect of one treatment is shown here.

will be described together. However, chameleons show behavioral modifications associated with their unique morphology that require a separate description.

When presented with a prey item, *S. undulatus* and *P. sinaitus* orient their head so that it faces the prey, then either move slowly forward and lunge towards the prey or lunge directly at the prey (Fig. 4A,B). Slow forward movement may be terminated at any time, and the feeding attempt aborted. However, if the lizard lunges, the animal rotates over the forelimbs and continues towards the prey. As it moves towards the prey, the mouth opens slightly and the tongue moves forward in the mouth. At the onset of the lunge, the tongue is protruded beyond the jaw margin, reaching maximum tongue protrusion at approximately the time of prey contact. Maximum gape usually occurred 25 ms after prey contact as the prey was pulled into the mouth. In all the feeding sequences observed during this study, the prey was either pulled into the mouth using only the tongue or pinned with the tongue and grabbed using the jaws. The size of the prey relative to the size of the animal determined whether the prey was pulled into the mouth using strictly the tongue or contacted with the tongue and grabbed with the jaws; similar behaviors have been observed in other lizards (Schwenk and Throckmorton, 1989; Schwenk, 2000). Of the sequences analyzed, prey size varied only slightly, and the animals always moved the prey into the mouth using the tongue. Once the prey has been captured, the animal rotates back to the resting position and begins processing it.

Prey capture in chameleons shows some basic similarities to that of both *S. undulatus* and *P. sinaitus*. When the prey is sighted, the chameleon orients the head towards the prey and directs both eyes at the prey item (Fig. 5A). Protraction begins with the tongue moving forward out of the mouth on the entoglossal process. As the tongue is protruded forward on the

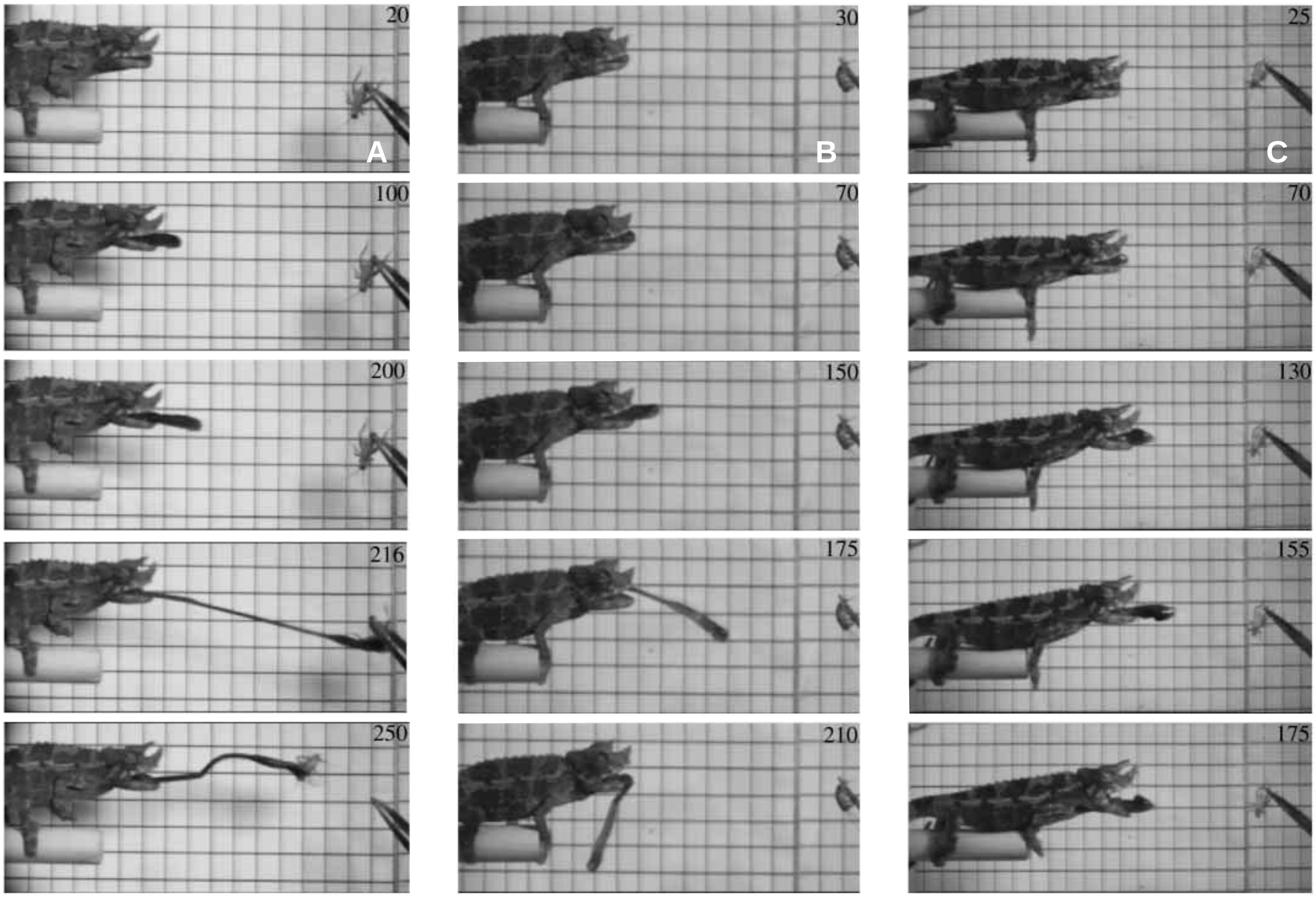

Fig. 5. Normal and post-surgery feeding sequences in *Chamaeleo jacksonii*. The prey is presented on the right using forceps. Selected frames of each feeding sequence were chosen to represent equivalent points in the feeding sequence. Time (in milliseconds) is given in the upper right-hand corner. Sequences A, B and C are for different individuals, but are representative sequences for each treatment. (A) Normal feeding sequence in *C. jacksonii*; frame 1, onset of hyobranchial protraction; frame 2, near-maximum hyobranchial protraction; frame 3, onset of tongue projection; frame 4, prey contact; frame 5, onset of retraction. (B) Feeding sequence after bilateral transection of the M. mandibulohyoideus. Note the position of the tongue at onset of tongue projection (frame 3) and the abrupt drop in the tongue trajectory (frame 4). (C) Feeding sequence after bilateral transection of the M. accelerator muscle. The tongue does not project off the entoglossal process, and the dorsal tongue pad can be seen sliding ventrally in frames 3 and 4.

entoglossal process, the M. accelerator contracts and creates a centrally directed force on the entoglossal process. This translates into elongation of the accelerator muscle and eventual projection off the entoglossal process (Wainwright and Bennett, 1992b). The onset of projection is accompanied by fast protraction of the hyobranchium, which may impart substantial forward momentum to the tongue (Wainwright et al., 1991). The tongue then contacts the prey and moves it back to the mouth. The tongue of chameleons is able to impart significant force during tongue retraction. On several occasions, the cricket was torn in half as it was held in the forceps. During normal feeding attempts, if the prey was captured, it was always captured using the tongue.

There are several notable changes that distinguish prey capture in chameleons. The first and most obvious is the ability to project the tongue ballistically off the hyobranchium. The tongue can be projected by 840 % of the upper jaw length, in contrast to *S. undulatus* and *P. sinaitus*, which only protrude the tongue by 30 and 35 % of the upper jaw length respectively. The tongue, riding on the entoglossal process of the hyobranchium, is moved into position for projection by forward movement of the hyobranchium. In chameleons, the hyobranchium is highly protrusible, reaching approximately 20 mm beyond the jaw margin. In *S. undulatus* and *P. sinaitus*, precise movements of the hyobranchium cannot be quantified using high-speed videography. However, cineradiography of radio-opaque markers placed in the tongue and hyobranchium of *Agama stellio* revealed only intraoral movements of the hyobranchium during prey capture (A. Herrel, personal communication).

Effects of denervation of the M. mandibulohyoideus

The effects of bilaterally denervating the M. mandibulohyoideus are variable among the three species. Qualitatively, it is difficult to see changes in the feeding behavior after denervation in both *S. undulatus* and *P. sinaitus*. In *C. jacksonii*, there are drastic differences in the ability to protract the hyobranchium and project the tongue after surgery. To compare equivalent events, only successful feeding attempts were used for both *S. undulatus* and *P. sinaitus*. However, chameleons could not capture prey at distances greater than 80 mm after surgery, but would still attempt to do so. Therefore, to compare equivalent projection efforts, we compared normal prey-capture events with post-surgery misses.

Of the 14 kinematic variables measured during prey capture, only head angle at prey contact was significantly different after surgery in *S. undulatus* (Table 2). At prey contact, the angle of the head to the horizon decreased from 23.91 to 15.64 °. Although not significantly different, the angle of the head at the onset of the lunge also decreased from 20.70 to 14.83 ° below the horizon. After denervation, these animals tended to lunge at the prey horizontally and ventroflex the craniovertrebral joint less. During prey capture, the tongue of *S. undulatus* is protruded on average by 0.25 cm. After denervation of the hyobranchium protractors, there is no change in the distance to which the tongue is protruded (Table 2). Prey-capture success decreased from 84 to 76 %.

The feeding kinematics of *P. sinaitus* also changed very little in response to the treatment (Table 3). Only tongue protrusion distance and head angle at prey contact differed significantly before and after denervation. After denervation, tongue protrusion distance decreased from 0.45 to 0.35 cm (Fig. 4C). This is in contrast to *S. undulatus*, in which tongue protrusion distance remained unchanged. The shape of the tongue also appears to change: it seems to decrease in thickness. This is most visible within 10 ms of prey capture. Although tongue reach decreases, there is no significant

Table 2. *Mean values of selected kinematic variables for normal feeding sequences and after bilateral M. mandibulohyoideus denervation in* Sceloporus undulatus

Variable	Normal	After M. mandibulohyoideus denervation	*F* value
Duration (ms)			
Mouth opening	194.00±31.20	190.00±15.51	0.02
Tongue protrusion	81.45±12.09	89.78±9.19	1.29
Tongue retraction	16.66±1.39	24.06±3.24	1.49
Mouth closing	21.28±2.02	29.62±4.19	0.87
Feeding sequence	243.00±27.59	229.00±16.26	0.53
Gape angle (degrees)	60.01±2.45	52.33±1.27	5.41
Head angle at onset of lunge (degrees)	20.70±0.97	14.83±1.99	7.67
Head angle at prey contact (degrees)	23.91±0.97	15.64±2.14	52.69*
Gape distance (cm)	0.78±0.02	0.69±0.02	3.00
Tongue protrusion distance (cm)	0.25±0.01	0.24±0.01	0.70

Values are means ± s.e.m., *N*=9 for each treatment (three trials from three individuals).
F values are the results of a two-way ANOVA.
*Indicates a significant difference at *P*=0.05.

Table 3. *Mean values of selected kinematic variables for normal feeding sequences and after bilateral M. mandibulohyoideus denervation in* Pseudotrapelus sinaitus

Variable	Normal	After M. mandibulohyoideus denervation	*F* value
Duration (ms)			
Mouth opening	196.00±20.32	316.00±57.23	1.98
Tongue protrusion	94.41±41.02	110.00±26.73	0.15
Tongue retraction	18.51±1.22	21.29±2.82	0.43
Mouth closing	22.31±1.96	23.13±2.31	0.08
Feeding sequence	224.91±20.12	342.00±57.99	1.88
Gape angle (degrees)	46.61±0.71	46.90±0.99	0.19
Head angle at onset of lunge (degrees)	9.42±1.55	4.83±1.46	15.37
Head angle at prey contact (degrees)	12.14±1.16	7.01±1.14	23.08*
Gape distance (cm)	1.01±0.02	0.99±0.02	2.00
Tongue protrusion distance (cm)	0.45±0.02	0.35±0.01	20.50*

Values are means ± S.E.M., *N*=9 for each treatment (represents three trials from three individuals).
F values are the results of a two-way ANOVA.
*Indicates a significant difference at *P*=0.05.

difference in the lizards' ability to capture prey. Average prey capture success decreased from 81 to 61 % after the treatment, but was highly variable among the individuals. During prey capture, both *S. undulatus* and *P. sinaitus* capture the prey using only the tongue. Neither species had any difficulties capturing, transporting or processing prey after nerve transection.

The effect of bilateral denervation of the M. mandibulohyoideus was most pronounced in *C. jacksonii* (Table 4). The most apparent effect is that the entoglossal process is no longer protruded out of the mouth. Before nerve transection, the average protrusion distance of the entoglossal process was 20.8 mm beyond the tip of the mandible. After transection, the entoglossal process does not move forward out of the mouth, and the tongue is projected off the entoglossal process while it is still in the mouth (Table 4). At the onset of mouth opening, the tongue can be seen moving forward in the mouth and is protruded beyond the mandible by only 5 mm (Fig. 5B). Since the M. mandibulohyoideus no longer protracts the entoglossal process, activation of the

Table 4. *Mean values of selected kinematic variables for normal feeding sequences and after bilateral M. mandibulohyoideus denervation in* Chamaeleo jacksonii

Variable	Normal	After M. mandibulohyoideus denervation	*F* value
Duration (ms)			
Mouth opening	1468.00±151.28	1683.00±124.39	0.43
Tongue protrusion	805.23±84.04	0±0	47.67*
Tongue projection	25.97±3.24	31.47±3.04	0.63
Tongue retraction	487.77±120.90	263.78±14.43	4.27
Mouth closing	104.59±8.11	116.62±11.53	42.07*
Feeding sequence	1636.00±149.19	1889.00±114.71	0.78
Gape angle (degrees)	58.04±1.81	56.80±1.98	0.12
Head angle (degrees)	45.52±5.00	47.69±5.85	0.08
Gape distance (cm)	1.59±0.08	1.57±0.06	0.08
Tongue projection distance (cm)	9.50±0.34	2.90±0.22	160.25*
Entoglossal process protrusion (cm)	2.08±0.06	0±0	574.27*

Values are means ± S.E.M., *N*=9 for each treatment (represents three trials from three individuals).
Note that, after denervation, the hyobranchium is not protruded and the tongue was projected from within the mouth.
F values are the results of a two-way ANOVA.
*Indicates a significant difference at *P*=0.05.

accelerator muscle is probably responsible for the forward movement of the tongue. The time to the onset of tongue projection was not significantly different post-transection and occurred approximately 1200 ms after mouth opening. At the onset of tongue projection, the tongue is only slightly protruded beyond the mandible, and the trajectory looks normal as the tongue projects out of the mouth. Once the tongue has been projected approximately 25 mm out of the mouth, horizontal movement stops abruptly, and the tongue falls ventrally (Fig. 5B, occurring between frames 3 and 4). A notable difference between the two treatments is that, in normal feeding sequences, the coils of the M. hyoglossus can be seen within the first 10 ms after projection. However, after transection, the M. hyoglossus is not coiled and appears taut during the same time period (Fig. 5B, frame 4).

After nerve transection, chameleons cannot capture prey items at distances greater than 30 mm, and prey capture success decreased from 68 % to zero. However, if the prey item is presented within 30 mm, the lizards are more successful at capturing prey, and the success rate returns to near normal levels (63 %). Prey capture at distances less than 30 mm is qualitatively similar to that of normally feeding animals at the same distance. At this distance, the tongue is usually not fully projected off the entoglossal process and the M. hyoglossus is not visible at maximum protrusion.

Effects of denervation of the M. verticalis or M. accelerator

Treatment effects differed among the three species; however, there were no kinematic variables for which the treatment effect was significant for all three species. Again *S. undulatus* and *P. sinaitus* were both able to capture prey items after nerve transection, but *C. jacksonii* could not. For this reason, normal prey-capture events were compared with misses after nerve transection in the latter species. Because the animals still attempted to capture prey at the pre-surgery distances, we were able to compare equivalent projection efforts.

In *S. undulatus*, the duration and timing events during prey capture generally increased after transection of the M. verticalis. However, only maximum gape angle was significantly different (Table 5). After transection, the gape angle increased on average by 4 ° for all three individuals. Gape distance also increased after nerve transection, but the difference was not significant. Head angle at prey contact did not decrease as it did when the M. mandibulohyoideus was transected, and lizards were equally successful at capturing prey whether lunging horizontally or from an erect position.

Maximum tongue protrusion distance in *S. undulatus* was variable, ranging from 2.6 to 3.3 mm in normally feeding animals. After transection, the average tongue protrusion distance decreased in all three individuals but, out of several feeding attempts, most individuals were able reach pre-surgery distances at least once. Post-surgery animals were still able to apprehend the prey and pull it into the mouth using only the tongue. Although not significantly different, there was a general decrease in prey-capture success for all three individuals, from 84 % pre-surgery to 73 % post-surgery. The duration of prey transport and processing did not differ between the treatments.

As with *S. undulatus*, duration and timing events generally increased after nerve transection in *P. sinaitus*. Gape distance was the only variable that was significantly different after bilaterally transecting the accelerator muscle (Table 6). The gape distance increased on average by 2 mm in all three animals. Although not significant, gape angle also increased after surgery in two of the three individuals. These results are similar to those found in *S. undulatus*, in which gape angle increases after bilateral m. verticalis denervation. In both

Table 5. *Mean values of selected kinematic variables for normal feeding sequences and after bilateral M. verticalis denervation in* Sceloporus undulatus

Variable	Normal	After M. mandibulohyoideus denervation	*F* value
Duration (ms)			
Mouth opening	214.00±43.95	377.00±78.93	10.20
Tongue protrusion	87.00±22.69	147.00±66.55	3.42
Tongue retraction	20.36±1.46	26.84±2.31	3.77
Mouth closing	26.84±4.11	32.39±2.93	4.00
Feeding sequence	251.00±43.98	417.00±78.91	10.93
Gape angle (degrees)	44.58±2.11	48.73±1.71	18.97*
Head angle at onset of lunge (degrees)	17.92±2.47	11.30±2.47	0.96
Head angle at prey contact (degrees)	16.86±2.71	13.11±1.88	0.60
Gape distance (cm)	0.67±0.03	0.74±0.02	9.00
Tongue protrusion distance (cm)	0.29±0.02	0.26±0.02	11.61

Values are means ± S.E.M., *N*=9 for each treatment (represents three trials from three individuals).
F values are the results of a two-way ANOVA.
*Indicates a significant difference at *P*=0.05.

Table 6. *Mean values of selected kinematic variables for normal feeding sequences and after bilateral M. verticalis denervation in* Pseudotrapelus sinaitus

Variable	Normal	After M. mandibulohyoideus denervation	*F* value
Duration (ms)			
Mouth opening	148.00±16.73	169.00±16.95	2.14
Tongue protrusion	80.52±10.30	63.86±14.16	2.77
Tongue retraction	19.44±1.39	23.14±2.70	1.00
Mouth closing	23.14±8.10	28.69±10.30	4.00
Feeding sequence	180.00±15.65	211.00±15.71	3.18
Gape angle (degrees)	47.06±1.81	52.14±1.51	3.08
Head angle at onset of lunge (degrees)	3.12±4.59	17.49±3.01	2.23
Head angle at prey contact (degrees)	5.88±6.06	25.68±4.26	3.15
Gape distance (cm)	1.05±0.07	1.21±0.07	128.00*
Tongue protrusion distance (cm)	0.48±0.02	0.21±0.05	5.67

Values are means ± S.E.M., *N*=9 for each treatment (represents three trials from three individuals).
F values are the results of a two-way ANOVA.
*Indicates a significant difference at *P*=0.05.

species, the mouth opens wider as the tongue moves the prey into the mouth.

Tongue protrusion distance decreased in all three individuals although this was not significant. In two individuals of *P. sinaitus*, tongue protrusion distance was reduced by 1.5 mm. In the third individual, there was no tongue protrusion after this treatment. The two animals that could still protrude the tongue were still able to capture the prey using only the tongue, while the others could capture prey only using the jaws. Prey-capture success decreased in all three individuals from 88 to 70 %, but the decrease was most pronounced in the individual that could not protrude its tongue. The duration of prey transport and processing did not change after surgery and resembled those in normal feeding sequences.

Again, the effect of nerve transection was most obvious in *C. jacksonii*. After bilateral denervation of the accelerator muscle, *C. jacksonii* was no longer able to project the tongue off the entoglossal process (Fig. 5C; Table 7). Normal tongue projection distances were as much as twice snout–vent length, but after transection there was no tongue projection (Table 7).

Table 7. *Mean values of selected kinematic variables for normal feeding sequences and after bilateral M. accelerator denervation in* Chamaeleo jacksonii

Variable	Normal	After M. mandibulohyoideus denervation	*F* value
Duration (ms)			
Mouth opening	1524.39±211.81	1709.00±219.97	3.98
Tongue protrusion	715.45±63.87	1103.26±146.48	46.73*
Tongue projection	27.76±1.96	0±0	300.00*
Tongue retraction	226.76±14.68	0±0	759.81*
Mouth closing	117.55±24.06	144.39±29.12	3.40
Feeding sequence	1744.00±217.49	1857.00±243.97	3.92
Gape angle (degrees)	53.25±1.39	51.97±2.47	0.22
Head angle (degrees)	75.40±36.71	36.03±2.51	1.12
Gape distance (cm)	1.33±0.04	1.42±0.06	3.25
Tongue projection distance (cm)	9.74±0.17	0±0	3218.84*
Entoglossal process protrusion (cm)	2.20±0.09	2.33±0.07	3.50

Values are means ± S.E.M., *N*=9 for each treatment (represents three trials from three individuals).
Note that the tongue is protruded out of the mouth on the hyobranchium but not projected off the entoglossal process.
F values are the results of a two-way ANOVA.
*Indicates a significant difference at *P*=0.05.

The early stages of the feeding sequence resemble a normally feeding animal. The tongue moves forwards out of the mouth on the entoglossal process, but this is due solely to hyobranchial protraction. As the tongue moves out of the mouth, there are obvious differences in gross tongue morphology. The tongue tip is pointed instead of rounded, which is probably because the entoglossal process is pushing into the tip of the tongue (Fig. 5C). The tongue pouch, which is normally everted during tongue protrusion, is not everted, and the characteristic shape of the tongue changes (compare Fig. 5B,C). As the accelerator muscle moves out of the mouth on the entoglossal process, the tongue pad rolls ventrally, and at maximum protraction the tongue pad lies ventrally on the entoglossal process. Once hyobranchial retraction begins, the tongue pad moves back to the original dorsal position.

After transection of the accelerator muscle, *C. jacksonii* is never able to capture prey using the tongue. During each new feeding bout, the animals attempted to project the tongue at prey items presented at 70 mm or more, but with each unsuccessful attempt they moved forward towards the prey item. If the prey was placed close enough for the animal to contact it with the tongue tip, it was still unable to grasp it. The tongue hit the prey and pushed it away without it sticking to the tongue. If the prey was placed close enough, the lizards eventually grabbed the prey using the jaws. This is in contrast to animals in which the M. mandibulohyoideus was transected. These animals grabbed the prey using the tongue even when it was presented at close range. Even if the lizards were able to capture the prey using the jaws, they had great difficulty processing the prey item. The duration of prey processing increased from an average of 30 s to more than 10 min in some cases.

Discussion

There have been numerous studies describing the kinematics of head and tongue movements during feeding in iguanid (Delheusy and Bels, 1992; Schwenk and Throckmorton, 1989; Smith, 1984), agamid (Herrel et al., 1995, 1996, 1997; Kraklau, 1991; Schwenk and Throckmorton, 1989; Smith, 1988) and chameleonid (Bell, 1990; Wainwright et al., 1991; So et al., 1992) lizards. Because these published accounts describe the feeding of iguanids, agamids and chameleonids, the kinematic variables discussed here are limited to those that are important to an understanding the effects of the denervation experiments. For a more detailed description of feeding movements, refer to the aforementioned studies.

Denervation of the M. mandibulohyoideus

The effect of denervation of the M. mandibulohyoideus was different in each species. In general, the duration events tended to increase and the linear and angular measurements decreased. The only significant difference for *S. undulatus* was a decrease in the head angle at prey contact. Before surgery, these animals stood high on their forelimbs and lunged downwards towards the prey; after surgery, the forelimbs were depressed and the lizards tended to lunge horizontally. It seemed at first that this difference might be attributable to the distance at which the prey was presented. Normally feeding animals positioned near a prey item tend to stand up on the forelimbs and lunge downwards, but if the prey is presented farther away, the animals run towards the prey and lunge horizontally. However, prey distance did not vary between treatments, and the reason for the change in behavior remains unclear.

In *S. undulatus*, there was no reduction in tongue protrusion after inactivation of the mandibulohyoideus complex. In normally feeding animals, the tongue was always protruded out of the mouth during prey capture. During the lunge, the tongue reaches maximum protrusion at prey contact, tongue retraction begins shortly after this, and the tongue moves the prey into the mouth.

The effect of denervation of the M. mandibulohyoideus on tongue protrusion distance in *P. sinaitus* was very different from that in *S. undulatus*. The overall feeding movements did not change, but the tongue was not protruded as far and it did not look as thick and fleshy. After denervation, there was a significant difference in tongue protrusion distance, which decreased from 0.45 to 0.35 cm. In *C. jacksonii*, the entoglossal process was normally protruded approximately 2 cm beyond the mandible. However, after nerve transection, the entoglossal process was no longer protracted. The M. accelerator can be seen moving the tongue forwards during slow opening of the jaws. Projection occurs while the tongue is still in the mouth, but the tongue is stopped short of its destination by the M. hyoglossus.

The results of M. mandibulohyoideus denervation in *S. undulatus* are surprising considering the anatomical arrangement and the hypothesized function of these muscles. Action of these muscles has long been thought to contribute to protraction of the hyobranchium and tongue (Gnanamuthu, 1937; Smith, 1984; Schwenk and Throckmorton, 1989). Only two studies have looked at the function of these muscles in iguanids. Smith (1984) compared activation patterns of various tongue and hyolingual muscles in *Ctenosaura similis*. The M. geniohyoideus (M. mandibulohyoideus) was active during both tongue protrusion and prey processing. Simultaneous displacements of radio-opaque markers revealed antero-dorsal movement of the hyobranchium. Electrical stimulation of the hyobranchial protractors caused forward movement of the entire hyobranchial apparatus in the iguanid *Oplurus cuvieri* (Delheusy et al., 1994). Both these studies support the aforementioned hypothesis about the function of these muscles, but do not show the extent to which these muscles are involved in protruding the tongue out of the mouth. The results of the present study suggest that the M. mandibulohyoideus is not necessary for normal tongue protrusion in *S. undulatus*.

Functional studies on the hyolingual system in agamids also support the role of the M. mandibulohyoideus in tongue protrusion. M. mandibulohyoideus slip I is active during both tongue protrusion (Herrel et al., 1995) and prey processing in *Agama stellio* (Herrel et al., 1997). In the agamid lizard *Pogona barbata*, Throckmorton et al. (1985) found that stimulation of the M. ceratomandibularis internus (M. mandibulohyoideus slip II) protracts the entire hyobranchium and of the ceratomandibularis externus (M. mandibulohyoideus slip I) abducts the ceratobranchial, causing the distal ends to swing anteriorly and laterally. In *Pogona barbata*, these muscles function in frill erection as well as hyobranchial protraction and tongue protrusion. Both studies suggest that the M. mandibulohyoideus is involved in protrusion the hyobranchium. In *P. sinaitus*, M. mandibulohyoideus denervation caused a 23 % decrease in tongue protrusion distance. Unlike *S. undulatus*, these muscles are necessary for normal tongue protrusion in *P. sinaitus*. Interestingly, most kinematic variables describing the feeding movements changed very little in *P. sinaitus* even though there was a definite reduction in the ability to protrude the tongue.

The arrangement of hyobranchial musculature in chameleons differs from that of other lizards in that both slips I and II of the M. mandibulohyoideus originate near the mandibular symphysis and run posteriorly to insert ventrally on the basibranchial. In *S. undulatus* and *P. sinaitus*, M. mandibulohyoideus slip I originates medially on the mandible and pulls the ceratobranchials anteriorly and laterally. In *C. jacksonii*, these muscles only provide anterior movement, allowing the entoglossal process to be protracted further out of the mouth. Several studies have looked at the role of these muscles during feeding. Electromyographic recordings in *C. jacksonii* showed that they are active from the onset of hyobranchial protraction until tongue projection (Wainwright and Bennett, 1992a). Zoond (1933) performed an extensive set of experiments on the mechanism of tongue projection in chameleons. In one experiment, the geniohyoideus muscle slips (M. mandibulohyoideus complex) were cut, preventing hyobranchial protraction. Tongue projection still occurred, but never to the same extent as in an intact animal. The results of transecting the nerves innervating the mandibulohyoideus muscles in *C. jacksonii* agree with the results of Zoond (1933). Both studies show that this muscle is responsible for hyobranchial protraction and is necessary for normal tongue projection.

An important effect of denervating the hyobranchial protractors was that the tongue was not projected more than 3 cm. The entoglossal process was not protracted out of the mouth, and tongue projection occurred while the tongue was still in the mouth. As the tongue was projected, it followed a straight trajectory towards the prey. However, as the tongue tip reached 3 cm beyond the mandible, the tongue stopped moving forward and fell ventrally (Fig. 5B). Zoond (1933) observed similar results when he cut the hyobranchial protractors and suggested that, since the hyobranchial protractors were no longer active, action of the hyobranchial retractor (M. sternohyoideus) began to pull the hyobranchium back during tongue projection. However, Wainwright and Bennett (1992a) showed that activation of the M. sternohyoideus does not start until the tongue is almost completely retracted onto the hyobranchium. Although our data cannot fully explain the differences in tongue protraction after M. mandibulohyoideus transection, we provide alternative hypotheses on how the system might work.

When the tongue is projected after M. mandibulohyoideus denervation, it appears that the M. hyoglossus actively restricts forward movement. One possible explanation is that transection of the rami of the hypoglossal nerves that innervate the M. mandibulohyoideus may have disrupted the sensory input that is important in timing the activation of the M. hyoglossus. Fast hyobranchial protraction and tongue projection occur almost simultaneously during normal projection, and coordination of these movements may require sensory information from the hyobranchium protractors. If this hypothesis is correct, then the M. hyoglosus may become active earlier in the feeding cycle in denervated animals than in intact animals. Simultaneous electromyography and nerve transection may help elucidate this mechanism. A similar hypoglossal feedback mechanism is found in the frogs *Bufo marinus* (Nishikawa and Gans, 1992) and *Rana pipiens* (Anderson and Nishikawa, 1993). In these animals, hypoglossal nerve transection prevents mouth opening, and sensory feedback is necessary to coordinate the simultaneous jaw and tongue movements.

Another possible explanation is that passive properties of the hyobranchial system restrict unraveling of the M. hyoglossus during projection. The coiled hyoglossus muscle originates on ceratobranchial II, running ventrally and then anteriorly to insert on the M. accelerator. After denervation of the M. mandibulohyoideus, the hyobranchium is unable to move to the protracted state (Fig. 6B). It is possible that this orientation does not allow the M. hyoglossus to unravel during projection and may prevent normal tongue projection.

Denervation of the M. verticalis or M. accelerator

Studies of the intrinsic tongue musculature have not fully explained its function in iguanid or agamid lizards. Smith (1984) put forth several hypotheses about how the intrinsic and extrinsic tongue musculature could be involved in tongue protrusion. For intrinsic muscles, she suggested that contraction of the M. verticalis might be involved in sliding the tongue forward on the entoglossal process. This mechanism relies on two factors: (i) the development of muscle fibers that form a ring around the entoglossal process, so that contraction would exert a normal force onto the entoglossal process; and (ii) an entoglossal process that is sufficiently tapered to facilitate forward movement on the entoglossal process (Smith, 1988). The intrinsic musculature of iguanian lizards is variable, but there are three general morphologies represented by iguanids, agamids and chamaeleonids.

In iguanid lizards, the muscle bordering the entoglossal process is the M. verticalis. The fibers of this muscle run dorso-ventrally on either side of the entoglossal process but never form a complete ring around the process (Fig. 3A). Schwenk (1986) looked at the outgroup to lizards, *Sphenodon punctatus*, and found that the fibers of the M. verticalis are arranged vertically in the foretongue and surround the entoglossal process in the hindtongue. Smith (1988) suggested that the

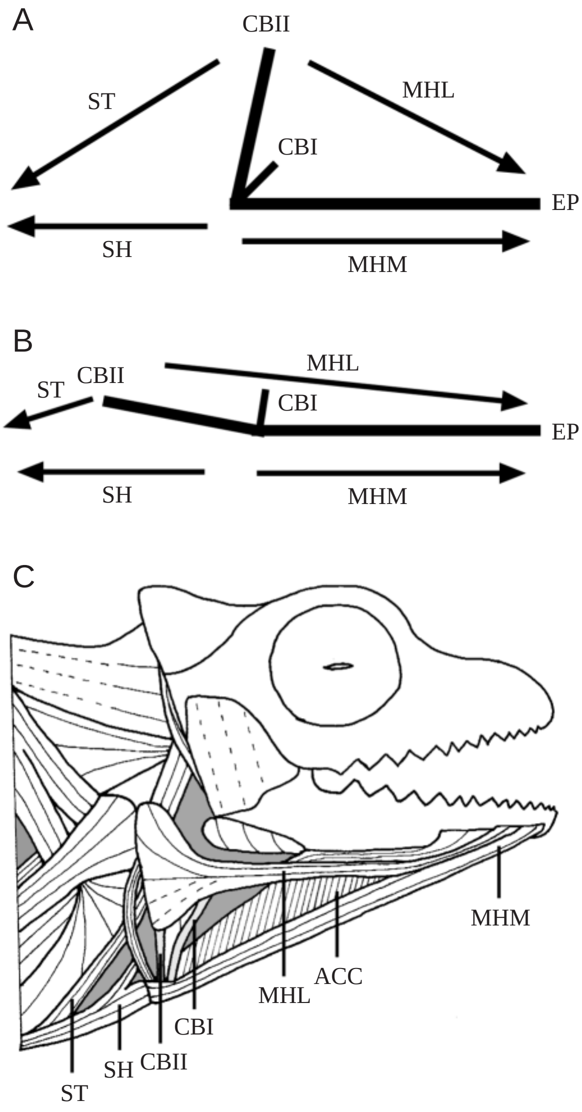

Fig. 6. (A) Position of the hyoid at rest in the mouth. Arrows approximate muscle placement and likely directions of action for the hyobranchial protractor and retractor muscles. (B) Direction of action of the lateral and medial slips of the M. mandibulohyoideus (MHL and MHM) during protraction and for the M. sternothyroideus (ST) and M. sternohyoideus (SH) during retraction. Action of the SH should pull the ceratobranchials down during hyobranchial protraction. (C) The tongue of chameleons is supported by the entoglossal process (EP) of the hyobranchium. The hyobranchium is connected to the mandible by the hyobranchial protractors [MH lateralis (MHL) and MH medialis (MHM)] and to the sternum by the hyobranchium retractor muscles (ST and SH). The tongue pad, riding on the M. accelerator (ACC) covers the anterior three-quarters of the entoglossal process and is connected to the hyobranchium by the M. hyoglossus. CBI, ceratobranchial I; CBII, ceratobranchial II.

arrangement of the muscle fibers in the hindtongue of *S. punctatus* is far more similar to that in agamids than to that in iguanids because it possesses posterior fibers that surround the entoglossal process. In the iguanid *Oplurus cuvieri*, Delheusy et al. (1994) found that the fiber arrangement of the M. verticalis was similar to that in agamids and *Sphenodon punctatus*. It is unclear whether the arrangement of this muscle in iguanids represents the retention of the primitive morphology, similar to that in *Sphenodon punctatus* (Schwenk, 1986), or is a derived condition for iguanids (Smith, 1988).

The effect of denervating the M. verticalis and M. accelerator was as different as the arrangement of the musculature. Unfortunately, there are no experimental data documenting the function of this muscle in iguanid lizards. In the iguanid lizard studied here, *S. undulatus*, the tongue is normally protruded approximately 3 mm beyond the mandible. After denervation, there was no difference in the distance the tongue was protruded and the ability to capture prey decreased only slightly. Generally, the kinematic variables tended to increase, yet only gape angle was significantly different. Denervation in *S. undulatus* has little effect on tongue protrusion or prey capture, suggesting that the M. verticalis is not necessary to protrude the tongue. These findings do not support the hypothesis of Smith (1984) that the M. verticalis is used to protract the tongue in iguanid lizards.

Several studies have addressed the role of the M. verticalis in agamid lizards. Observations of tongue protrusion during prey capture in the agamid *Phrynocephalus helioscopus* revealed an unusual degree of tongue protrusibility. These animals can protract the tongue by more than 50 % of the mandible length, and the form of the tongue during protraction looks very similar to that of chameleons during the protrusion phase. Tongue protrusion distance and overall similarities in tongue morphology during protrusion led Schwenk and Bell (1988) to propose that an evolutionary precursor to the chameleon tongue may be found in agamids. Smith (1988) showed that most agamids have a well-developed ring muscle and a sufficiently tapered hyobranchium to allow tongue protraction by contraction of the M. verticalis. Electromyographical recordings of this muscle in an agamid showed that the M. verticalis is indeed active during tongue protrusion (Herrel et al., 1995). When the M. verticalis is denervated in *P. sinaitus*, there is a definite reduction in tongue protrusion. In intact animals, the tongue can often be protruded 4.8 mm beyond the mandible. After transection, the mean tongue protrusion distance is 2.1 mm, a 43 % decrease in protrusibility. Unlike *S. undulatus*, the M. verticalis in *P. sinaitus* is necessary for normal tongue protrusion. This supports the hypotheses put forth by Smith (1984, 1988) and Schwenk and Bell (1988).

The function of the accelerator muscle in chameleons has been the subject of numerous studies. Zoond (1933) found that stimulation of the hypoglossal nerve innervating the M. accelerator caused the tongue to be projected off the entoglossal process. From the results of nerve transection and from watching feeding behavior, he came to the conclusion that the accelerator muscle is responsible for tongue projection. Zoond (1933) suggested that the M. hyoglossus restrains the contracting accelerator muscle until sufficient force is built up to project the tongue. However, phasic activity in the M. hyoglossus does not start until after tongue projection

(Wainwright and Bennett, 1992a). It is thought that elongation of the accelerator muscle during contraction moves the muscle over the tapered region of the entoglossal process, eventually causing projection (Wainwright and Bennett, 1992b). In this study, we denervated the accelerator muscle and found that the tongue was no longer projected. Hyobranchial protraction occurs normally but at maximum hyobranchium protrusion, and the tongue appears flaccid and often rolls ventrally on the entoglossal process. These findings support the hypothesis of Zoond (1933) that the accelerator muscle is necessary to project the tongue off the entoglossal process.

Because all forward movement of the tongue after this treatment was due to hyobranchial protraction, the rapid hyobranchial protraction occurring at the onset of tongue projection was more visible than during normal feeding. Wainwright et al. (1991) calculated the velocity of the hyobranchium during rapid hyobranchial protraction and provided a conservative estimate of hyobranchium protraction velocity of approximately $0.75\,\mathrm{m\,s^{-1}}$. This represents approximately 15 % of the tongue's maximum velocity. They suggested that movement of the hyobranchium might impart substantial inertial force to the tongue during projection. However, after denervation of the accelerator muscle, rapid hyobranchium protraction never resulted in the tongue moving forward on the entoglossal process. This suggests that passive properties of the tongue may hold it on the hyobranchium.

The mechanism of rapid hyobranchium protraction remains unclear. Wainwright and Bennett (1992a) found that electromyographic recordings of the M. mandibulohyoideus did not show differences in either intensity or frequency at the onset of rapid hyobranchial protraction. This implies that the muscles are not solely responsible for the change in hyobranchium velocity. During our dissection of the tongue and hyobranchium, we discovered some interesting properties of the hyobranchium that might help to explain its rapid protraction. The presence of a joint between ceratobranchial II and the entoglossal process was first described by Gnanamuthu (1937). However, it was unclear how this might aid in tongue projection. We noticed that the joint between ceratobranchial II and the entoglossal process allows the hyobranchium to snap rapidly between two configurations. At rest, ceratobranchial II sits perpendicular to the entoglossal process (Fig. 6A). However, as the hyobranchium reaches an elongated or protracted state, the ceratobranchials roll through the joint more easily, allowing the hyobranchium to be protruded faster. There appears to be a 'catch' that resists rotation until they reach a certain configuration. At full protrusion, the ceratobranchials lie parallel to the entoglossal process (Fig. 6B). This mechanism would be emphasized by the action of the M. mandibulohyoideus slips. The lateral slip of the M. mandibulohyoideus pulls the ceratobranchials forward, whereas the medial slip moves the basihyoid forward (Fig. 6C). Action of the lateral slip would store energy at the joint until the hyobranchium was pulled past the catch point, at which time the hyobranchium rapidly unfolds. Energy storage is probably aided by the M. sternothyroideus, which pulls the ceratobranchials postero-ventrally. Thus, rapid hyobranchial protraction is probably due to the morphology of the hyobranchium, specifically the joint between ceratobranchial II and the entoglossal process, as well as the arrangement and action of the M. mandibulohyoideus slips and possibly the M. sternothyroideus.

Evolution of a projectile tongue

On the basis of the morphological characters of the tongue, Smith (1988) suggested that the tongues of iguanids and agamids have evolved in parallel, with the tongue morphology of agamids more closely resembling *Sphenodon puntatus*. Iguanids, then, do not necessarily represent an evolutionary step in the development of the chameleon tongue, but instead may represent an alternative solution that is functionally similar to the first step of the three-part transformation series proposed by Schwenk and Bell (1988). The three-part transformation series consists of: (i) tongue protrusion produced mainly by the M. genioglossus, (ii) additional protrusion accomplished by sliding of the M. verticalis on the entoglossal process, and (iii) projection of the tongue off the entoglossal process due to force exerted by the M. accelerator.

Our findings in *S. undulatus* correspond nicely to the first step in the transformation series. In *S. undulatus*, neither the M. mandibulohyoideus complex nor the M. verticalis is necessary for normal tongue protraction. The only other muscle that could protract the tongue is the M. genioglossus, which is probably primarily responsible for tongue protrusion in *S. undulatus*. In agamids, the development of the M. verticalis (step ii) into a ring-shaped muscle has allowed the tongue to be protracted further out of the mouth. In *P. sinaitus*, the combined effort of the M. mandibulohyoideus and the M. verticalis provides approximately 50 % of the lingual protrusion. The other 50 % may be attributed to the activity of the M. genioglossus, but this has yet to be tested. In contrast to *S. undulatus*, the mandibulohyoideus muscles of *P. sinaitus* are also involved in protruding the tongue beyond the mandibles. Greater protraction of the hyobranchium allows the entoglossal process to clear the jaw margin, which facilitates sliding of the ring muscle on the process and results in greater lingual protrusion. In *C. jacksonii*, this mechanism is taken to the extreme, and the M. accelerator is used for ballistic tongue projection (step iii). However, this is associated with other morphological changes that are necessary to project the tongue. In chameleons, the M. genioglossus is reduced (Bell, 1989) and no longer functions in tongue protraction, so hyobranchial protrusion is due solely to the M. mandibulohyoideus. Reduction of the M. genioglossus frees the tongue from the floor of the mouth and allows the M. mandibulohyoideus to protrude the entoglossal process beyond the mandibles.

The results of these experiments support the transformation series put forward by Schwenk and Bell (1988). Although iguanids may not represent the primitive morphology, they may be viewed as functionally analagous. An agamid precursor to the chameleon mechanism is also supported. Although the relationships within the Agamidae are unclear (whether chameleons are more closely allied with the Leiolepidinae or the Agaminae), leiolepidines do not possess a M. verticalis but agamines do (Smith, 1988), providing some support for agamine–chameleon affinities.

There are several important findings of this study. First, as the M. verticalis develops into a ring-like structure, it becomes increasingly important in moving the tongue out of the mouth. The M. mandibulohyoideus has also become specialized, and its function may have co-evolved with the M. verticalis and M. accelerator. Second, a change in the form and function of one component of a system is likely to result in a corresponding change in associated parts. In *P. siniatus*, the anatomical arrangement of the mandibulohyoideus muscles did not imply any significant differences in function compared with *S. undulatus*. However, denervation experiments revealed how mandibulohyoideus function has changed in these morphologically similar groups.

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