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Respiratory Physiology & Neurobiology 154 (2006) 73–88

RESPIRATORY PHYSIOLOGY & NEUROBIOLOGY

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Functional morphology and evolution of aspiration breathing in tetrapods

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Accepted 12 June 2006

Abstract

In the evolution of aspiration breathing, the responsibility for lung ventilation gradually shifted from the hyobranchial to the axial musculoskeletal system, with axial muscles taking over exhalation first, at the base of Tetrapoda, and then inhalation as well at the base of Amniota. This shift from hyobranchial to axial breathing freed the tongue and head to adapt to more diverse feeding styles, but generated a mechanical conflict between costal ventilation and high-speed locomotion. Some "lizards" (non-serpentine squamates) have been shown to circumvent this speed-dependent axial constraint with accessory gular pumping during locomotion, and here we present a new survey of gular pumping behavior in the tuatara and 40 lizard species. We observed gular pumping behavior in 32 of the 40 lizards and in the tuatara, indicating that the ability to inflate the lungs by gular pumping is a shared-derived character for Lepidosauria. Gular pump breathing in lepidosaurs may be homologous with buccal pumping in amphibians, but non-ventilatory buccal oscillation and gular flutter have persisted throughout amniote evolution and gular pumping may have evolved independently by modification of buccal oscillation. In addition to gular pumping in some lizards, three other innovations have evolved repeatedly in the major amniote clades to circumvent the speed-dependent axial constraint: accessory inspiratory muscles (mammals, crocodylians and turtles), changing locomotor posture (mammals and birds) and respiratory-locomotor phase coupling to reduce the mechanical conflict between aspiration breathing and locomotion (mammals and birds).

Keywords: Breathing mechanics; Buccal pump; Aspiration; Gular pump; Buccal oscillation; Gular flutter; Evolution; Comparative method; Extant phylogenetic bracket; Homology; Comparative respiratory physiology; Tetrapoda; Amphibia; Amniota; Mammalia; Testudines; Lepidosauria; Squamata; Archosauria; Crocodilia; Aves

1. Introduction

The functional morphology and evolution of aspiration breathing have been active areas of research in the past two decades. Earlier studies tended to

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doi:10.1016/j.resp.2006.06.003

focus on mechanics of breathing at rest (reviewed in Liem, 1985), but more recent work has focused on lung ventilation during trackway, treadmill or wind-tunnel locomotion (e.g., Carrier, 1987a; Boggs et al., 1997a; Wang et al., 1997; Owerkowicz et al., 1999; Farmer and Carrier, 2000b; Nassar et al., 2001; Deban and Carrier, 2002; Frappell et al., 2002; Landberg et al., 2003; Klein et al., 2003; Klein and Owerkowicz, 2006). Progress toward reconstructing the evolutionary history of aspiration breathing has been made by the application of comparative phylogenetic methods (e.g., Brainerd et al., 1993; Brainerd, 1999; Perry and Sander, 2004), and considerable recent work has also focused on reconstructing the breathing mechanisms of non-avian dinosaurs (Ruben et al., 1999; Carrier and Farmer, 2000; Claessens, 2004b; O'Connor and Claessens, 2005) and on the evolution of respiratory rhythm generators (Straus et al., 2003; Vasilakos et al., 2005).

Here, we briefly review some of the older findings, but focus primarily on new developments since the last review (Brainerd, 1999), add some new data on the phylogenetic distribution of gular pumping in lepidosaurs, and explore the concepts of constraint and innovation in the evolution of tetrapod respiratory pumps.

2. Lung ventilation in air-breathing fishes and amphibians

Integral to the evolution of aspiration breathing from buccal pumping was a complete shift in the respiratory pump mechanism from the head region to the thoracoabdominal region of the tetrapod body. The primitive condition, pure buccal pumping, is found in most air-breathing fishes, including lungfishes. In pure buccal pumping, axial musculature does not contribute to expiration or inspiration; expiration is powered by various combinations of elastic recoil, hydrostatic pressure and buccal suction, and inspiration takes place by air being drawn into the buccal cavity and pumped under positive pressure into the gas bladder (Liem, 1988; Brainerd, 1994).

Extant amphibians have long been known to use a buccal pump for lung inflation (Fig. 1). Most of the early tetrapod fossils exhibit well-developed ribs with clear costo-vertebral articulations (Carroll, 1988), which led some experts in functional morphology (e.g., Gans, 1970) and vertebrate paleontology (e.g., Romer, 1972) to posit that these forms were capable of aspiration breathing. However, while rib mobility is necessary for aspiration breathing, rib mobility does not necessarily make an animal an aspiration breather. Among extant amphibians, frogs and salamanders possess only short ribs fused to vertebrae, which effectively precludes any costal movements in these taxa, but caecilians possess mobile ribs (Wake, 2003), yet use only the buccal pump for inspiration (Carrier and Wake, 1995). The possibility remains that aspiration breathing was present in some early tetrapods, but the evidence from extant amphibians suggests that aspiration breathing did not arise until the appearance of the early amniotes.

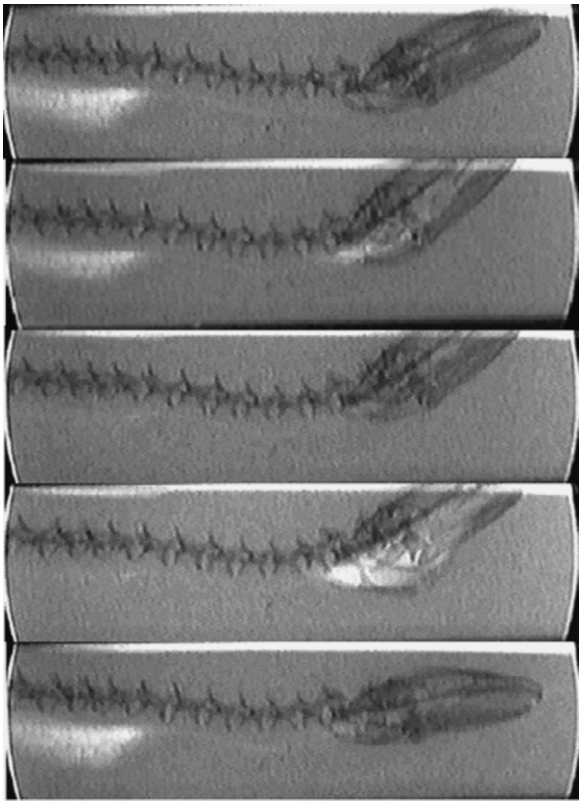

Fig. 1. Buccal pump breathing in an aquatic salamander, *Amphiuma tridactylum*. A time series of X-ray positive images from videofluoroscopy is shown in which air in the buccal cavity and lungs appears white and bones appear dark. In the first two frames, the transverse abdominal muscle contracts to power active exhalation, and in the last two frames hyobranchial depression draws air into the buccal cavity and then hyobranchial elevation pumps air into the lungs under positive pressure.

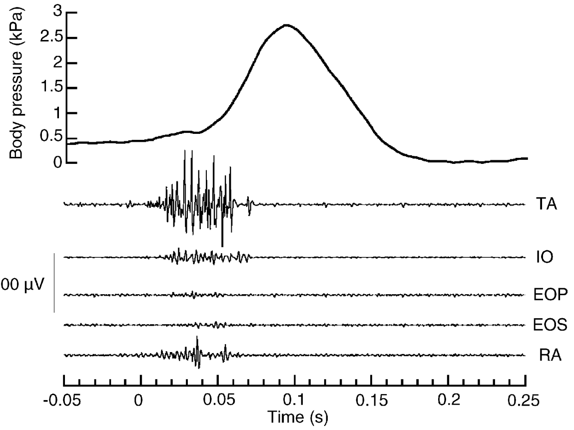

Fig. 2. The expiration pump in a larval tiger salamander, *Ambystoma tigrinum.* Electromyograms of the lateral hypaxial musculature were recorded simultaneously with pressure in the body cavity during exhalation. Strong activity in the transverse abdominal (TA) muscle increases body cavity pressure for active exhalation. *Muscle abbreviations*: TA, transverse abdominal; IO, internal oblique; EOP, external oblique profundus; EOS, external oblique superficialis; RA, rectus abdominis. From Brainerd, 1998.

Although extant amphibians inflate their lungs with a buccal pump, work in the past decade on salamanders has shown that the transverse abdominal (TA) layer of the lateral hypaxial musculature is active and generates increased body cavity pressure during exhalation (Fig. 2). Active expiration has been documented in five species from five families of salamanders: Proteidae, *Necturus maculosus* (Brainerd et al., 1993); Sirenidae, *Siren lacertina* (Brainerd and Monroy, 1998); Ambystomatidae, *Ambystoma tigrinum*, larval and adult stages (Brainerd, 1998; Simons et al., 2000); Amphiumidae, *Amphiuma tridactylum* (Brainerd and Landberg, 2001); Cryptobranchidae, *Cryptobranchus alleganiensis* (Brainerd, 1999). This widespread pattern of TA activity indicates that the "expiration pump" is primitive for salamanders.

An expiration pump powered by the TA muscle is probably a shared-derived character of Tetrapoda (Brainerd, 1999). Despite diverse inspiratory mechanisms in amniotes, the primary expiratory muscle in mammals, turtles, squamates, birds and crocodylians is the transverse abdominal (Gaunt and Gans, 1969; Gans and Clark, 1976; De Troyer et al., 1990; Fedde, 1987; Carrier, 1989). The expiratory pump is clearly primitive for Caudata (salamanders) and for Amniota, but passive exhalation occurs in most (De Jongh and Gans, 1969; Bennett et al., 1999), but not all (De Jongh, 1972), members of the other two lissamphibian clades, Anura (frogs) and Gymnophiona (caecilians). These results shed some doubt on whether the expiration pump is primitive for all tetrapods, but it should be noted that, relative to salamanders, frogs and caecilians have highly derived body forms (shortened and elongated trunks, respectively) and locomotor modes (saltatorial and fossorial, respectively), which likely affect their TA function. Since the presence of the TA muscle itself is a tetrapod character, it being absent in ray-finned fishes and lungfishes, these findings are best explained by the expiration pump being a shared-derived character of Tetrapoda. The expiration pump theory suggests that aspiration breathing may have evolved in two steps: first, from pure buccal pump breathing to the use of axial muscles for expiration and a buccal pump for inspiration; second, to pure aspiration breathing, in which axial muscles are used for both expiration and inspiration. Thus, the recruitment of axial muscles for the expiration pump may have been the first step in the transfer of lung ventilation from the head region to the trunk region of the tetrapod body (Brainerd, 1999).

3. Aspiration breathing in amniotes

Costal aspiration is the primitive aspiration breathing mechanism for amniotes (Gans, 1970), and costal aspiration is retained as the primary ventilation mechanism in lepidosaurs and birds. In Lepidosauria (lizards, snakes and tuataras), the intercostal muscles produce craniolateral rotation of the ribs, thereby expanding the thoracoabdominal cavity, reducing thoracoabdominal pressure and drawing air into the lungs (Carrier, 1989).

In birds, inflation of the air sacs takes place by rotation of the ribs and depression of the caudal end of the sternum (Claessens, 2004a). Elevation and depression of the pelvis has been proposed to assist lung ventilation in birds (Baumel et al., 1990; Carrier and Farmer, 2000), but cineradiographic analysis of breathing in free-standing cursorial bird species (emu, guinea fowl and tinamou) found no evidence of pelvic rotation (Claessens, 2004a). A recent study demonstrated the function of the previously enigmatic uncinate processes of bird ribs (Codd et al., 2005). The plate-like uncinate processes, through the action of the appen-

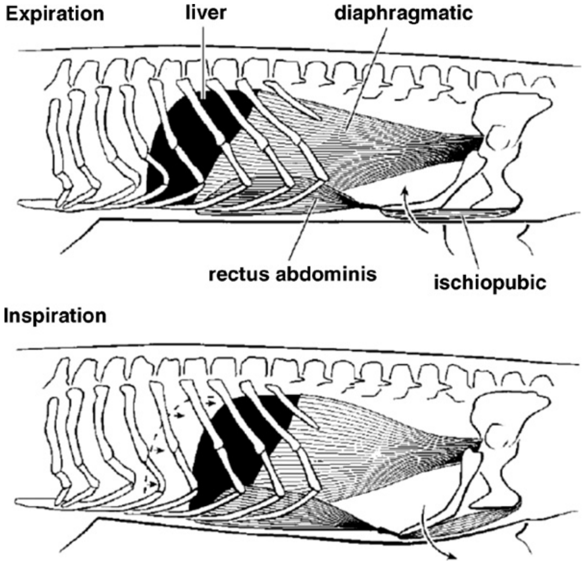

Fig. 3. Aspiration breathing by a combination of hepatic piston pumping (via the diaphragmaticus muscle) and pubic bone rotation (via the ischiopubis muscle) in *Alligator mississippiensis*. From Carrier and Farmer (2000).

dicocostalis muscle, were shown to increase lateral rib rotation in the Canada goose. The respiratory function of the uncinate processes was demonstrated definitively when the birds were resting on their breasts and sternal rotation was constrained, and this finding suggests that the uncinate processes and the appendicocostalis muscle probably contribute to ventilation in other species and during other behaviors as well.

Crocodylians, mammals and turtles have all evolved accessory respiratory muscles that reduce or eliminate their reliance on costal aspiration. These are all sometimes called diaphragm muscles, presumably by analogy with the mammalian diaphragm muscle, but they are not homologous structures. In crocodylians, the primitive thoracoabdominal cavity is divided by the liver into an anterior thoracic cavity and a posterior abdominal cavity (Fig. 3). The diaphragmaticus muscle originates from the pelvis and caudal gastralia and inserts onto a thick connective-tissue sheet surrounding the liver. This connective tissue merges with the visceral pleura, thereby attaching the lungs firmly to the cranial surface of the liver. The diaphragmaticus acts to retract the liver, thereby expanding the thoracic cavity and aspirating air into the lungs (Naifeh and Huggins, 1970; Gans and Clark, 1976). This lung ventilation mechanism is called hepatic piston pumping because the liver movement is similar to a piston sliding in a cylinder. Rib rotation and rotation of the paired pubic plates have also been shown to contribute to lung ventilation in crocodylians (Farmer and Carrier, 2000a) (Fig. 3).

In mammals, the diaphragm muscle divides the thoracoabdominal cavity into thorax and abdomen. In most mammals, the diaphragm is a flat sheet with muscle fibers radiating outward from a central tendon, and the diaphragm's apposition to the cranial surface of the liver gives it a dome-shape. Muscle fiber contraction reduces the curvature of the dome, thereby expanding the thoracic cavity and aspirating air into the lungs. Rib rotation is relatively small in mammals, when compared with rib rotation in lizards that rely only on costal aspiration, and the main ventilatory function of the rib cage in mammals is to prevent collapse of the thoracic wall when the diaphragm contracts and decreases thoracic pressure (Perry, 1989).

The flattening of dome-shaped muscles is also the mechanical basis of ventilation in turtles and tortoises, but there the similarity with mammalian ventilation ends. The so-called diaphragmaticus is an expiratory muscle that assists the TA, and the internal oblique (IO), which is an expiratory muscle in most other amniotes, becomes the primary inspiratory muscle in Testudines (Gaunt and Gans, 1969). The right and left halves of the IO form a pair of curved domes spanning the open region between the carapace and plastron through which the hindlimbs emerge. Contraction of the IO decreases the curvature of these muscle domes, thereby increasing the volume of the thoracoabdominal cavity and aspirating air into the lungs (Landberg et al., 2003).

4. Buccal pumping, gular pumping and buccal oscillation in tetrapods

It was long thought that aspiration and buccal pump breathing do not co-exist in extant tetrapods (Gans, 1970; Liem, 1985), a view that was reinforced by studies that looked for and failed to find buccal pumping in a crocodylian (Naifeh and Huggins, 1970) and a lacertid lizard (Cragg, 1978). These and other studies focused on the mechanics of quiescent breathing in resting animals, when the aerobic demand is minimal. More recently, however, savannah monitor lizards, *Varanus*

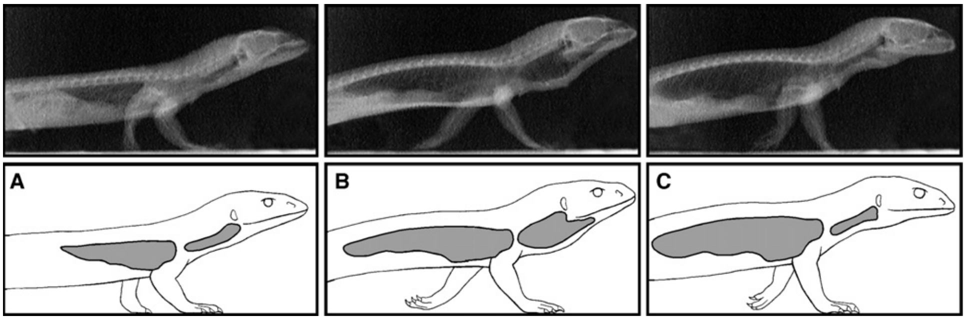

Fig. 4. Costal aspiration and gular pumping during locomotion in a savannah monitor lizard, *Varanus exanthematicus*. In the upper X-ray negative images, air in the lungs appears dark and bones appear white. In the lower schematic diagrams, the laterally projected areas of the lungs and gular cavity are shown in grey. (A) Costal expiration. (B) Costal inspiration and gular cavity filling. (C) Hyobranchial elevation pumps air into the lungs. A single or multiple pumps may occur between each large expiration. From Owerkowicz et al. (1999).

exanthematicus, were found to use gular pumping to supplement costal inspiration during high-speed locomotion (Owerkowicz et al., 1999, 2001). This behavior resembles the buccal pump of air-breathing fishes and amphibians in that the hyobranchial skeleton is retracted and depressed to draw air into the oropharynx, the nares and mouth are sealed, and then the hyobranchium is protracted and elevated to pump air into the lungs (Fig. 4). The homology of buccal pumping and gular pumping is unclear, and the separate term gular pumping has been used for this behavior in amniotes to reflect this uncertainty and also to reflect the more caudal position of the hyobranchium in the neck region or “*gula*” in amniotes rather than in the buccal region as in fishes and amphibians (Table 1).

Here, we present a phylogenetic analysis of buccal and gular pumping in tetrapods based on a new, broadly comparative study of lepidosaurs (presented here for the first time) and some recent studies on lung ventilation during treadmill locomotion in crocodylians and turtles. Two key factors in all of these studies are: (1) they looked for evidence of gular pumping under a variety of conditions, including locomotion, recovery from exercise and simulated predator attack/restraint, rather than only at rest and (2) they used airflow measurements to distinguish clearly between buccal oscillation (*sensu* De Jongh and Gans, 1969), in which air is rhyth-

Table 1
Terminology for rhythmic hyobranchial movements in tetrapods

Term	Definition	Phylogenetic distribution
Buccal pump	Oropharyngeal pump used for lung inflation in air-breathing fishes and amphibians	Actinopterygii, Dipnoi, Lissamphibia
Gular pump	Pharyngeal pump used as an accessory lung inflation mechanism in “lizards” and tuataras	Lepidosauria
Buccal oscillation	Non-ventilatory expansion and compression of the buccal cavity, performed with the mouth closed and usually serving an olfactory function	Lissamphibia, Testudines, Lepidosauria, Crocodylia
Gular flutter	Non-ventilatory expansion and compression of the buccal cavity, performed with the mouth open and usually serving a thermoregulatory function	Lepidosauria, Archosauria, Mammalia[a]

[a] We interpret the panting behavior of mammals to be a form of gular flutter since the hyoid oscillates up and down and it serves a thermoregulatory function. Panting, however, is a derived behavior relative to gular flutter because panting incorporates lung ventilation whereas gular flutter in lepidosaurs and archosaurs does not.

mically pumped in and out of the oropharynx, and gular pumping, in which air is pumped into the lungs (see Table 1 for terminology). Treadmill locomotion studies of lung ventilation in two turtles, *Trachemys* and *Terrapene* (Landberg et al., 2002, 2003) and two crocodylians, *Alligator* and *Crocodylus* (Farmer and Carrier, 2000b; TO, unpublished data) recorded buccal oscillation in these species, but no gular pumping was observed despite rigorous airflow measurement techniques and vigorous treadmill locomotion.

Table 2 shows the results of our survey of gular pumping in 40 species and 17 families of "lizards" (non-serpentine squamates). Almost half of the species (19 of 39) exhibited some accessory lung inflation by gular pumping during treadmill locomotion, and more showed gular pumping during recovery immediately after exercise (24 of 39, with one ambiguous). The largest proportion of species (32 of 38) showed gular pumping when trapped against a flat surface, and a large proportion (26 of 38) also showed gular pumping when restrained by the tail. The latter two restraint methods were chosen as appropriate simulations of a predator attack, which may focus on the tail of the lizard (grabbing and tugging at it) or on the trunk (pinning the animal to the ground).

This survey significantly expands on earlier reports by several workers that certain lizard species use gular pumping for lung inflation (Salt, 1943; Templeton and Dawson, 1963; Templeton, 1964; Deban et al., 1994; Bels et al., 1995; Al-Ghamdi et al., 2001). It further provides evidence that gular pumping is used by lizards under a variety of physiological conditions. Our observations indicate that gular pumping during locomotion is most strongly expressed in varanids. Varanidae (with nine cervical vertebrae) have longer necks than other lizards (eight or fewer cervical vertebrae) and the varanid hyobranchial apparatus, while slender, allows for greater volumetric changes of the gular cavity than in other lizards (Owerkowicz et al., 2001). Varanids are also better able than other lizards to utilize the additional oxygen from air pumped from the pharynx into the lungs (Bennett, 1994; Wang and Hicks, 2004), given their highly partitioned lung structure and functionally separate pulmonary and systemic circulation (Perry, 1989; Burggren and Johansen, 1982; Ishimatsu et al., 1988). That gular pumping can provide a respiratory advantage to varanids has been demonstrated in *V. exanthematicus* by a finding that exercise endurance and the rate of oxygen consumption during treadmill locomotion both decreased when animals were prevented from gular pumping (Owerkowicz et al., 1999). Whether other lizard species derive a respiratory benefit from gular pumping remains to be tested.

The widespread distribution of gular pumping in lizards indicates that this behavior is primitive for Squamata. One of us (TO) observed defensive body inflation in a tuatara (*Sphenodon guntheri*) under hand restraint. This observation indicates that the ability to perform gular pumping is primitive for Lepidosauria. We have not tested snakes extensively for gular pumping. Observations of a few snake species suggest that gular pumping is not expressed in this clade. Despite the lack of consensus among systematists on the exact position of Serpentes within Squamata, it is clear that the clade is deeply nested, and hence lack of gular pumping among snakes does not affect our conclusions that gular pumping is a primitive character for Squamata, and probably for all of Lepidosauria.

The capacity for rhythmic hyobranchial movements is present in all tetrapods, either in the form of non-ventilatory buccal oscillation and gular flutter or in buccal and gular pumping that contribute to lung inflation (Table 1; Fig. 5). In buccal oscillation, the mouth and glottis remain closed and hyobranchial depression and elevation cause air to oscillate in and out of the oropharynx through the nares. The primary function of buccal oscillation in amniotes is thought to be ventilation of the olfactory receptors (McCutcheon, 1943; Naifeh and Huggins, 1970; Dial and Schwenk, 1996). In gular flutter, the mouth is held open and hyobranchial depression and elevation ventilate the oropharyngeal mucosa for evaporative cooling when an animal is under heat stress (Heatwole et al., 1973). Gular flutter has been observed in squamates, turtles, birds and crocodylians (Heatwole et al., 1973; Sturbaum and Riedesel, 1974; Weathers and Schoenbaechler, 1976; ELB, unpublished observations of *Alligator mississippiensis*). Panting in mammals differs from gular flutter in that panting includes shallow lung ventilation by aspiration, but panting also includes hyoid and tongue oscillations (Biewener et al., 1985) that are remarkably similar to gular flutter. Thus, the capacity for rhythmic hyobranchial movements appears to have been retained throughout tetrapod evolution, and it is likely that the motor patterns and rhythm generators for these movements were retained from the gill ventilation behavior

Table 2
Survey of gular pumping behaviors in “lizards” (non-serpentine squamates)

Species name	Family	*N*	Exercise	Recovery	Tailhold	Trap
Iguania						
Agama persimilis	Chamaeleonidae	2	0	0	1	0
Physignathus cocincinus	Chamaeleonidae	2	0	1	1	1
Pogona vitticeps	Chamaeleonidae	3	0	1	1	1
Leiolepis belliana	Chamaeleonidae	2	0	0	1	0
Uromastyx maliensis	Chamaeleonidae	2	1	1	1	1
Basiliscus plumifrons	Corytophanidae	3	1	1	1	1
Basiliscus vittatus	Corytophanidae	1	0	1	0	1
Corytophanes cristatus	Corytophanidae	1	0	0	0	0
Ctenosaura quinquecarinata	Iguanidae	1	0	1	1	1
Iguana iguana	Iguanidae	4	1	1	1	1
Oplurus quadrimaculatus	Opluridae	2	1	1	1	1
Sceloporus poinsetti	Phrynosomatidae	5	1	1	1	1
Anolis biporcatus	Polychridae	2	1	1	1	1
Leiocephalus leiocephalus	Tropiduridae	2	0	0	1	1
Gekkota						
Christinanus marmoratus	Gekkonidae	3	×	×	1	1
Eublepharis macularius	Gekkonidae	2	1	1	×	×
Gekko stentor	Gekkonidae	2	1	1	0	1
Homopholis boivinii	Gekkonidae	2	0	1	1	1
Lialis jicari	Pygopodidae	1	0	0	0	0
Scincomorpha						
Cordylus warreni	Cordylidae	2	1	1	1	1
Gerrhosaurus major	Cordylidae	2	0	0	0	1
Platysaurus platysaurus	Cordylidae	2	0	0	0	1
Zonosaurus quadrilineatus	Cordylidae	2	0	0	0	1
Lacerta agilis	Lacertidae	2	1	1	1	1
Takydromus sexlineatus	Lacertidae	2	1	1	1	1
Chalcides ocellatus	Scincidae	2	0	1	0	1
Eumeces schneideri	Scincidae	2	0	0	1	0
Mabuya quinquetaeniata	Scincidae	2	0	0	0	1
Tiliqua scincoides	Scincidae	2	0	0	0	1
Tropidophorus apulus	Scincidae	1	0	0	0	0
Ameiva ameiva	Teiioidea	4	1	1	1	1
Tupinambis nigropunctatus	Teiioidea	3	0	0	1	1
Lepidophyma flavimaculata	Xantusiidae	2	1	1	1	1
Anguimorpha						
Elgaria coerulea	Anguidae	2	1	1	1	1
Ophisaurus apodus	Anguidae	1	0	0	0	1
Heloderma suspectum	Helodermatidae	2	1	?	×	×
Varanus albigularis	Varanidae	3	1	1	1	1
Varanus exanthematicus	Varanidae	>10	1	1	1	1
Varanus niloticus	Varanidae	1	1	1	1	1
Varanus rosenbergi	Varanidae	>10	1	1	1	1

The number one (1) indicates that gular pumping was observed in at least one individual; zero (0) indicates no observed gular pumping. Question mark (?) means that the observed behavior was ambiguous and × means not tested. *N* = number of individuals tested. *Exercise* is treadmill locomotion. *Recovery* is standing still immediately after exercise. *Tailhold* is holding the base of the tail such that the lizard cannot escape. *Trap* is restraining the lizard by pressing a hand down on top of it and trapping it against a flat surface.

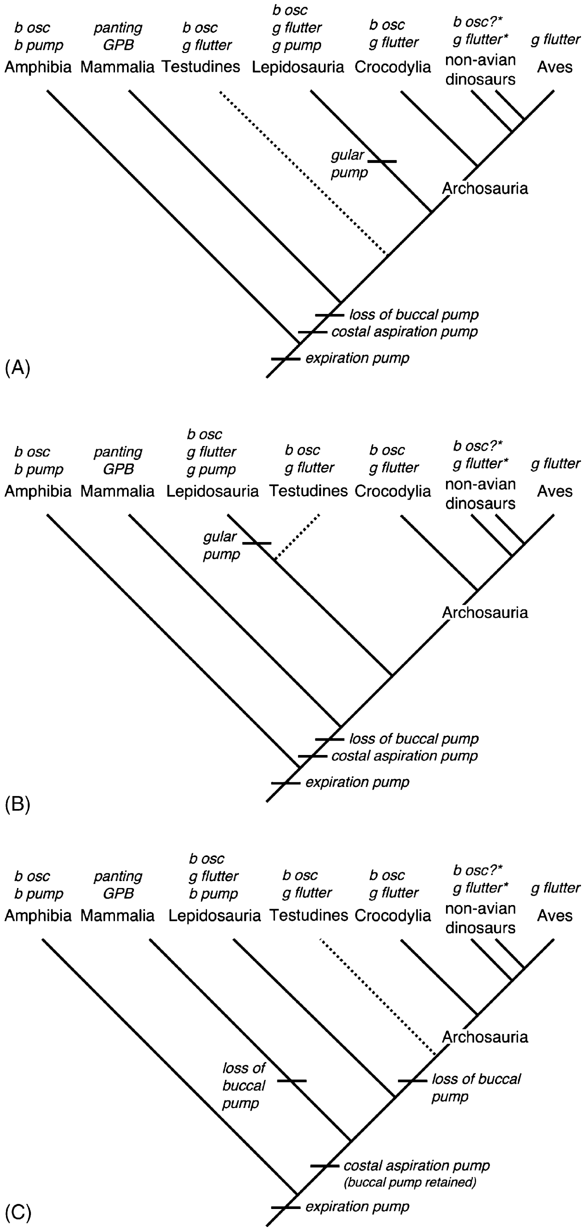

Fig. 5. Phylogenetic distribution of rhythmic hyobranchial movement behaviors in tetrapods. Amniote phylogeny remains controversial, so here we take a conservative approach and explore all three possible arrangements of an unresolved trichotomy between Testudines, Lepidosauria and Archosauria: (A) from Gauthier et al. (1988); (B) from Rieppel and deBraga (1996); (C) from Rest et al. (2003) (and citations therein). See Table 2 for definitions of buccal pump (b pump), gular pump (g pump), buccal oscillation (b osc) and gular flutter (g flutter). Glossopharyngeal breathing (GPB) is a learned breathing behavior in humans (Collier et al., 1956).

of fishes (McMahon, 1969; Smatresk, 1990; Brainerd, 1994; Perry et al., 2001, 2005; Straus et al., 2003; Vasilakos et al., 2005).

Amphibians and lepidosaurs are the only tetrapod groups in which rhythmic hyobranchial movements have been shown to contribute to lung ventilation by buccal or gular pumping (Fig. 5). As described above, recent studies have tested rigorously for the presence of gular pumping in turtles and crocodylians during exercise, and found buccal oscillation but no evidence that hyobranchial movements pump air into the lungs (Farmer and Carrier, 2000b; Landberg et al., 2002, 2003; TO, unpublished data on *Crocodylus porosus*). To our knowledge, no study has ever specifically looked for gular pumping in birds during locomotion or recovery, but it seems unlikely, based on their derived respiratory and hyobranchial morphology, that birds use gular pumping for lung ventilation.

The presence of gular flutter for evaporative cooling in crocodylians and birds suggests that this behavior was present in at least some non-avian dinosaurs (Fig. 5). Buccal oscillation may also have been present, particularly if respiratory rates were low and more frequent olfactory sampling was beneficial, but the absence of buccal oscillation in birds adds greater uncertainty. The absence of buccal/gular pumping in birds and crocodylians suggests that pharyngeal pump breathing was not present in non-avian dinosaurs, unless it evolved independently within some of the dinosaurian clades.

Mammals have not been found to use pharyngeal pump breathing under any natural conditions recorded to date. However, some humans have a remarkable capacity to use glossopharyngeal breathing (GPB) when the intercostal and diaphragm muscles are paralyzed by poliomyelitis or high-level cervical spinal injury (Collier et al., 1956; Warren, 2002; Bianchi et al., 2004). Lung ventilation by GPB is remarkably similar to buccal pumping in amphibians and gular pumping in lizards (Fig. 6; cf. Fig. 8). Tongue depression and retraction combined with jaw depression expand the oropharynx to inspire a small volume of air, and then jaw and tongue elevation pump air

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[*] Behaviors for non-avian dinosaurs are inferred from avian and crocodylian character states, with high confidence in the presence of gular flutter for evaporative cooling and lower confidence (b osc?) in the presence of buccal oscillation because it is absent in birds.

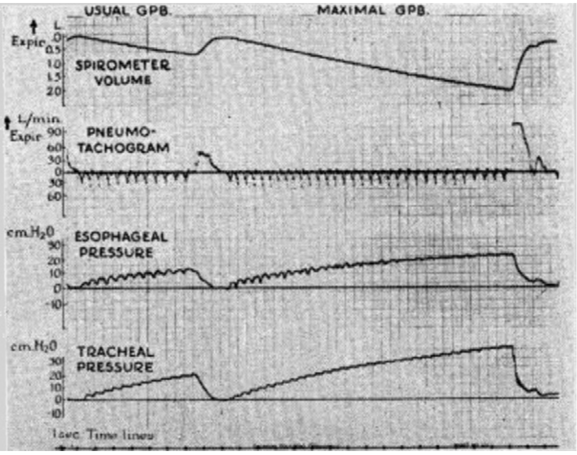

Fig. 6. Mechanics of glossopharyngeal breathing (GPB) in a human with paralysis of the respiratory muscles by poliomyelitis (Collier et al., 1956). The top trace shows lung volume measured with a spirometer, and the lower two traces show esophageal and tracheal pressure, as labeled. Second from the top is a pneumotach trace with many small buccal inspirations and buccal pumps followed by a long, passive expiration. From Collier et al. (1956).

into the lungs. Given the highly constricted pharynx of mammals (when compared to other amniotes), many small buccal pumps are required for each lung inflation. In Fig. 6, 12 buccal pumps produce the "usual GPB", 32 produce the maximal GPB (Collier et al., 1956), and another study reported that a highly proficient GPB patient routinely used three to eight pumps per normal breath (Bianchi et al., 2004). Each series of small inspirations is then followed by a large passive expiration (Fig. 6, trace labeled "pneumotachogram"). Some ventilator-dependent patients (particularly children) discover GPB spontaneously, but most need specific training to learn GPB. Highly proficient GPB patients can breathe without a ventilator for up to 12 h per day, and the behavior seems to become unconscious (Bianchi et al., 2004). Ventilator support is required, however, during sleep. Thus, under pathological conditions, a breathing behavior that is remarkably similar to buccal pumping can be expressed in humans.

It should be noted that GPB, while functionally similar to buccal or gular pumping, is unlikely to be homologous with them. GPB has never been reported in studies of lung ventilation in non-human mammals. GPB is a secondarily learned behavior, found only when aspiration breathing is limited. Interestingly, it may be the unique pharyngeal morphology of humans that makes GPB possible. The descent of the larynx in humans creates an expanded pharyngeal space between the mouth cavity and the glottis, which is then expanded and compressed in a manner analogous to the buccal pump. In other mammals, the larynx remains in contact with the soft palate (to maintain patency between the nasal cavity and the trachea) and the resulting oropharyngeal volume is small. This suggests that non-human mammals would derive negligible benefit from glossopharyngeal pumping. Altogether, this suggests that GPB is a derived, learned behavior found only in humans, but it is possible that people who learn GPB are able to recruit a primitive respiratory rhythm generator for GPB (see Vasilakos et al., 2005 for an evolutionary analysis of respiratory rhythm generators).

Buccal and gular pumping for lung ventilation are present under normal conditions in amphibians and lepidosaurs, and as discussed above, the underlying buccal oscillation pattern seems to be primitive for tetrapods. All that is required to convert buccal oscillation into a lung inflation mechanism is a change in the narial and glottal valving in the buccal compression phase, such that the nares are sealed and the glottis opens to let air into the lungs. From a neural control perspective this may seem like a minor change, but from a functional perspective it is important that the use of pharyngeal pumping *for lung ventilation* appears only in two major groups of tetrapods. The question then arises—was buccal pump breathing retained continuously as aspiration breathing evolved in the lineage leading to amniotes, or was buccal pump breathing lost at the base of amniotes and then gular pump breathing evolved at the base of lepidosaurs as an accessory mechanism?

Phylogenetic relationships among the major reptilian groups remain controversial, with morphological and paleontological studies placing Testudines variably as the sister group to Lepidosauria (Rieppel and deBraga, 1996) or to Archosauria + Lepidosauria (Gauthier et al., 1988). Early molecular studies nested turtles within archosaurs (e.g., Hedges and Poling, 1999), but additional sequence data now place turtles as the sister group to archosaurs and confirm the monophyly of Testudines, Lepidosauria (including *Sphenodon*) and Archosauria (e.g., Rest et al., 2003). Here, we conservatively treat the three primary reptilian lineages as an unresolved trichotomy, and map the evolution of breathing mechanisms onto all three possible resolutions of the trichotomy (Fig. 5).

Table 3
Constraints and innovations in the evolution of tetrapod respiratory pumps

Constraint	Innovation	Phylogenetic distribution
Tongue and head shape constrained by buccal pump	(1) Cutaneous respiration	Plethodontid salamanders
	(2) Aspiration breathing	Amniota
Locomotor endurance constrained by costal aspiration breathing	(1) Gular pump	Lepidosauria
	(2) Accessory inspiratory muscles	Mammalia, Testudines, Crocodylia
	(3) Locomotor posture	Mammalia, Aves
	(4) Respiratory-locomotor phase coupling	Mammalia, Aves

Parsimony mapping of the loss of buccal pumping and the gain (if necessary) of gular pumping onto the three possible cladograms indicates that buccal pumping was lost at the base of Amniota and gular pumping gained at the base of Lepidosauria in two of the three possible phylogenies (Fig. 5A and B). In the third phylogeny, parsimony analysis indicates that buccal pumping was retained in early amniotes and that gular pumping in lepidosaurs is homologous with buccal pumping in amphibians (Fig. 5C). However, even if the trichotomy were resolved, all of these interpretations of character evolution are weak because only one additional character change on any of these cladograms would reverse the conclusion.

One other potential clue to resolve the question of buccal/gular pump homology comes from fundamental differences in the mechanisms for sealing the nares in amphibians and lizards. During buccal pumping in amphibians, the external nares are sealed by narial musculature (Gans and Pyles, 1983), whereas during gular pumping in lizards, the internal nares are sealed by the tongue (Deban et al., 1994) or tissue surrounding the tongue (Owerkowicz et al., 2001). This difference may be significant because buccal oscillation in amphibians and lizards appears to be a homologous behavior, and buccal pumping may have been lost when aspiration breathing evolved. Then accessory gular pumping could have arisen independently by adding the tongue-based narial sealing mechanism.

This question of buccal and gular pump homology is important because it bears on our interpretation of the behavior and physiology of extinct amniotes. If they are homologous, then stem amniotes would most likely have been able to breathe with both buccal and aspiration pumps, whereas if they are homoplasious, then stem amniotes were limited to aspiration breathing (unless they evolved their own gular pumping mechanism independently). With the currently available evidence, we prefer the interpretation that buccal and gular pumping are not homologous, but we offer this conclusion tentatively and with the caveat that the available evidence is weak.

5. Constraint and innovation in the evolution of tetrapod respiratory pumps

The concept of mechanical constraint has been a frequent theme in the literature on tetrapod respiratory pumps (Table 3). The musculoskeletal units responsible for breathing also serve other functions, such as feeding or locomotion, and the conflicting mechanical requirements of multiple functions have been proposed to constrain the performance and evolution of one or both functions (e.g., Wake and Larson, 1987; Carrier, 1991; Janis and Keller, 2001).

Buccal pump breathing has been proposed to constrain the evolution of tongue morphology and head shape in extant and extinct amphibians (Wake and Larson, 1987; Janis and Keller, 2001). In the evolution of plethodontid salamanders, cutaneous gas exchange became the sole respiratory mode and the hyolingual system was no longer required to participate in buccal pumping. Feeding by extreme hyolingual projection evolved independently in two plethodontid lineages, presumably made possible by the decoupling of buccal pumping from feeding, as well as the loss of a suction feeding larval stage in plethodontid lineages with the most extreme tongue projection (Wake and Larson, 1987). Buccal pumping may also have constrained the head shapes of early tetrapods to be relatively broad and flat (Szarski, 1962), and the onset of aspiration breathing is correlated with the appearance of more narrow and deep head shapes in amniotes in the fossil record (Janis and Keller, 2001).

The evolution of aspiration breathing may have allowed the musculoskeletal systems of the head and tongue of amniotes to diversify, but the ribs and intercostal musculature became constrained by their dual function in aspiration breathing and high-speed locomotion (Carrier, 1987b). In lizards, tidal volume decreases to almost zero during trackway sprinting and then increases during pauses in locomotion and during recovery from exercise (Carrier, 1987a). The intercostal and abdominal muscles are active bilaterally and in synchrony with breathing movements when lizards are at rest, but then are recruited unilaterally to bend the body and stiffen the trunk against ground reaction forces during locomotion (Carrier, 1990, 1991). The mechanical conflict between breathing and locomotion becomes more severe with increasing speed, and oxygen consumption may even decrease at high speeds (Wang et al., 1997). In tetrapods that are subject to this speed-dependent axial constraint, the highest rates of ventilation occur during recovery, whereas in unconstrained species, ventilation increases with speed up to a maximum and then decreases rapidly during recovery (Fig. 7).

In various tetrapod groups, at least four types of innovation have arisen to circumvent the speed-dependent axial constraint: gular pumping, diaphragm-like muscles, changes in locomotor posture and phase coupling of ventilation and locomotion (Table 3). Gular pumping during locomotion in savannah monitor lizards circumvents the constraint by more than doubling the inspired volume over costal aspiration alone (Owerkowicz et al., 1999; Munns et al., 2004). Gular pumping generates a distinctive pattern of airflow in which numerous small inspirations are associated with gular cavity filling, but then little or no exhalation follows each gular inspiration (Fig. 8). A series of gular pumps is followed by a single large exhalation, a small

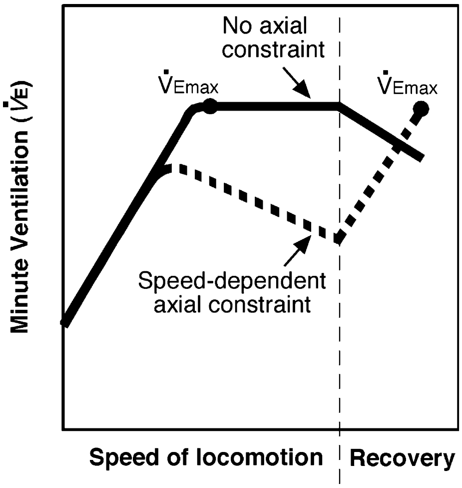

Fig. 7. Predictions of the speed-dependent axial constraint hypothesis. When there is a mechanical conflict between locomotion and breathing, as in most lizards, ventilation and oxygen consumption decrease at high speeds and maximum ventilation ($V_{E_{\max}}$) is reached only during recovery (dashed line). In mammals and other tetrapod groups that have circumvented the constraint, ventilation and oxygen consumption increase with speed and $V_{E_{\max}}$ is reached during, not after, locomotion (solid line). Modified from Owerkowicz et al. (1999).

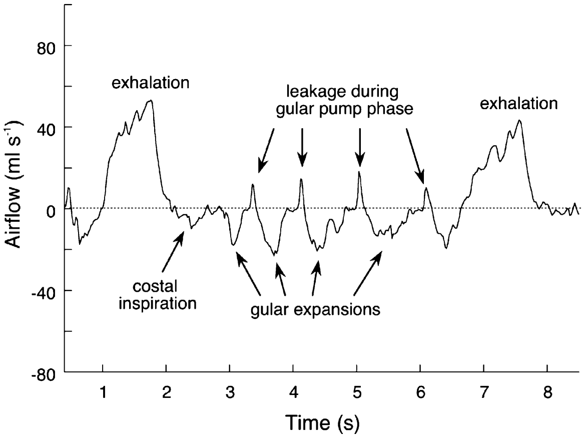

Fig. 8. Airflow (pneumotach) trace showing gular pumping in juvenile savannah monitor lizard during locomotion on a treadmill at 2 km h^{-1}. Each breath begins with a large exhalation, followed immediately by a small costal inspiration. In this trace, a series of four gular pumps can be seen between two large exhalations. Note that a small amount of air leaks out during the gular pumping phase, but that the volume of each gular inflation is considerably larger than the leakage, indicating that air is being pumped into the lungs. The dotted line is zero flow. Compare with the pneumotachogram trace in Fig. 6.

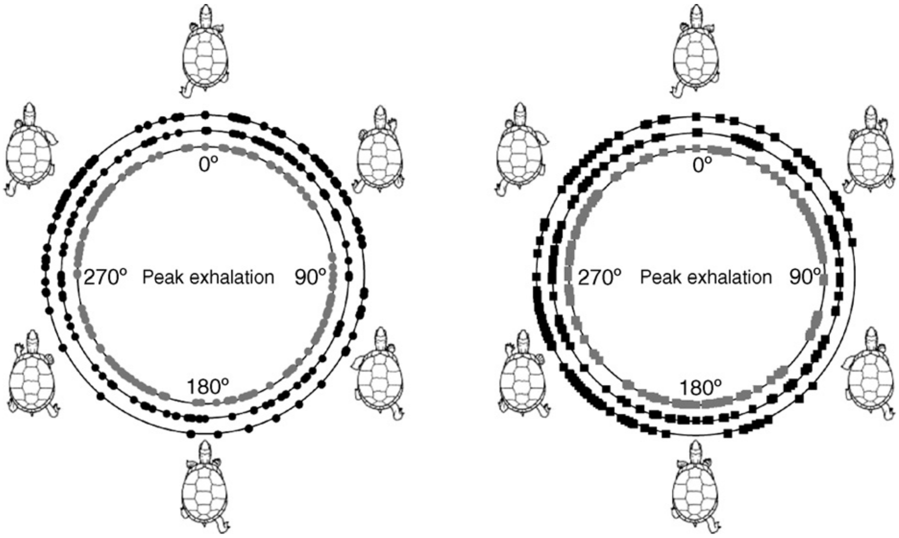

Fig. 9. Polar plots of the phase relationship between the locomotor stride cycle and peak inspiratory (left) and peak expiratory (right) airflow for three individual box turtles, *Terrapene carolina* (individual 01, black; individual 02, dark grey; individual 03, light grey). Breathing and locomotion appear to be completely decoupled in turtles. From Landberg et al. (2003).

costal inspiration and then another train of gular pumps. The contribution of gular pumping to lung ventilation in other monitor lizards has been questioned (Frappell et al., 2002), partially based on the observation that 11–49% of the expired gasses in four varanid species exit through the mouth rather than through the nares during exercise (Schultz et al., 1999). This result could mean that the lizards hold their mouths open most of the time during exercise, which would disrupt the narial seal and prevent gular pumping (Frappell et al., 2002). We have observed that savannah monitors sometimes close the mouth visibly before a gular pump, so it is possible that expiration can be through the mouth but that gular pumping can still occur. Gular pumping is best diagnosed by examination of flow traces: expiratory volumes alone are not informative, but a pattern of small inspirations that are not balanced by equal-sized expirations indicate gular pumping (Fig. 8).

A second type of innovation to circumvent the axial constraint is the evolution of accessory inspiratory muscles (Table 3). The dome-shaped diaphragm muscle in mammals contributes to the relatively high locomotor stamina of mammals relative to lepidosaurs (Carrier, 1987b). A recent study confirmed that hepatic piston pumping in American alligators effectively decouples their terrestrial locomotor mechanics from breathing (Farmer and Carrier, 2000b).

In turtles and tortoises, the internal oblique muscle has been recruited as a dome-shaped, accessory inspiratory muscle. Testudines are encased in a rigid shell and therefore should not be subject to the same mechanical constraint as lizards, but it is reasonable to hypothesize that movements of the legs in and out of the shell during locomotion might affect lung ventilation (Gans and Hughes, 1967). However, recent studies have found that breathing and treadmill locomotion are decoupled in two turtle species, *Trachemys scripta* and *Terrapene carolina* (Landberg et al., 2002, 2003). Gait and ventilation volume data show no relationship between peak expiratory or inspiratory flow rates and phase of the locomotor cycle (Fig. 9).

Some groups of lizards have intracoelomic septa that divide the lungs from the rest of the body cavity, as in the post-pulmonary septum of varanids, or the lungs and liver from the rest of the body cavity, as in the post-hepatic septum of some teiids (Klein et al., 2003; Klein and Owerkowicz, 2006). These septa contain smooth muscle or no muscle; therefore unlike a mammalian diaphragm they do not participate actively in lung ventilation. However, recent studies have shown that removal of the septa decreases tidal volume during locomotion in both varanids and teiids, probably by artificially permitting the paradoxic (cranial) movement of the viscera in response to low intrathoracic pressures during inspiration (Klein and Owerkowicz, 2006).

A third type of innovation is a marked change in locomotor posture, which allows the animal to reduce the need for hypaxial muscles to produce locomotor forces (Carrier, 1987b). In birds, the evolution of flight was associated with stiffening the trunk and large changes in loading patterns during locomotion. In quadrupedal mammals, changes in locomotor posture from a sprawling gait with pronounced lateral body bending to a more adducted limb posture and sagittal bending may have reduced the ground reaction moments acting on the trunk, but many hypaxial muscles still contribute to locomotion (Deban and Carrier, 2002). In dogs, the abdominal portions of the internal and external oblique muscles act as expiratory muscles when dogs stand at rest, but during locomotion their electrical activity pattern changes to match the locomotor rhythm and they act primarily to stabilize the trunk against sagittal shearing torques (Fife et al., 2001; Deban and Carrier, 2002). The electromyographic activity patterns of the external and internal intercostals, the thoracic part of the external oblique and the transverse abdominis indicate that they all serve both respiratory and locomotor functions simultaneously (Fife et al., 2001), and the only hypaxial musculature that can be shown to have a purely respiratory role during locomotion in dogs is the parasternal portion of the internal intercostal muscles (Carrier, 1996). These results show that there is still a mechanical conflict between the respiratory and locomotor functions of hypaxial muscles in mammals, and that the diaphragm muscle and respiratory-locomotor phase coupling are the best explanations for the high ventilatory performance and endurance of mammals during locomotion.

A fourth type of innovation that has been proposed to reduce respiratory-locomotor mechanical conflicts is phase coupling (Bramble and Carrier, 1983). Timing inhalation or exhalation to coincide with a particular phase of the locomotor cycle may recruit locomotor forces to assist ventilation, as in galloping and hopping mammals (Bramble and Carrier, 1983; Baudinette et al., 1987; Young et al., 1992a), or reduce the negative effects of locomotor forces on ventilation, as in flying birds (Boggs et al., 1997a,b; Funk et al., 1997). Running birds and trotting mammals often couple at one breath per stride (1:1) or one breath per step (2:1) (Bramble and Jenkins, 1993; Nassar et al., 2001). Flying birds also show respiratory-locomotor coupling, but with a wider range of coupling ratios ranging from 1:1 to 1:5, with the most common ratio being 1:3 (Boggs, 2002). Putative direct effects of respiratory-locomotor coupling on ventilation volumes and cost of ventilation are difficult to measure (Lee and Banzett, 1997), but the discovery that the natural frequency of the locomotor cycle and the resonant frequency of the respiratory system are closely matched in both birds and mammals during running (Young et al., 1992b; Nassar et al., 2001) provides indirect evidence that respiratory-locomotor coupling bestows some selective advantage (Deban and Carrier, 2002). The inherent mechanical conflict between costal aspiration breathing and high-speed sprinting remains evident in some lizards, particularly when gular pumping is not recruited to assist ventilation, but accessory inspiratory muscles, changes in locomotor posture and respiratory-locomotor phase coupling are innovations that have circumvented the speed-dependent axial constraint in most amniotes.

Acknowledgements

Thanks to Dave Carrier and Frietson Galis for inviting ELB to participate in their symposium “Constraint and Innovation in Vertebrate Evolution” at the 6th International Congress of Vertebrate Morphology, for which some of the ideas in this review were developed. We are grateful to M. Wake and J. O’Reilly for information on caecilian ribs. For lending us lizard species for the gular pumping survey we thank: H. Blacker, K. Druzisky, T. Hsieh, C. Infante, K. Jackson, C. Kidd, N. Kley, K. Schwenk, M. Shapiro, J. Sheen. Thanks to Donal Boyer and the San Diego Zoo for the opportunity to observe defensive gular pumping in a tuatara. This material is based in part on work supported by the National Science Foundation under Grant No. 9875245.

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