
New perspectives on the evolution of lung ventilation mechanisms in vertebrates

E.L. Brainerd

Department of Biology and Program in Organismic and Evolutionary Biology,
University of Massachusetts, Amherst MA 01003, USA

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Abstract. In the traditional view of vertebrate lung ventilation mechanisms, air-breathing fishes and amphibians breathe with a buccal pump, and amniotes breathe with an aspiration pump. According to this view, no extant animal exhibits a mechanism that is intermediate between buccal pumping and aspiration breathing; all lung ventilation is produced either by expansion and compression of the mouth cavity via the associated cranial and hyobranchial musculature (buccal pump), or by expansion of the thorax via axial musculature (aspiration pump). However, recent work has shown that amphibians exhibit an intermediate mechanism, in which axial muscles are used for exhalation and a buccal pump is used for inhalation. These findings indicate that aspiration breathing evolved in two steps: first, from pure buccal pumping to the use of axial musculature for exhalation and a buccal pump for inspiration; and second, to full aspiration breathing, in which axial muscles are used for both inhalation and exhalation. Furthermore, the traditional view also holds that buccal pump breathing was lost shortly after aspiration breathing evolved. This view is now being challenged by the discovery that several species of lizards use a buccal pump to augment costal aspiration during exercise. This result, combined with the observation that a behavior known as „buccal oscillation“ is found in all amniotes except for mammals, suggests that a reappraisal of the role of buccal pumping in extant and extinct amniotes is in order.

Key words. Aspiration – Buccal pump – Functional morphology
Physiology – Respiration

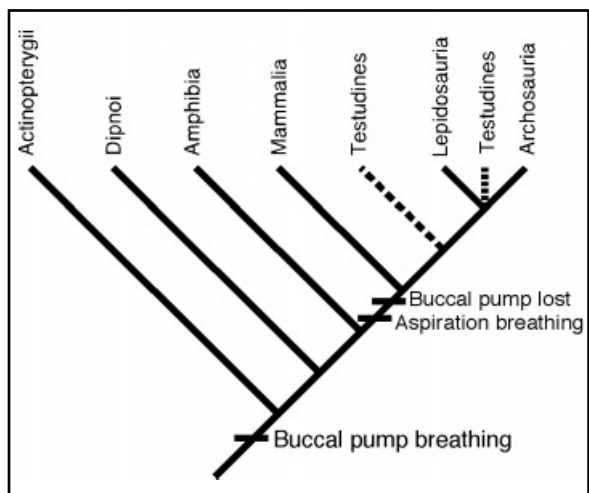
Introduction

In most comparative physiology and comparative anatomy textbooks, the evolution of vertebrate lung ventilation is treated in only a few sentences, if at all (e.g. Kardong, 1997; Kent and Miller, 1997; Schmidt Nielsen, 1994; Withers, 1992). Such brevity is possible because the traditional view of lung ventilation is quite simple: air-breathing fishes and amphibians breathe with a buccal pump, whereas amniotes breathe with an aspiration pump. According to this view, all extant vertebrates are either buccal pumpers or aspiration breathers; no intermediate mechanisms are found. The perceived lack of intermediate forms has impeded attempts to study the evolution of respiratory mechanisms (Gans, 1970a). Paleontologists, functional morphologists and comparative physiologists have argued for decades over the breathing mechanisms of extinct tetrapods, with little consensus emerging (Clack, 1989; Coates and Clack, 1991; Gans, 1970b; Hicks and Farmer, 1998; Packard, 1976; Romer, 1972; Ruben *et al.*, 1997).

In recent years, however, a more complex and informative view of lung ventilation has begun to emerge from detailed studies of living vertebrates (Brainerd, 1994a; Brainerd and Monroy, 1998; Carrier, 1991; Liem, 1985, 1988, 1989). The goal of this paper is to review the information available from studies of extant animals, and then to combine these data with phylogenetic and paleontological information to reconstruct the evolutionary history of respiratory mechanisms in vertebrates.

Two distinct lung ventilation mechanisms are found in vertebrates: (1) buccal pump breathing, in which the mouth cavity expands and compresses to pump air into the lungs under positive pressure; and (2) aspiration breathing, in which the thorax expands to generate sub-atmospheric pressure, thereby drawing air into the lungs. Buccal pump breathing is found in air-breathing,

Fig. 1. Traditional view of the evolution of lung ventilation mechanisms in vertebrates. Buccal pumping is found in ray-finned fishes (Actinopterygii), lungfishes (Dipnoi) and amphibians. Aspiration breathing is found in mammals, turtles (Testudines), lizards and snakes (Lepidosauria) and birds and crocodilians (Archosauria). Buccal pumping is assumed to have been lost at the base of the Amniota, shortly after aspiration breathing evolved. (Cladogram after Gauthier *et al.*, 1988; Hedges and Poling, 1999)



ray-finned fishes, such as *Amia* and *Lepisosteus*, in lungfishes, *Protopterus*, *Lepidosiren* and *Neoceratodus*, and in the extant amphibians, Anura, Caudata and Gymnophiona. Aspiration breathing is found in all amniotes (mammals, turtles, lizards, snakes, birds and crocodilians). From the phylogenetic distribution of lung ventilation mechanisms, it is clear that buccal pump breathing evolved before aspiration breathing (Fig. 1). In the traditional view of the evolution of lung ventilation mechanisms, the transition from buccal pumping to aspiration breathing occurred quite abruptly. No extant animals possess an intermediate mechanism, and aspiration breathers lost the ability to buccal pump for lung ventilation shortly after aspiration breathing evolved (Fig. 1; Gans, 1970a, 1970b).

Buccal pump breathing

Two types of buccal pump breathing have been described: two-stroke and four-stroke (named by analogy with two-stroke and four-stroke engines; Brainerd, 1994a; Brainerd *et al.*, 1993). A two-stroke breath begins as the mouth cavity expands to draw fresh air into the buccal cavity (Fig. 2). Exhalation of gas from the lungs occurs during this buccal inhalation, and the exhaled air mixes with the fresh air. Some of this mixture is then pumped into the lungs, and the rest exits via the mouth and gill slits. Because some of the expired gas is rebreathed, two-stroke breathing seems as though it would be quite inefficient. However, a study of lung ventilation in larval tiger salamanders (*Ambystoma tigrinum*) found that 80% of the inspired gas is fresh air and only 20% is rebreathed (Brainerd, 1998). The high proportion of fresh air results from the large volume of air that is drawn into the buccal cavity to mix with a relatively smaller volume of gas exhaled from the lungs. Only a small proportion of this mixed gas is pumped into the lungs, and the rest is exhaled from the mouth and gill slits (Fig. 2). Two-stroke breathing is found in most frogs, in terrestrial phase salamanders, and in some aquatic salamanders (*Siren*, *Necturus* and the larvae of ambystomatids, salamandrids and dicamptodontids).

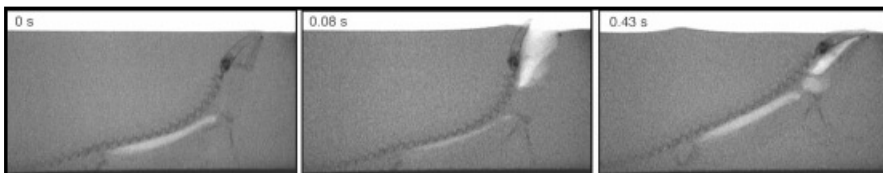
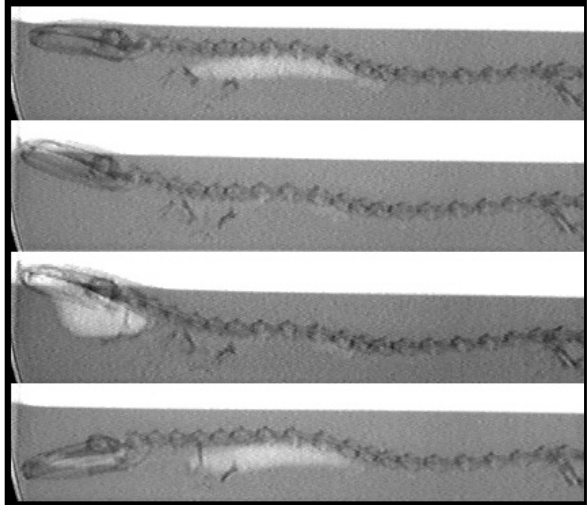


Fig. 2. Videoradiograph of two-stroke buccal pumping in an aquatic phase, larval salamander, *Ambystoma tigrinum* (10× slow motion). The video clip shows X-ray positive images in which X-ray dense materials such as bone and water appear dark, and air appears white. A breath begins as

the mouth cavity expands to draw fresh air into the buccal cavity. Exhalation occurs during this buccal inhalation, and the exhaled air mixes with the fresh air. Some of this mixture is then pumped into the lungs, and the rest exits via the mouth and gill slits

Fig. 3. Videoradiograph of four-stroke buccal pumping in an aquatic salamander, *Amphiuma tridactylum*. The video clip shows X-ray positive images in which X-ray dense materials such as bone and water appear dark, and air appears white. Artificial breathing sounds have been added to emphasize the air movements. Note that the well-ossified elements of the hyobranchial apparatus are retracted and depressed to expand the buccal cavity and are protracted and elevated for compression



In four-stroke breathing, the buccal cavity expands during exhalation, and then compresses fully to expel all of the exhaled gas (Fig. 3). The buccal cavity then expands for a second time, and compresses to pump fresh air into the lungs. Because the buccal cavity compresses fully after exhalation, no mixing of expired and inspired air occurs in the buccal cavity, and no expired gas is rebreathed. Four-stroke buccal pumping is found in basal ray-finned fishes and in a few aquatic amphibians (*Xenopus*, *Amphiuma* and *Cryptobranchus*, Brainerd and Dumka, 1995; Brainerd *et al.*, 1993; Brett and Shelton, 1979).

In both two-stroke and four-stroke breathing, additional buccal pumps may occur after the first expiration-inspiration cycle. These accessory buccal pumps are purely inspiratory and they serve to add extra air to the lungs. In some aquatic salamanders, *Siren* and *Cryptobranchus*, and in lungfishes, *Lepidosiren* and *Protopterus*, one or two accessory buccal pumps per breath are commonly observed. In an elongated salamander, *Amphiuma tridactylum*, up to seven accessory buccal pumps have been observed (T. Landberg and E. Brainerd, unpublished observations), and caecilians routinely use 10–20 buccal pumps to fill their lungs (Bennett *et al.*, 1999; Carrier and Wake, 1995). The large number of accessory buccal pumps seen in *A. tridactylum* and in caecilians may result from the relatively large lung volume and small buccal volume of these highly elongate animals.

Aspiration breathing

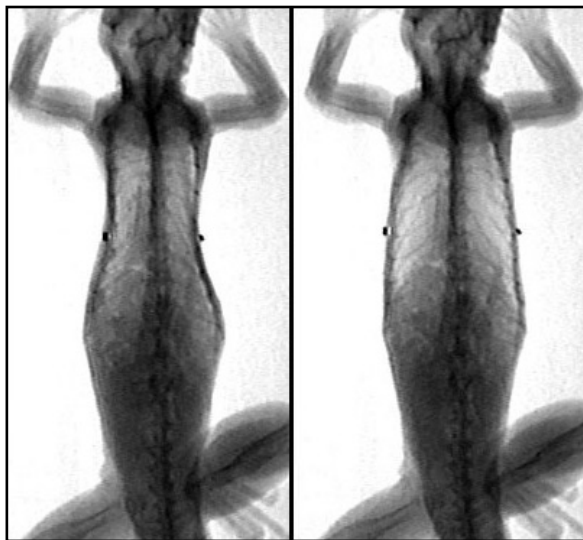
All extant amniotes use some form of aspiration pump for lung ventilation (Gans, 1970b; Liem, 1985). In the primitive condition, aspiration breathing was probably powered entirely by rotation of the ribs (costal aspiration). This condition is retained in extant lepidosaurs (lizards and snakes), as exemplified by

the green iguana, *Iguana iguana* (Fig. 4). Exhalation is powered by the transversus abdominis and retrahens costarum muscles, and inhalation is powered by the external and internal intercostal muscles (Carrier, 1989). In green iguanas, as in most lepidosaurs, the body cavity is not divided into separate pleural and peritoneal spaces. Instead, the lungs and viscera are contained within a single, „pleuroperitoneal“ cavity. This condition is also seen in air-breathing fishes and amphibians, indicating that an undivided body cavity is the primitive condition for amniotes.

In mammals, a muscular diaphragm muscle is present in addition to the primitive costal aspiration system. The diaphragm is a dome-shaped muscle which divides the body cavity into separate pleural and peritoneal (thoracic and abdominal) cavities. Contraction of the diaphragm causes the dome to flatten, thereby increasing the volume of the thoracic cavity. The ribs contribute little to volume change during resting ventilation, but they serve a critical role in stiffening the chest wall. If ribs were not present, the action of the diaphragm would cause the chest to collapse, rather than producing an increase the volume of the lungs (Perry, 1983, 1987). Exhalation in mammals is driven partially by the passive elastic recoil of the lungs, and partially by activity of the abdominal musculature (transversus abdominis, rectus abdominis and external and internal obliques; Abe *et al.*, 1996; De Troyer and Loring, 1986).

In turtles, the ribs and vertebral column have become incorporated into a dorsal carapace, and the venter is covered with a bony plastron. The ribs are immovably fixed to the carapace; therefore costal aspiration is not possible in turtles. Instead, turtles use movements of the plastron and the limbs and girdles to ventilate their lungs (Gaunt and Gans, 1969). For exhalation, the transversus abdominis and diaphragmaticus muscles elevate the plastron toward

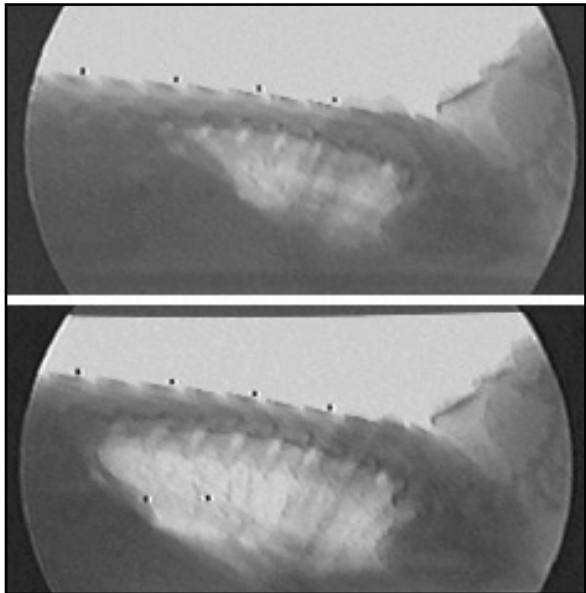
Fig. 4. Videoradiograph of aspiration breathing in a lizard, *Iguana iguana* (dorsoventral projection). The ribs rotate craniolaterally for inhalation and caudo-medially for exhalation. Artificial breathing sounds have been added to emphasize air flow



the carapace, thereby decreasing the volume of the pleuroperitoneal cavity (note that the „diaphragmaticus“ muscle in turtles is not homologous with either the mammalian or the crocodilian diaphragmaticus muscles). During inhalation, the obliquus abdominis and testocoracoideus muscles act to increase the volume of the pleuroperitoneal cavity, thereby aspirating air into the lungs. Movements of the limbs and girdles and deformation of the carapace can also produce changes in lung volume, but these movements do not seem to contribute significantly to routine lung ventilation.

Crocodylians ventilate their lungs with a hepatic piston pump in addition to the primitive costal aspiration pump (Gans and Clark, 1976). The liver divides the body cavity of crocodylians into separate pleural and peritoneal cavities. A unique muscle, found only in crocodylians, originates from the pelvis and caudal gastralia and inserts onto a thick collagenous septum that adheres to the liver. This muscle is called diaphragmaticus, but it is not homologous with the mammalian diaphragm muscle (or with the turtle diaphragmaticus). The collagenous sheet covering the liver also merges with the collagenous covering of the lungs, thereby attaching the lungs firmly to the cranial surface of the liver. In *Alligator mississippiensis*, a breath begins with exhalation, followed by inhalation and then a pause with the lungs full before the next breath (Fig. 5; this is the pattern seen in most ectothermic vertebrates which, unlike birds and mammals, do not breathe continuously). During exhalation, abdominal muscles (transversus abdominis and external and internal obliques) contract to increase abdominal pressure thereby driving the liver forward and forcing air out of the lungs. The diaphragmaticus muscle then pulls the liver caudally, similar to a piston in a cylinder, thereby expanding the thorax and

Fig. 5. Videoradiograph of hepatic piston breathing in an American alligator, *Alligator mississippiensis*. The head is toward the right of the picture. The *small dark spots* are lead markers glued onto the skin (video footage courtesy of E.H. Wu). The liver moves like a piston within a cylinder to ventilate the lungs



aspirating air into the lungs (Fig. 5). Costal aspiration also contributes to lung ventilation in crocodylians, as does a recently discovered mechanism called pelvic aspiration (C. G. Farmer and D.R. Carrier, Pelvic aspiration in the American alligator (*Alligator mississippiensis*). Submitted to *J. Exp. Biol.*). The pelvic aspiration mechanism is made possible by the odd structure of the pelvis in crocodylians – the pubis does not contribute to the acetabulum, but rather it is a mobile element which is connected by a joint to the cranial aspect of the ischium. The two spatula-shaped pubes can be rotated dorsally during exhalation and ventrally during inspiration, thereby compressing and expanding the abdominal cavity.

Birds possess a highly specialized ventilation system in which flow between flexible, avascular air sacs generates a unidirectional flow of air through a rigid lung structure. This system allows greater extraction of oxygen because air and blood can flow in cross-current directions (Bretz and Schmidt-Nielsen, 1972). The air sacs are ventilated by rocking motions of the enlarged sternum coupled with costal movements. During flight, pressure changes in the air sacs are coupled with the wing beat cycle, indicating that flight can have a substantial mechanical effect on ventilation (Boggs *et al.*, 1997a, 1997b).

Aspiration breathing has also been reported in Polypteridae, a family of fresh-water, air-breathing fishes from Africa (Brainerd *et al.*, 1989). These fishes have fixed, immobile ribs and no evident specializations for aspiration breathing. However, it is clear from videoradiography that they do breathe by aspiration (Fig. 6). A breath begins as they approach the surface and exhale. Then they open their mouths and air rushes down into the lungs before the mouth is closed. Because air flows into the lungs while the buccal cavity is still

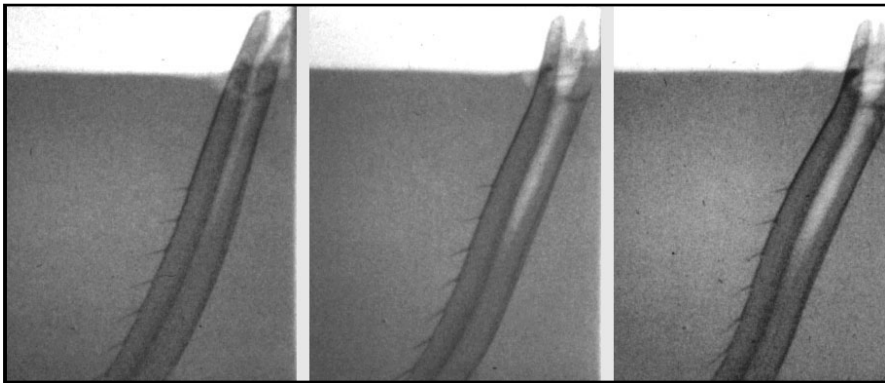
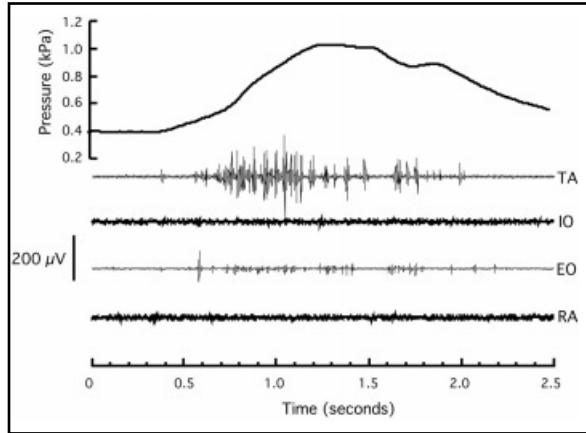


Fig. 6. Videoradiograph of lung ventilation in *Polypterus senegalis* (20× slow motion). The video clip is set to pause at the end of recoil aspiration to demonstrate that air

has flowed into the lungs before the mouth is sealed. After the pause, the mouth seals and one buccal pump adds more air to the lungs

Fig. 7. Body pressure and hypaxial muscle EMGs during exhalation in a hellbender salamander, *Cryptobranchus alleganiensis*. Inspiration is not shown here, but no consistent EMG activity is seen during inspiration in salamanders. Muscle abbreviations: *TA* transversus abdominis, *IO* internal oblique, *EO* external oblique, *RA* rectus abdominis

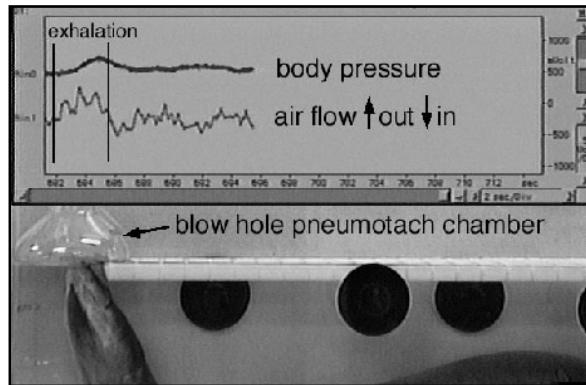


expanding, buccal pumping cannot be responsible for this initial air flow. After aspiration occurs, the mouth closes and extra air is buccal pumped into the lungs. Aspiration in these fishes is produced by the elastic recoil of their interlocking-scale integument (Brainerd, 1994b). Active exhalation, powered by intrinsic lung musculature, deforms the scale jacket; inhalation occurs when the glottis is opened and the scale jacket recoils to its original shape. It is likely that this recoil aspiration mechanism evolved independently of costal aspiration breathing; therefore, it probably has little bearing upon the transition between buccal pump breathing and aspiration in tetrapods (although some early tetrapods did retain ventral, interlocking integument which could have contributed to lung ventilation by recoil aspiration; Brainerd, *et al.*, 1989).

Lung ventilation in amphibians and the evolution of aspiration breathing

Recent work on the mechanics of breathing in salamanders has yielded new insight into the evolution of aspiration breathing in tetrapods (Brainerd, 1998; Brainerd and Dumka, 1995; Brainerd and Monroy, 1998; Brainerd *et al.*, 1993, 1997). Prior to this work, our understanding of amphibian respiratory mechanics was based almost entirely on studies of frogs (Gans *et al.*, 1969; Jones, 1982; de Jongh and Gans, 1969; Vitalis and Shelton, 1990; West and Jones, 1974). These studies concluded that the basic breathing mechanism of frogs is very similar to that found in lungfishes (Bishop and Foxon, 1968; McMahon, 1969). Exhalation is passive, driven by elastic recoil of the lungs and body wall. Inspiration is via a two-stroke buccal pump. In this model, only cranial and hyobranchial muscles contribute to lung ventilation; axial musculature does not contribute at all. In this view, the evolution of aspiration breathing involved a sudden shift of the responsibility for lung ventilation from the cranial and hyobranchial musculoskeletal systems to the axial system, with no extant, intermediate forms (Gans, 1970a).

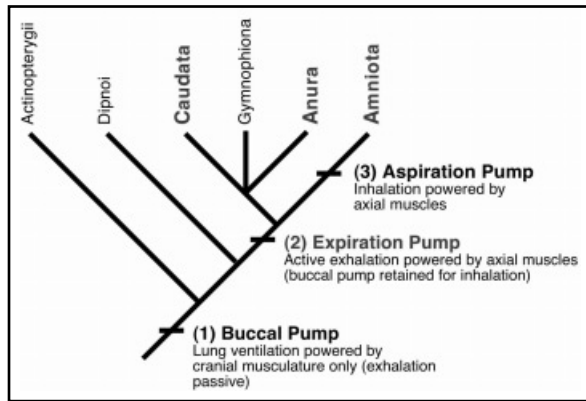
Fig. 8. Lung ventilation in *Amphiuma tridactylum*, a large, nearly limbless, aquatic salamander. Real-time digital oscilloscope traces are superimposed onto a live video of the animal breathing in a blowhole pneumotach chamber made from a plastic drink bottle. Video overlay is produced by replacing a background colour on the computer screen with live video (using a scan converter with genlock), and then outputting the mixed video to a VCR. Flow trace is red, pressure trace is blue



Studies of lung ventilation in salamanders have found, however, that salamanders use axial musculature to power active exhalation (Brainerd, 1998; Brainerd and Dumka, 1995; Brainerd and Monroy, 1998; Brainerd *et al.*, 1993, 1997). Simultaneous measurements of body cavity pressure and muscle activity (by electromyography, EMG) show that the transversus abdominis (TA) muscle is active to increase body pressure and force air out of the lungs (Fig. 7). In some salamanders, such as *Necturus maculosus* and larval *Ambystoma tigrinum*, exhalation is very fast and is produced primarily by activity of the hypaxial musculature; elastic recoil of the lungs contributes little to exhalation (Brainerd, 1998; Brainerd *et al.*, 1993). In other salamanders, such as *Amphiuma tridactylum* and *Siren lacertina*, exhalation is slower, and the first half of exhalation is passive (Brainerd and Dumka, 1995; Brainerd and Monroy, 1998). This passive exhalation is produced primarily by elastic recoil of the lungs, and exhalation is probably assisted by the hydrostatic pressure difference between the caudal end of the lungs and the glottis. Air flow traces from a blowhole pneumotach system combined with pressure measurements from the body cavity show that body pressure does not increase during the first, passive part of exhalation, but that it does increase during the second half of exhalation when the remaining air is actively squeezed out of the lungs (Fig. 8).

Five species from five families of salamanders have now been studied, and all were found to use hypaxial musculature, primarily the TA, for active exhalation (Sirenidae, *Siren lacertina*; Cryptobranchidae, *Cryptobranchus alleganiensis*; Proteidae, *Necturus maculosus*; Ambystomatidae, *Ambystoma tigrinum*; Amphiumidae, *Amphiuma tridactylum*). This widespread distribution indicates that use of the TA for exhalation (an „expiration pump“) was present in the common ancestor of all salamanders. The TA has also been shown to contribute to exhalation in all amniotes, in one aquatic frog during routine lung ventilation (*Xenopus*; de Jongh, 1972) and in all frogs during vocalization

Fig. 9. Simplified cladogram of vertebrate relationships with the acquisition of aspiration breathing characters mapped onto it. Clades in which hypaxial musculature contributes to active exhalation (expiration pump) are shown in red



(e.g. Martin and Gans, 1972; Marsh and Taigen, 1987). No muscles appear to be active during exhalation in the one caecilian which has been studied, but this may be related to the high, resting body pressure of these animals which contributes to their internal concertina mode of locomotion (Bennett *et al.*, 1999; Carrier and Wake, 1995; O'Reilly *et al.*, 1997; Summers and O'Reilly, 1997). Lungfishes (Dipnoi) and ray-finned fishes (Actinopterygii) use passive exhalation with no contribution from hypaxial musculature (Brainerd *et al.*, 1993; Deyst and Liem, 1985). This phylogenetic distribution of the expiration pump indicates that the ability to recruit axial musculature for exhalation is a shared-derived character found only in tetrapods (Fig. 9).

The discovery that an axial muscle-based, expiration pump is primitive for tetrapods suggests the following scenario for the evolution of aspiration breathing (Fig. 9): (1) prior to the evolution of tetrapods, a buccal pump was used for lung ventilation, with no contribution from axial musculature; (2) in the common ancestor of tetrapods, hypaxial musculature was recruited for exhalation and the buccal pump was retained for inspiration; (3) in amniotes, intercostal musculature and ribs were recruited for inhalation by costal aspiration. In this scenario, aspiration breathing did not evolve by a sudden, complete shift of the responsibility for lung ventilation from the cranial and hyobranchial musculoskeletal systems to the axial system. Instead, the transfer occurred in two stages: initially, axial musculature was only used for exhalation; later, axial musculature came to be used for both exhalation and inhalation.

Buccal pumping and buccal oscillation in amniotes

In the traditional view of the evolution of lung ventilation, not only does aspiration breathing evolve all at once, but this view has also held that buccal pumping was lost shortly after the evolution of aspiration breathing (Fig. 1). However, recent studies of lizards have shown that, in addition to costal aspi-

Fig. 10. Videoradiograph of lung ventilation in a leopard gecko (*Eublepharis macularius*) immediately after cessation of locomotion on a treadmill. Each breath begins with exhalation, followed by costal aspiration. The buccal cavity compresses during exhalation to eject the expired gas, and then expands to fill with fresh air during costal aspiration. The buccal cavity then compresses to pump more air onto the lungs

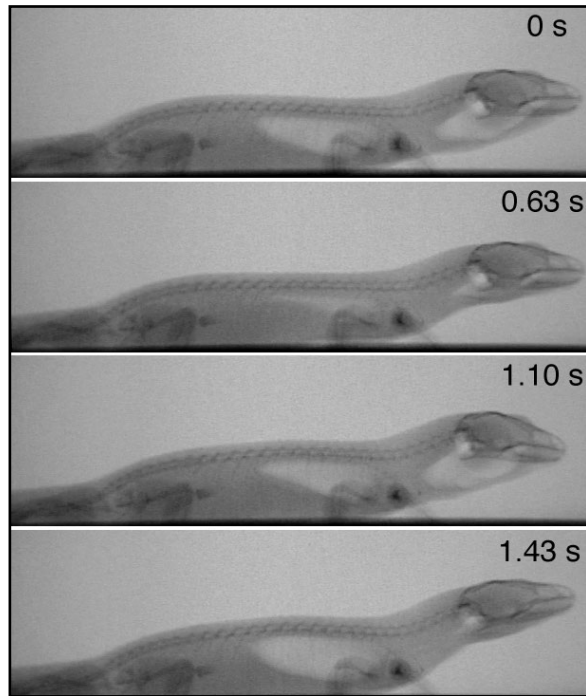
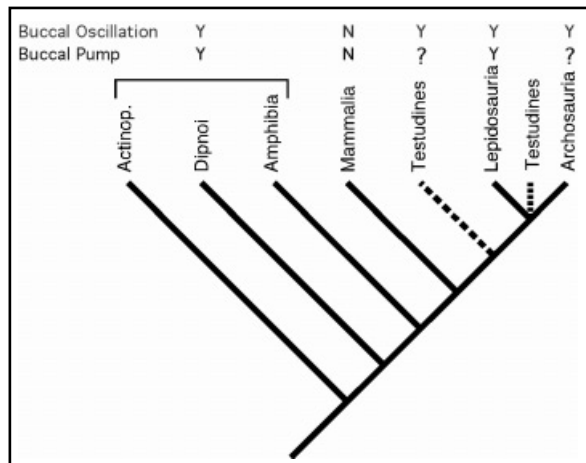


Fig. 11. The distribution of buccal oscillation and buccal pumping in vertebrates. Definition of group names: Actinopterygii, ray-finned fishes; Dipnoi, lungfishes; Amphibia, salamanders, frogs and caecilians; Mammalia, mammals; Testudines, turtles; Lepidosauria, lizards and snakes; Archosauria, crocodilians and birds



ration, some lizards also use a buccal pump to assist lung ventilation (Brainerd and Owerkowicz, 1996; Owerkowicz and Brainerd, 1997; note that the buccal pump is sometimes called a gular pump in lizards because the hyobranchial apparatus is located in the neck region). Lizards do not normally buccal pump while at rest, but some species do buccal pump during locomotion and during the first few breaths taken while standing still after locomotion. Leopard geckos, for example, utilize a buccal pump extensively during

and after locomotion (Fig. 10). Each breath begins with exhalation, followed by costal aspiration which only partially fills the lungs. The buccal cavity also compresses during exhalation to expel the exhaled gas, and then expands to fill with fresh air during costal aspiration. The internal nares are then sealed, probably by the tongue, and the buccal cavity compresses to pump more air into the lungs. The first buccal pump is either followed by exhalation, or by more buccal pumps in series before exhalation occurs again. Buccal pumping during and after locomotion has also been observed in a monitor lizard (*Varanus exanthematicus*, Brainerd and Owerkowicz, 1996; Owerkowicz and Brainerd, 1997), and preliminary observations indicate that other lizards, particularly highly active species, may also supplement costal aspiration with buccal pumping (unpublished data, Brainerd and Owerkowicz).

The discovery of buccal pumping in lizards represents the first time that any amniote has been shown to buccal pump for lung ventilation. Two studies looked for and failed to find buccal pumping in a lacertid lizard and in a crocodylian, but these studies did not look for buccal pumping during locomotion (Cragg, 1978; Naifeh and Huggins, 1970). Various movements which are called buccal pumping, gular pumping or gular flutter have previously been reported in lizards, but these movements have always been attributed to defensive and aggressive displays, thermoregulation, olfaction, or buccopharyngeal gas exchange. In defensive and aggressive displays, the lungs are hyperinflated with a buccal pump (sometimes called gular pump) in order to increase the apparent size of the animal (Bels *et al.*, 1995; Deban *et al.*, 1994; Salt, 1943). For olfaction, air is oscillated in and out of the nares (across the olfactory epithelium) without being pumped into the lungs (Dial and Schwenk, 1996; Weldon and Ferguson, 1993). For thermoregulation, air is oscillated rapidly in and out of the mouth – this is a form of panting which is sometimes called gular flutter (Heatwole *et al.*, 1973). One study suggests that significant gas exchange can occur across the buccal epithelium in *Trachysaurus rugosus*, and oscillation of air in and out of the buccal cavity may ventilate this epithelium (Drummond, 1946). It would be interesting to examine other species to determine whether significant buccopharyngeal gas exchange also occurs in other lizards.

It is important to distinguish clearly between buccal oscillation, in which air moves in and out of the buccal cavity without being pumped into the lungs, and buccal pumping, in which the mouth and nares are sealed and air is pumped into the lungs from the buccal cavity (as distinguished by de Jongh and Gans, 1969). Buccal pumping is associated with lung ventilation and inflation of the lungs for display. Buccal oscillation is associated with thermoregulation (panting), olfaction (sniffing), and buccopharyngeal respiration. In both behaviours, the hyobranchial apparatus is retracted and depressed and then protracted and elevated. However, during buccal oscillation, the nares are open and the glottis is closed, whereas during buccal pumping, the nares are closed and the glottis is open.

Fig. 12. Buccal oscillation in an aquatic turtle, *Staurotypus triprocatus*. A real-time digital oscilloscope trace of air flow is superimposed onto a live video of the animal breathing in a blowhole pneumotach chamber made from a plastic drink bottle. Buccal expirations are the same volume as the buccal inspirations, indicating that these oscillations are not pumping any air into the lungs

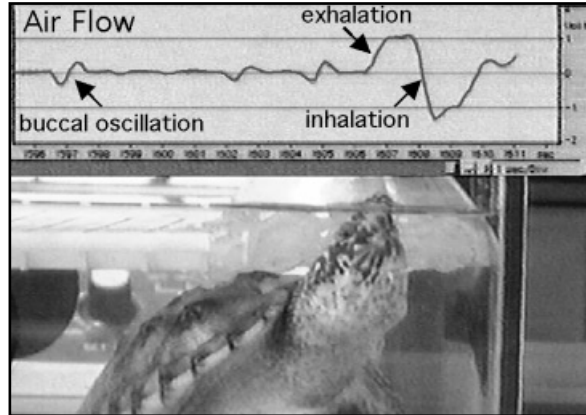
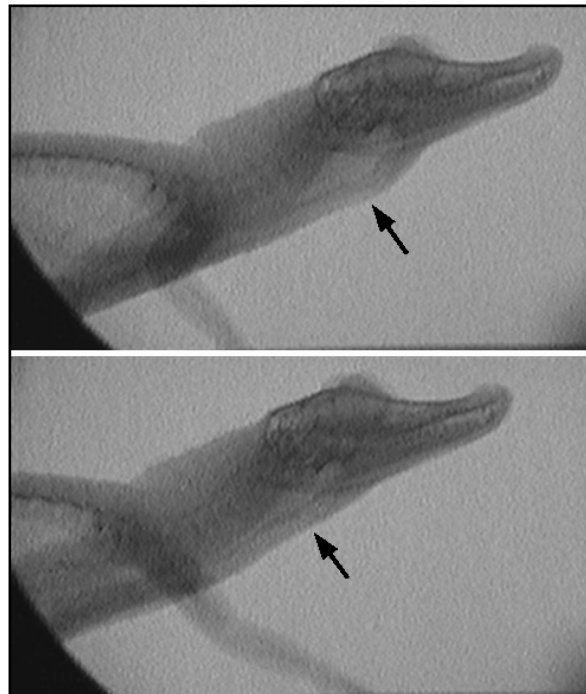


Fig. 13. Videoradiograph of buccal oscillation in an American alligator, *Alligator mississippiensis*. Air flows in and out of the mouth cavity without being pumped into the lungs



Buccal oscillation is present in all major groups of vertebrates, with the exception of mammals (Fig. 11). Buccal oscillation is also absent in snakes, but because it is present in diverse lizards, it is likely that buccal oscillation is primitive for lepidosaurs. The loss of buccal oscillations in snakes and mammals may be due to specialization of the tongue and hyoid for tongue flicking in snakes and for intraoral food manipulation in mammals. Turtles perform buccal oscillations both in air and underwater (Hansen, 1941; Root, 1949), and it is possible that they also buccal pump for lung inflation under certain cir-

cumstances. However, preliminary studies of two aquatic turtles, *Staurotypus triproctatus* and *Emydoidea blandingii*, found no evidence that air is pumped from the buccal cavity into the lungs (Fig. 12; unpublished data, T. Landberg and E. Brainerd). Crocodylians buccal oscillate extensively during activity (Fig. 13). It is also possible that they buccal pump for lung ventilation, but a recent study found no evidence for this, even during locomotion (C. G. Farmer and D.R. Carrier, Pelvic aspiration in the American alligator (*Alligator mississippiensis*). Submitted to *J. Exp. Biol.*). Birds perform buccal oscillations for cooling (gular flutter), but it seems unlikely that they would use buccal pumping because their hyoid and buccal cavity are so small relative to the ventilation needs.

Both buccal oscillation and buccal pumping are found in air-breathing fishes, amphibians and Lepidosauria (Fig. 11). It is somewhat unclear from this distribution whether buccal pumping evolved independently in lepidosaurs, or was retained from non-amniote ancestors. However, it is clear that buccal oscillation has been retained from anamniotes within most of the amniote lineages, and it seems likely that any animal which is capable of buccal oscillation could buccal pump relatively easily by changing the valving sequence of the nares and glottis. Therefore, in contrast to the traditional scenario in which buccal pumping was lost shortly after the evolution of aspiration breathing (Fig. 1), it is now clear that buccal oscillation and possibly buccal pumping have been retained as accessories to aspiration breathing in most amniotes (mammals being the exception, which is probably why the ubiquity of buccal oscillation has been overlooked in the past).

The discovery that buccal pumping was probably not lost with the advent of aspiration breathing forces a reappraisal of lung ventilation mechanisms and the function of the hyobranchial apparatus in extinct vertebrates. The longstanding debate about whether the earliest amphibians were buccal pumpers or aspiration breathers must now include the possibility that they used both mechanisms (Coates and Clack, 1991; Gans, 1970*b*; Packard, 1976; Romer, 1972). In addition, large hyoid bones have been preserved in the fossils of some pterosaurs and theropod dinosaurs (Dal Sasso and Signore, 1998; Padian, 1984). It is intriguing to consider that these structures may have assisted lung ventilation in these and possibly other extinct amniotes.

Conclusions

Aspiration breathing evolved in two steps: initially, axial muscles were recruited for exhalation; later, axial muscles and ribs were recruited for inspiration by costal aspiration.

Buccal pumping was not abandoned when costal aspiration breathing evolved. Both mechanisms are present in extant lizards, and both mechanisms may well have been present in some groups of extinct amphibians and amniotes.

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