The Journal of Experimental Biology 203, 3255–3263 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JEB2942



# THE MECHANICS OF PREY PREHENSION IN CHAMELEONS

ANTHONY HERREL[1,*], JAY J. MEYERS[2], PETER AERTS[1] AND KIISA C. NISHIKAWA[2]

[1]*University of Antwerp, Department of Biological Sciences, Universiteitsplein 1, B-2610 Belgium* and
[2]*Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, USA*

*e-mail: aherrel@uia.ua.ac.be

*Accepted 7 August; published on WWW 9 October 2000*

## Summary

**Iguanian lizards generally use their tongue to capture prey. Because lingual prehension is based on surface phenomena (wet adhesion, interlocking), the maximal prey size that can be captured is small. However, published records show that prey items eaten by chameleons include small vertebrates such as lizards and birds, indicating that these lizards are using a different prey prehension mechanism. Using high-speed video recordings, cineradiography, electromyography, nerve transection and stimulation experiments, we investigated the function of the tongue during prey capture. The results of these experiments indicate that chameleons have modified the primitive iguanian system by including a suction component in their prehension mechanism. Suction is generated by the activity of two modified intrinsic tongue muscles that pull the tongue pad inwards. Moreover, we demonstrate that the mechanism described here is a prerequisite for successful feeding.**

Key words: prey capture, lizard, *Rhampholeon spectrum*, *Chameleo* spp., *Brookesia* spp., functional morphology, nerve transection, suction, hyolingual apparatus.

## Introduction

Two prey capture strategies are generally recognised in lizards: tongue and jaw prehension (Schwenk and Throckmorton, 1989). Tongue prehension is the predominant prey capture mode in all members of the most primitive lizard clade, the Iguania (i.e. Iguanidae, Agamidae and Chameleonidae). The mechanism by which the prey adheres to the tongue of iguanid lizards during capture is thought to be based on adhesive bonding and/or interlocking (Bramble and Wake, 1985). Since the chameleon tongue pad contains a large number of epithelial glands and possesses numerous papillae that can lock into surface irregularities on the prey (Schwenk, 1983), both wet adhesion and interlocking presumably play an important role during prey capture.

Although the chameleon tongue is generally considered to be an example of an adhesive bonding system (Bramble and Wake, 1985), suction was suggested as a possible mechanism that would enable chameleons to capture large, smooth prey (Schwenk, 1983). Indeed, because the strength of the adhesive bond is limited by the surface area of the tongue contacting the prey (Emerson and Diehl, 1980), this system places severe limitations on the maximal prey size that can effectively be transported by the tongue. However, personal observations (A. Herrel and J. J. Meyers) and published records indicate that chameleons are successful in capturing large prey such as lizards (up to 10 % of their own body mass) and birds (Broadley, 1973; Schleich et al., 1996). Schwenk (1983) inferred from the morphology and position of the paired pouch retractor muscle (first described by Houston in 1828) that it could play an important role in capturing large prey. He proposed that, after the tongue is splayed open upon impact, it is retracted into its original concave state by activation of the pouch retractor muscles, thus creating suction on the prey.

Here, we test the previously cited hypotheses concerning the mechanics of tongue prehension in chameleons, using high-speed video recordings, cineradiography and electromyography, and investigate the function of the pouch retractor muscle during feeding using denervation experiments. In addition, we investigate quantitatively the contribution of the tongue prehension mechanisms proposed in the literature (i.e. adhesive bonding, interlocking and suction) to the total prehensile forces.

## Materials and methods

### *Animals*

The data represented in this study are based on a wide variety of chameleon species. Species were selected to represent a wide array of body sizes, varying from less than 40 mm in snout–vent length for the smallest, *Rhampholeon spectrum*, to over 200 mm snout–vent length for the largest, *Chameleo melleri*. We also included species of the major radiations of chameleons such as the Madagascar lineage of true chameleons including *C. pardalis* and *C. oustaleti*, the African mainland radiation of true chameleons including *C. calyptratus* and *C. fischeri*, the Madagascar radiation of dwarf chameleons including *Brookesia supraciliaris* and *B. armorata*

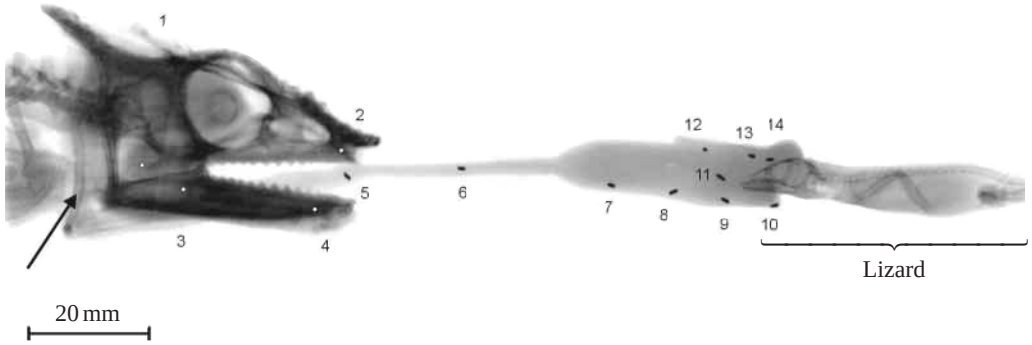


Fig. 1. Static X-ray of the head of an anaesthetized *Chameleo pardalis* with lead markers implanted into the jaws and tongue (see Materials and methods). At the back of the head, the hyoid apparatus is clearly visible (arrow). The tongue has been placed over the head of a dead *Anolis* lizard to illustrate the shape of the tongue and the position of the pouch marker (11) just after prey contact. The numbering shows the positions of the markers used to analyse tongue movements (see Materials and methods).

(morphological data only) and the African mainland radiation of dwarf chameleons including *Rhampholeon spectrum* (Klaver and Böhme, 1986). Some species were chosen because of their availability as captive-bred animals readily available commercially (*C. jacksonii*, *C. calyptratus*). All animals were obtained commercially.

### *Morphology*

Morphological data were gathered by dissection of individuals of the species *C. jacksonii*, *C. oustaleti*, *C. fischeri*, *C. pardalis* and *C. calyptratus*. Drawings were made of all stages of the dissection using a Zeiss dissecting microscope with *camera lucida*. The tongues of specimens of *C. jacksonii* were sectioned (cross, sagittal and frontal sections) and stained using standard histological techniques (trichrome stain; Humason, 1979). Two *C. jacksonii*, one *B. armorata* and one *B. supraciliaris* were cleared and stained to identify bone and cartilage using a modified Taylor and Van Dyke (1978) stain. An additional three specimens of *C. jacksonii* were cleared and stained to identify nerves with Sudan Black (Nishikawa, 1987). The nerves providing motor input to the hyolingual musculature were traced using both cleared and stained and preserved specimens, and were then drawn using a Zeiss dissecting microscope with *camera lucida*.

### *High-speed and X-ray filming*

Chameleons of the species *C. jacksonii* (*N*=5), *C. oustaleti* (*N*=5), *C. pardalis* (*N*=5), *C. fischeri* (*N*=5), *C. melleri* (*N*=2), *C. calyptratus* (*N*=1) and *Rhampholeon spectrum* (*N*=5) were filmed using high-speed video systems (D.I.T. 660 video system set at 120 or 180 frames $s^{-1}$; NAC-1000 video system set at 500 frames $s^{-1}$; Redlake Motionscope digital high-speed camera set at 500 frames $s^{-1}$). At least five successful feeding attempts were recorded for each individual capturing crickets, large grasshoppers and/or *Anolis* lizards. Animals of the species *C. fischeri* (*N*=1) and *C. pardalis* (*N*=2) were also filmed after implantation of small lead markers under anaesthesia (intramuscular injection of Ketalar; 150 mg $kg^{-1}$ body mass) using a Siemens Tridoros-Optimatic 880 X-ray apparatus equipped with a Sirecon 2 image intensifier and an Arriflex 16 mm camera. Again, at least five successful feeding attempts were recorded for each individual capturing crickets of varying sizes and/or large grasshoppers.

High-speed films were analysed by digitising external markers on the jaws and tongue; X-ray films were analysed by digitising the radio-opaque markers implanted into the jaws (markers 1–4) and tongue (markers 5–14; Fig. 1). Several additional variables were calculated on the basis of the positions of the original markers: the horizontal displacement of the tongue tip (*x*-coordinate of marker 10), of the pouch (*x*-coordinate of marker 11), of the anterior tongue pad (*x*-coordinate of marker 14), of the middle tongue pad (*x*-coordinate of marker 13) and of the m. accelerator (*x*-coordinate of marker 7); the shape change of the tongue pad (both the entire pad, distance 12–14, and the anterior part separately, distance 13–14), the tongue thickness (distance 9–13), the distance between the markers in the m. accelerator and the pouch (distance 7–11) and the shape changes in the m. accelerator (distance 7–10). Only those sequences in which the animals kept their head in the vertical plane (i.e. without rolling or sideways head movements) were analysed.

### *Electromyography*

Bipolar electrodes (Gans and Gorniak, 1980) prepared from Formvar-insulated Ni/Cr wire were inserted under anaesthesia into selected jaw (m. depressor mandibulae and m. adductor mandibulae externus medialis) and hyolingual (m. accelerator, m. hyoglossus and m. pouch retractor) muscles of two *C. pardalis*. Since one animal refused to eat after surgery, data were gathered for only one animal. Signals were amplified (Gould Bio-electric, model 13-6615-58 preamplifiers and Honeywell Accudata 117DC amplifiers) and recorded on a Honeywell 96 FM 14-channel tape recorder at a speed of 19.05 cm $s^{-1}$. Simultaneously the animal was video-taped using a NAC-1000 video system set at 500 frames $s^{-1}$. The output of a signal generator, sent to a light-emitting diode kept in the visual field and recorded on the tape-recorder, allowed us to synchronise video and electromyographic recordings. Electrode placement was checked surgically upon implantation and on dorso-ventral and lateral static X-rays (Siemens Tridoros Optimatic 880 X-ray apparatus). Three successful feeding sequences of one of the *C. pardalis* eating large

grasshoppers (48±8 mm; mean ± S.E.M.) were recorded before the animal removed the electromyographic electrodes.

### *Denervation experiments*

The hypoglossal nerve branch providing motor input to the pouch retractor muscles was bilaterally transected under gas anaesthesia (5 % fluothane, AErrane; see Meyers and Nishikawa, 2000) in two specimens of *C. fischeri*. The animals were filmed feeding normally before surgery and within the first 5 days after surgery using a D.I.T. 660 high-speed video system set at 120 or 180 frames $s^{-1}$. Before surgery, at least five successful capture events were recorded for each individual using crickets of varying sizes (5–15 mm). After surgery, at least five prey capture attempts during which the tongue touched the prey were recorded for each animal using crickets of varying sizes.

### *Stimulation experiment*

Stimulation experiments were performed using a Grass (S48) stimulator. Bipolar electrodes 50 cm long prepared from Teflon-coated stainless-steel wire (Gans and Gorniak, 1980) were inserted bilaterally in the pouch retractor muscles of two anaesthetised (Ketalar, 150 mg $kg^{-1}$ body mass) *C. melleri* and one *C. calyptratus*. The muscles were tetanically stimulated using 10 V, 1 s stimulation trains at 70 Hz (see Herrel et al., 1999). A glass tube attached to a Kistler piezo-electric force transducer (type 9203) connected to a portable Kistler charge amplifier (type 5995) was inserted into the pouch. During stimulation, the tongue was immobilised and the glass tube withdrawn. Three different tests were performed. When not stimulating the muscles, all the forces measured upon retraction of the tube are due to adhesive bonding and interlocking. When stimulating the muscles, but using an open tube (i.e. no suction can be created), the force recorded is due to increased adhesive bonding; when repeating this experiment with a sealed tube, suction forces come into play as well. During this last experiment, the tube was sealed at both ends using Plasticine.

By comparing different conditions, the relative contribution of each prehensile mechanism can be deduced. All tests were repeated at least five times for each animal (see Table 1). The data were analysed using a two-way analysis of variance.

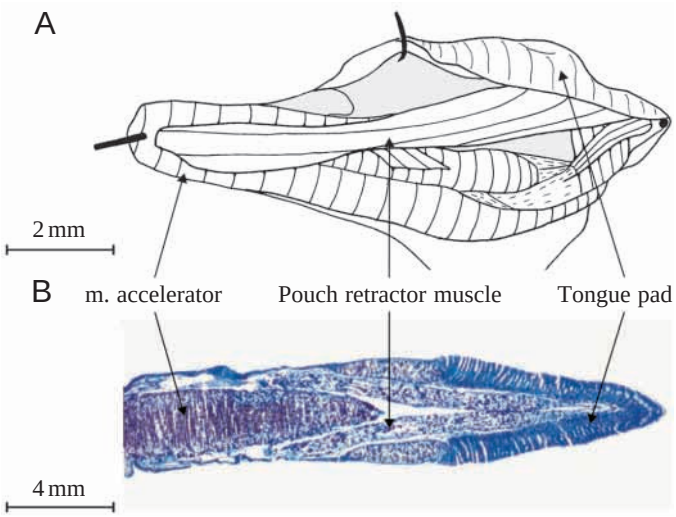


Fig. 2. (A) Anatomical drawing (lateral view) of the dissected tongue of an adult *Chameleo fischeri*. (B) Frontal section (trichrome-stained) through the tongue of an adult *C. oustaleti*. A and B clearly illustrate the position of the pouch retractor muscle relative to the m. accelerator and the tongue pad. The paired pouch retractor muscle is firmly attached to the connective tissue sheet surrounding the m. accelerator and inserts at the inner side of the tongue pad at the so-called pouch or dimple.

## Results

### *Morphology*

Only a short description of the relevant structures within the chameleon hyolingual apparatus will be presented here; a detailed account of the hyolingual morphology in chameleons will be published elsewhere. Detailed descriptions of the extrinsic tongue musculature are given by Houston (1828), Mivart (1870) and Gnanamuthu (1930, 1936) and of the intrinsic muscles by Houston (1828), Bell (1989) and Schwenk (2000).

The chameleon tongue is composed of a central cylindrical accelerator muscle, which sits on the entoglossal process of the hyoid. The tongue retractors (m. hyoglossi) insert posteriorly on the connective tissue sheet surrounding the accelerator muscle. Anteriorly, the tongue pad sits on top of the accelerator muscle and is firmly attached at its anterior, lateral and posterior sides to the thick connective tissue sheet of the m.


Fig. 3. (A) Photograph in lateral view of a chameleon (*Chameleo oustaleti*) while capturing a cricket. Note how the tongue has almost completely engulfed the prey. (B) Head-on view of a chameleon tongue (*C. fischeri*) immediately before prey capture, clearly showing the formation of a dorsal (upper) and a ventral (lower) 'lip'. (C) Lateral view of a chameleon tongue (*C. oustaleti*) capturing a large (5 cm) locust. Note how the tongue has completely engulfed the head of the locust. e, eye; l, lower tongue 'lip'; p, prey (cricket); u, upper tongue 'lip'.

accelerator (Fig. 2). In addition, several pairs of intrinsic muscles connect the tongue pad to the accelerator muscle. Of these, the pouch retractor (see Houston, 1828) is the largest muscle (up to 7% of the total hyolingual muscle mass in small chameleons; A. Herrel and J. J. Meyers, personal observations). This paired muscle runs from its origin at the dorsolateral side of the m. accelerator to the inner aspect of the tongue pad and inserts at the internal aspect of the postero-ventral side of the so-called pouch or dimple (see Fig. 2). In the animals examined in this study, no fibres of the m. hyoglossus were observed to be continuous with those of the pouch retractor muscle.

The pouch retractor muscle has a separate and distinct innervation, provided by a branch of the hypoglossal nerve running along the accelerator muscle and providing motor input to this muscle (see Meyers and Nishikawa, 2000).

### *Kinematics*

Chameleons were filmed in lateral, ventral and frontal views using high-speed video and cine systems while eating a variety of prey (crickets, grasshoppers and lizards). A first qualitative analysis of these recordings clearly showed that chameleons do not use the typical iguanid or agamid adhesive-based tongue prehension system. Upon tongue protraction (the slow opening

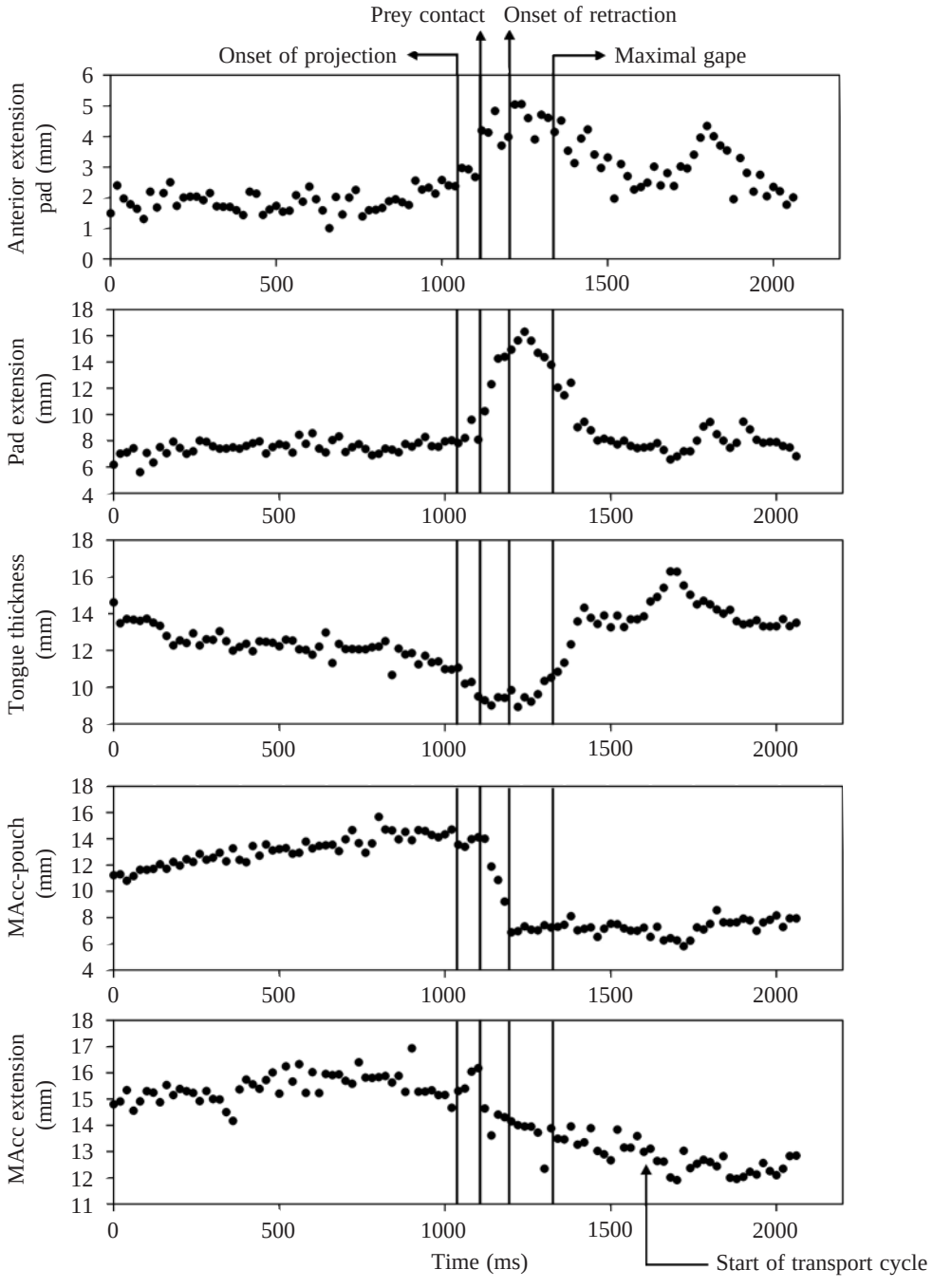


Fig. 4. Changes in selected kinematic variables during a capture and subsequent transport cycle in a *Chameleo pardalis*. The vertical lines indicate distinct kinematic phases during the capture cycle (indicated at the top of the figure). From top to bottom, the shape change of the anterior part of the tongue pad (anterior pad extension; distance between markers 13 and 14 in Fig. 1), extension of the whole pad (distance between markers 12 and 14), changes in tongue thickness (distance between markers 13 and 9), changes in the distance between the markers implanted in the pouch and the accelerator muscle (distance 7–11) and extension of the m. accelerator (distance between markers 7 and 9) are shown. MAcc, m. accelerator.

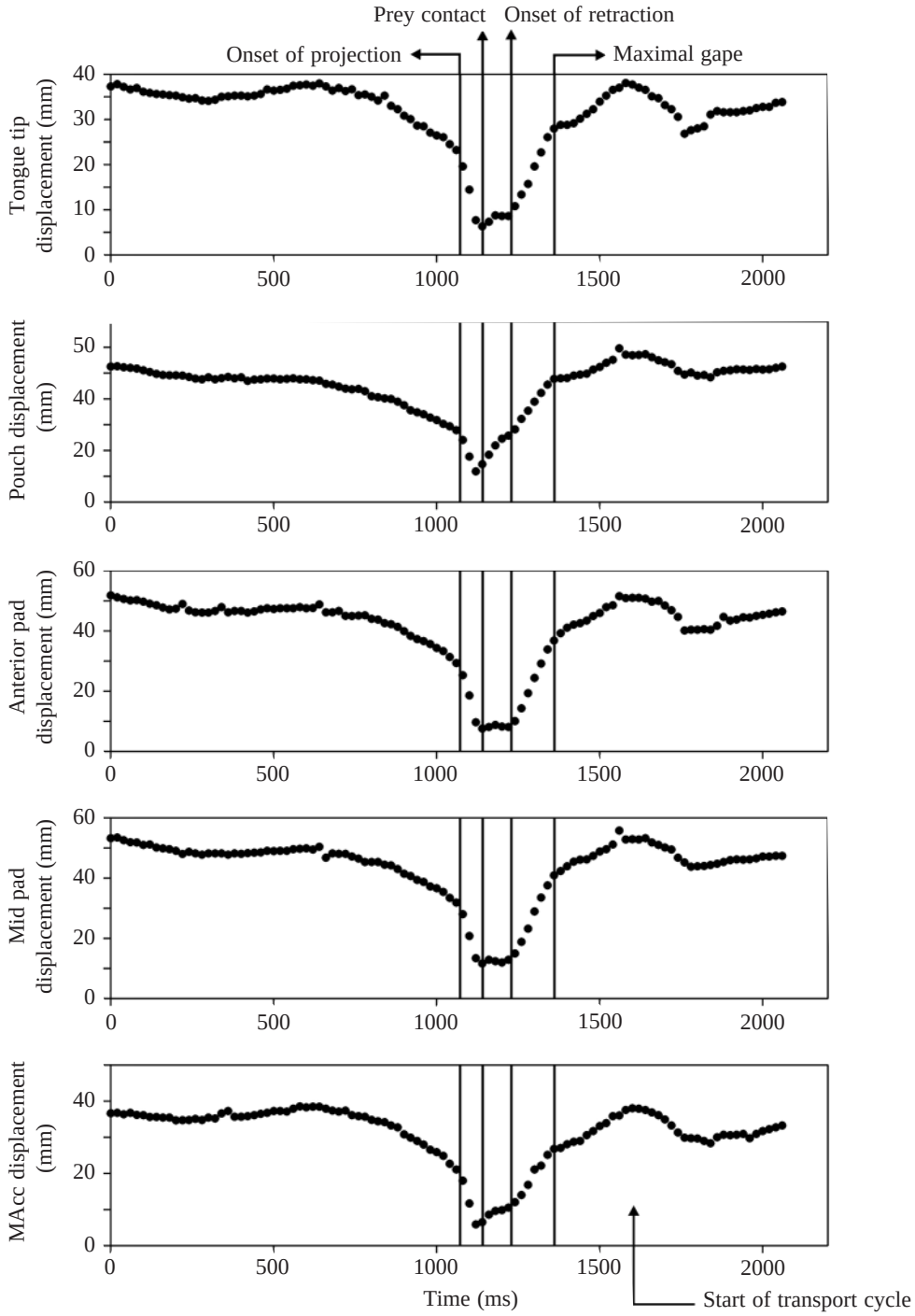


Fig. 5. Horizontal displacement (*versus* time) of selected markers implanted in the tongue of a *Chameleo pardalis* during a capture and subsequent transport cycle. The vertical lines indicate distinct kinematic phases during the capture cycle (indicated at the top of the figure). From top to bottom, the displacements of the tongue tip (marker 10 in Fig. 1), the pouch (marker 11), the anterior tongue pad (marker 14), the mid tongue pad (marker 13) and the accelerator muscle (marker 7) are represented. Anterior displacements correspond to a ventral deflection of the curve. MAcc, m. accelerator.

phase), the pouch or dimple (which is usually observed in anaesthetised animals) has disappeared and the tongue pad appears rounded. After projection, but before prey contact, the tongue deforms, and two lip-like structures are created (one ventral and one dorsal; Fig. 3). Next, the tongue is decelerated and the lips are positioned over the prey. Once the prey has been grabbed, the tongue appears to continue to move over the prey, with the dorsal and ventral lips (mainly the ventral lip) rolling inwards towards the lumen of the pouch in a conveyer-belt-like way. At the onset of tongue retraction, the entire head of large prey, or the entire prey when it is small, is engulfed in the tongue (Fig. 3). Quantification of the high-speed recordings shows that the first inward motion of the pad (in frontal view) is observed between 18 and 6 ms prior to prey contact. The first shape change in the tongue pad (in lateral view) is observed 4–6 ms before that.

The analysis of X-ray films of prey capture events (Figs 4, 5) indicates that, at rest, the anterior tip of the tongue is invaginated to form a so-called pouch. Upon tongue protraction, this pouch is everted. During the preparatory phase

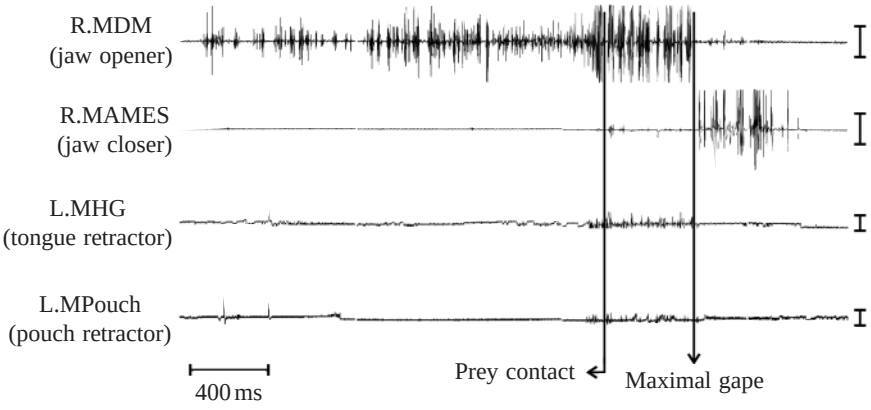


Fig. 6. Representative original electromyogram of the activity in selected jaw and hyolingual muscles in an adult *Chameleo pardalis* eating a large (50 mm) grasshopper. The jaw opener (right m. depressor mandibulae, R.MDM) is active throughout mouth opening. After maximal gape, the jaw closer (right m. adductor mandibulae externus superficialis, R.MAMES) becomes active and closes the jaws. Just before prey contact, the tongue retractors (left and right mm. hyoglossi, L.MHG+R.MHG) and the pouch retractor (M.Pouch) become active. Vertical scale bars represent 0.5 mV.

(the slow opening phase), the tongue is protruded from the mouth (Wainwright et al., 1991) and its thickness gradually decreases. In the anterior tongue pad, a slight elongation can be observed, and the pouch is maximally everted. Tongue projection results in a rapid elongation of the entire tongue pad and the invagination of the pouch (Fig. 4). During prey contact, the tongue pad is kept stationary (as indicated by the position of the anterior pad marker), but the pouch and tongue tip move backwards, thus engulfing the prey (Fig. 5). The accelerator muscle, which was extended during protraction, relaxes at prey contact, reducing the distance between the anterior and posterior markers (Fig. 4). The inward movement of the pouch marker is considerably larger (e.g. up to 1 cm in an adult *C. pardalis*; Figs 4, 5) than that of the tongue tip (up to 3 mm) or anterior tongue pad (1–2 mm). During tongue retraction, the thickness of the tongue increases again, and the tongue pad shortens to its original length. Tongue retraction consists of a rapid initial and slower second phase (Fig. 5). During the subsequent intraoral transport cycle, the prey is released from the pouch (opening of the pouch, reflected in an increase in tongue thickness), and shape changes are restricted to the anterior tongue area.

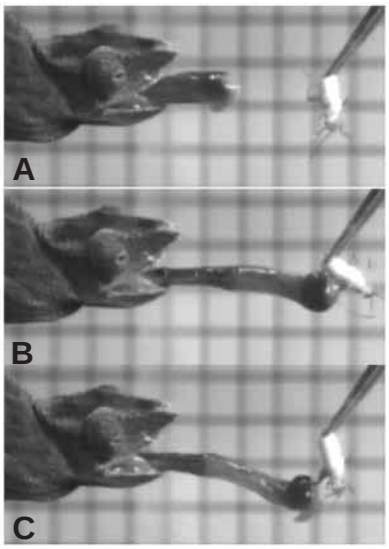


Fig. 7. A prey capture sequence after denervation of the pouch retractor muscle in a female *Chameleo fischeri* attempting to capture a cricket. Note how the pouch is still everted during projection (A), but pushes the prey away upon contact (B,C). However, neither at prey contact nor during tongue retraction are the characteristic 'lips' (see Fig. 3) formed.

### *Electromyography*

To investigate whether the pouch retractor muscle is indeed responsible for the observed invagination of the pouch just before or at prey contact, electrodes were implanted bilaterally into this and several other jaw and hyolingual muscles. The electromyographic recordings show that the pouch retractor muscle is indeed active during prey capture. The activity in this muscle always begins shortly before prey capture and lasts until the tongue has been retracted within the oral cavity (Fig. 6). As reported previously (Wainwright and Bennett, 1992), jaw opening is associated with activity in the m. depressor mandibulae, jaw closing with activity in the external adductor musculature, tongue projection with activity in the m. accelerator and tongue retraction with activity in the m. hyoglossus. Quantification of the electromyographic recordings indicates that activity in the pouch retractor muscle starts between 23 and 5 ms before the onset of tongue projection. However, the first movements of the pouch do not begin until immediately before or at the moment of prey contact. This implies a delay of up to 50 ms between the onset of activity in the pouch retractor and the first inward movement of the pouch. Simultaneously, the tongue retractor and the m. accelerator show a burst of activity (see also Wainwright and Bennett, 1992), and they may be acting as antagonists to the pouch retractor.

However, as data were gathered for only one animal, the observed recruitment patterns should be regarded as indicative only; further electromygraphic experiments are needed to confirm or reject these patterns.

### *Nerve transections*

A critical test of the observed muscle activity pattern (i.e. the activity of the pouch retractor muscle) was performed by bilaterally transecting the hypoglossal nerve branch innervating the pouch retractor muscle in two *C. fischeri*. Qualitative analyses of prey capture sequences show that the

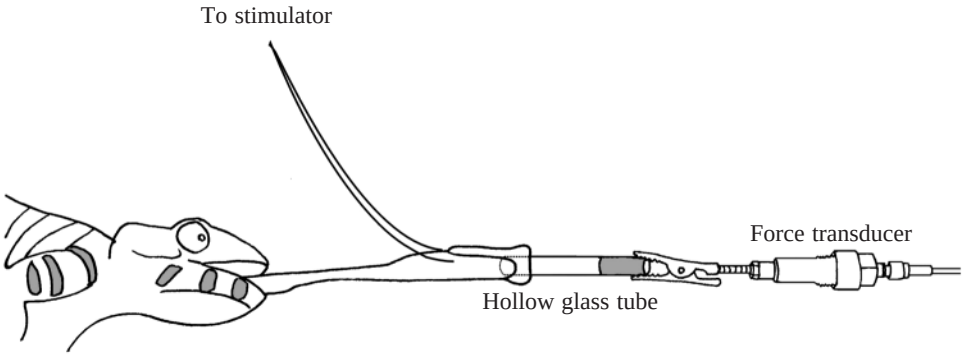


Fig. 8. Schematic drawing illustrating the stimulation experiment. A hollow glass tube, attached to a force transducer, was inserted into the tongue of an anaesthetised and immobilised chameleon (*Chameleo melleri* or *C. calyptratus*). Stimulation electrodes were inserted bilaterally into the pouch retractor muscles. Upon retraction of the glass tube, adhesive forces are measured as the maximal tensile force recorded with the force transducer.

surgery did not affect the general tongue projection kinematics in these animals. The kinematic profiles and the projection distance or duration were unaffected by this surgery. The results of this experiment clearly show that, after nerve transection, the pouch is no longer formed during tongue projection. Instead, the animals push the prey away with their tongue upon contact (Fig. 7). These results thus confirm the preliminary electromygraphic data and show that the pouch retractor muscle is indeed responsible for the invagination of the pouch during prey capture. After surgery, the animals were no longer able to capture any prey successfully, regardless of the size (small *versus* large crickets) of the prey offered.

### *Stimulation experiment*

One final test was performed to investigate whether the observed increase in prehensile capacity was due (i) to simple friction (i.e. adhesive bonding and interlocking), (ii) to an increase in adhesive properties resulting from the activation of the pouch retractor muscle (reshaping of the tongue pad) or (iii) to suction created by the tongue on the prey after the lips are put in place. During a first test, a hollow glass tube attached to a force transducer was inserted into the pouch of anaesthetised animals and retracted. Next, the tube was replaced, the pouch retractor muscles stimulated and the tube pulled back (Fig. 8). In a third test, the tube was sealed at both ends and the previous experiment was repeated. A two-way analysis of variance performed on the raw data indicated significant individual ($F_{2,45}$=5.14; $P$<0.01) and treatment ($F_{2,45}$=5.14; $P$<0.001) effects. Interaction effects were not significant ($F_{4,45}$=1.42; $P$>0.05). Subsequent *post-hoc* tests (Duncan multiple-range tests) indicated that the ‘sealed with stimulation’ treatment was significantly different from the other two treatments (both $P$<0.01). The difference in force required to pull the tube out of the mouth in the three conditions indicates that approximately 13 % of the total prehensile force is due to adhesive properties, approximately 17 % to increased adhesive properties and the remaining 70 % to suction forces (Table 1). Although the relative contribution of different mechanisms will probably depend on the texture and shape of the prey item, this experiment clearly demonstrates that suction plays an important role during prey prehension.

## Discussion

The development of a tongue and a tongue-based mechanism of food transport are undoubtedly two of the key innovations associated with the evolutionary transition from an aquatic to a terrestrial environment. The development of a tongue was probably constrained by the drastic differences in the physical properties of the new medium in which feeding had to take place. Tongue transport is largely based on suface phenomena that create friction between the prey and the tongue (Bramble and Wake, 1985), so the use of a tongue-based mechanism of food transport in itself strongly constrained the available prey spectrum of the first terrestrial vertebrates. Scaling relationhips between mass and area dictate that tongue size would have to grow disproportionately to prey size if larger (and thus heavier) prey were to be transported. Obviously, tongue-based prey capture mechanisms (and especially ballistic ones) are constrained in similar ways because both the gravitational and inertial forces acting on the prey during tongue retraction have to be countered.

It is generally thought that chameleons, like other iguanians, rely on serous and mucous secretions and on interlocking to hold the prey on the tongue after capture (Bramble and Wake, 1985; Bell, 1989; Bels et al., 1994). On the basis of

Table 1. *The force required to release a test object from the tongue*

| Specimen | Force (N) | *N* |
|---|---|---|
| Open tube, no stimulation | | |
| *Chamaelio melleri* 1 | 0.03±0.02 | 5 |
| *C. melleri* 2 | 0.05±0.04 | 7 |
| *C. calyptratus* | 0.02±0.02 | 5 |
| Open tube, stimulation of the pouch retractor muscle | | |
| *C. melleri* 1 | 0.06±0.02 | 5 |
| *C. melleri* 2 | 0.11±0.06 | 7 |
| *C. calyptratus* | 0.07±0.04 | 7 |
| Sealed tube, stimulation of the pouch retractor muscle | | |
| *C. melleri* 1 | 0.21±0.14 | 5 |
| *C. melleri* 2 | 0.37±0.14 | 6 |
| *C. calyptratus* | 0.24±0.10 | 9 |

Values are means ± S.D.

morphological and photographic data, Schwenk (1983) suggested that, during prey capture, the tongue hits the prey and is splayed, resulting in the forcible discharge of mucous. Interlocking by free-standing cells on the tongue surface and suction (for large smooth prey) were also put forward as possible adhesive mechanisms (Schwenk, 1983). However, we demonstrate here that the shape of the tongue is actively controlled by muscular action (indicated by the absence of shape changes after denervation of the pouch retractor muscles), and that shape changes begin before prey contact. The tongue is thus not splayed upon impact, making the forcible discharge of mucous through impact phenomena unlikely. Instead, the tongue pad, which is evaginated during projection, is actively invaginated immediately before prey contact, is placed over the prey and is responsible for pulling the prey inwards. Once contact has been made with the prey, adhesive mechanisms (i.e. wet adhesion and interlocking) become important in maintaining a grip on the prey while it is being transferred further into the pouch.

Our data confirm the suggestion of Schwenk (1983) that suction plays an important role in the mechanics of chameleon tongue prehension. More than two-thirds of the total force generated by the tongue in chameleons is due to suction, thus enabling the animals to capture much larger prey (up to 15 % of their body mass) than would be possible using surface phenomena alone (the maximum size of prey effectively transported with the tongue in a generalized agamid lizard, *Plocederma stellio*, was approximately 5 % of its body mass; but, regarding the strength of adhesive bonding in *Phrynocephalus helioscopus*, see Schwenk, 2000). Clearly, a suction process is enabled by the rearrangement of the intrinsic tongue musculature in chameleons so that the tongue pad can be withdrawn to form a pouch-like structure. Interestingly, an evolutionary precursor for this unique arrangement of the intrinsic musculature (a modified arrangement of the fibres of the m. hyoglossus) may be present in agamid lizards (K. Schwenk, personal communication; note that this depends on the nature of the relationship between chameleons and agamids).

The withdrawal of the tongue pad and the subsequent formation of a pouch not only create suction forces on the prey, but also increase the adhesive properties of the tongue considerably, presumably by increasing the contact surface area and possibly by reorientating the tongue papillae (resulting in increased interlocking). Moreover, in addition to allowing chameleons to capture large prey, the action of the pouch retractor muscle is essential for prey capture in general. Denervation of the motor nerve branch innervating the pouch retractor muscles showed that the animals could still project their tongue but were unable to capture prey. Instead, the prey was pushed away upon contact (even for short projection distances with low tongue accelerations) and did not stick to the tongue upon retraction. Given this constraint, both the prehensile tongue and the novel prehension mechanism probably evolved simultaneously in the ancestor of the present-day chameleons.

Although it has been suggested that similar suction-based capture systems may be present in other vertebrates, such as toads and lungless salamanders (see also Gans and Gorniak, 1982; Bramble and Wake, 1985) that make use of a ballistic tongue to capture prey, we suggest that active suction, as seen in chameleons, is probably only present in arboreal organisms. Terrestrial animals such as toads can use inertia-based mechanisms to slap the tongue onto the prey (positioned on the ground) and thus maximise contact surface area. However, arboreal organisms with ballistic tongues (e.g. lungless salamanders) are likely to push potential prey off branches when relying on impact mechanisms, and such animals would be expected to have evolved ‘prehensile’ mechanisms.

### *Evolutionary considerations*

Tongue prehension is the predominant mode of prey capture in the most primitive group of lizards, the Iguania (Schwenk and Throckmorton, 1989; Schwenk, 2000). All representatives of this large group use their tongue to capture small prey items (Schwenk, 2000). Generally, these animals are ambush predators, relying on crypsis to reduce the possibility of being detected by potential prey or predators. Within the Iguania, chameleons have taken this behavioural strategy to an extreme by developing a ballistic tongue projection mechanism, enabling them to capture prey from a distance without needing to move their body. Here, we demonstrate that, in concert with the development of a ballistic tongue, chameleons have evolved a novel, suction-based prey prehension mechanism allowing them to capture even large prey. Both the prehensile tongue and the novel prehension mechanism probably evolved simultaneously, since without an active pouch retraction system chameleons cannot capture prey. The novel prey prehension mechanism described here was observed in several different species from both chameleon subfamilies (both the *Chameleo* and the *Brookesia*/*Rhampholeon* radiations; see Klaver and Böhme, 1987) and probably represents a primitive trait for the entire family. Given the importance of the mechanism we describe for tongue prehension in chameleons, it presumably originated early in their evolutionary history.

We thank Professor Dr F. De Vree for allowing us to use the equipment in his laboratory, J. Fret for excellent technical assistence, the entire Nishikawa laboratory for helpful comments and K. D’Août for taking the photograph used in Fig. 3. P.A. is research director and A.H. is a postdoctoral research associate of the Fund for Scientific Research, Flanders (FWO-Vl). Supported by NSF grant 9809942 and NIH grant R25-GM56931 to K.C.N.

## References

**Bell, D. A.** (1989). Functional anatomy of the chameleon tongue. *Zool. Jb. Anat.* **119**, 313–336.

**Bels, V. L., Chardon, M. and Kardong, K. V.** (1994). Biomechanics of the hyolingual system in Squamata. In *Advances in Comparative*

*and Environmental Physiology*, vol. 18 (ed. V. L. Bels, M. Chardon and P. Vandewalle), pp. 197–240. Berlin: Springer.

**Bramble, D. M. and Wake, D. B.** (1985). Feeding mechanisms of lower tetrapods. In *Functional Vertebrate Morphology* (ed. M. Hildebrand, D. M. Bramble, K. F. Liem and D. B. Wake), pp. 230–261. Cambridge, MA: Harvard University Press.

**Broadley, D. G.** (1973). Predation on birds by reptiles and amphibians in South-Eastern Africa. *The Honeyguide* **76**, 19–21.

**Emerson, S. B. and Diehl, D.** (1980). Toe pad morphology and mechanisms of sticking in frogs. *Biol. J. Linn. Soc.* **13**, 199–216.

**Gans, C. and Gorniak, G. C.** (1980). Electromyograms are repeatable: precautions and limitations. *Science* **210**, 795–797.

**Gans, C. and Gorniak, G. C.** (1982). Functional morphology of lingual protrusion in marine toads (*Bufo marinus*). *Am. J. Anat.* **163**, 195–222.

**Gnanamuthu, C. P.** (1930). The anatomy and mechanism of the tongue of *Chameleon carcaratus* (Merrem). *Proc. Zool. Soc. Lond.* **1**, 467–486.

**Gnanamuthu, C. P.** (1936). Comparative study of the hyoid and tongue of some typical genera of reptiles. *Proc. Zool. Soc. Lond. B* **107**, 1–63.

**Herrel, A., Aerts, P., Fret, J. and De Vree, F.** (1999). Morphology of the feeding system in agamid lizards; ecological correlates. *Anat. Rec.* **254**, 496–507.

**Houston, J.** (1828). On the structure and mechanism of the tongue of the chameleon. *Trans. R. Irish Acad.* **15**, 177–201.

**Humason, G. L.** (1979). *Animal Tissue Techniques*. San Fransisco, CA: Freeman.

**Klaver, C. and Böhme, W.** (1986). Phylogeny and classification of the Chamaeleonidae (Sauria) with special reference to hemipenis morphology. *Bonn. Zool. Mon.* **22**, 1–64.

**Meyers, J. J. and Nishikawa, K. C.** (2000). Comparative study of tongue protrusion in three iguanian lizards, *Sceloporus undulatus*, *Pseudotrapelus sinaitus* and *Chamaeleo jacksonii*. *J. Exp. Biol.* **203**, 2833–2849.

**Mivart, S. G.** (1870). On the myology of *Chameleo parsonii*. *Proc. Sci. Meet. Zool. Soc. Lond.* **57**, 850–890.

**Nishikawa, K. C.** (1987). Staining amphibian peripheral nerves with sudan black B: progressive vs. regressive methods. *Copeia* **1987**, 489–491.

**Schleich, H. H., Kästle, W. and Kabisch, K.** (1996). *Amphibians and Reptiles of North Africa*. Koenigstein, Germany: Koeltz Scientific Publishers.

**Schwenk, K.** (1983). Functional morphology and evolution of the chameleon tongue tip. *Am. Zool.* **23**, 1028.

**Schwenk, K.** (2000). Feeding in lepidosaurs. In *Feeding in Tetrapod Vertebrates: Form, Function, Phylogeny* (ed. K. Schwenk). London: Academic Press (in press).

**Schwenk, K. and Throckmorton, G. S.** (1989). Functional and evolutionary morphology of lingual feeding in squamate reptiles: phylogenies and kinematics. *J. Zool., Lond.* **219**, 153–175.

**Taylor, W. R. and Van Dyke, G. C.** (1978). *Revised Procedures for Staining and Clearing Small Fishes and Other Vertebrates for Bone and Cartilage Study*. Privately printed.

**Wainwright, P. C. and Bennett, A. F.** (1992). The mechanism of tongue projection in chameleons. I. Electromyographic tests of functional hypothesis. *J. Exp. Biol.* **168**, 1–21.

**Wainwright, P. C., Kraklau, D. M. and Bennett, A. F.** (1991). Kinematics of tongue projection in *Chameleo oustaleti*. *J. Exp. Biol.* **159**, 109–133.