

New insight into the evolution of the vertebrate respiratory system and the discovery of unidirectional airflow in iguana lungs

Robert L. Cieri^a, Brent A. Craven^b, Emma R. Schachner^a, and C. G. Farmer^{a,1}

^aDepartment of Biology, University of Utah, Salt Lake City, UT 84112; and ^bApplied Research Laboratory, Department of Mechanical and Nuclear Engineering, The Pennsylvania State University, University Park, PA 16802

Edited by Neil H. Shubin, The University of Chicago, Chicago, IL, and approved October 17, 2014 (received for review March 19, 2014)

The generally accepted framework for the evolution of a key feature of the avian respiratory system, unidirectional airflow, is that it is an adaptation for efficiency of gas exchange and expanded aerobic capacities, and therefore it has historically been viewed as important to the ability of birds to fly and to maintain an endothermic metabolism. This pattern of flow has been presumed to arise from specific features of the respiratory system, such as an enclosed intrapulmonary bronchus and parabronchi. Here we show unidirectional airflow in the green iguana, a lizard with a strikingly different natural history from that of birds and lacking these anatomical features. This discovery indicates a paradigm shift is needed. The selective drivers of the trait, its date of origin, and the fundamental aerodynamic mechanisms by which unidirectional flow arises must be reassessed to be congruent with the natural history of this lineage. Unidirectional flow may serve functions other than expanded aerobic capacity; it may have been present in the ancestral diapsid; and it can occur in structurally simple lungs.

diapsid | evolution | lung | lizard | respiratory system

Energetically demanding forms of locomotion, such as powered flight, require a great capacity for gas exchange and selection for aerobic stamina may underlie many unique features of the avian respiratory system (1, 2). The avian respiratory system consists of highly vascularized lungs and avascular air sacs, which are membranous structures that effect ventilation and, in some species, extend between the muscles and even enter the bones (3). The topography of the conducting airways is complex; they form a circular system of tubes, analogous to the loop formed by the blood circulatory system in which arteries connect to veins through numerous small diameter vessels, the capillaries. Likewise, the avian conducting airways connect to each other through numerous tubules, the parabronchi, to form a circular path for respiratory gases (3). Gases flow through most of the parabronchi in the same direction during both inhalation and exhalation (unidirectional flow). This is due to the presence of aerodynamic valves (4–10). In contrast, the mammalian conducting airways arborize with the branch tips ending in blind sacs, there are no valves, and gases travel in the opposite direction along the conducting airways during expiration from the direction followed during inspiration (tidal flow). The presence of aerodynamic valves and unidirectional flow has generally been thought to be a highly derived feature found, among extant animals, only in birds and having evolved either in the crown group with flight or somewhere along the saurischian lineage leading to birds (11), perhaps as a mechanism to meet the high energetic demands of endothermy.

The discovery of unidirectional flow in the lungs of alligators (12, 13) and the savannah monitor lizard (14) indicates that we do not understand the distribution of this phenomenon among different lineages of vertebrates and raises questions about its underlying value. It is possible that unidirectional flow evolved convergently in crocodylians and monitor lizards and serves to expand aerobic capacity. Although monitor lizards are ectotherms,

their lifestyles are largely convergent with small predatory mammals (15) and they have high aerobic capacities compared with other lizards (16). In contrast, extant alligators have limited aerobic stamina (17) but their common ancestor with birds may have had a great aerobic capacity (18) or may have been endothermic (19, 20). Crocodylians and monitor lizards also share a suite of features of their pulmonary and cardiac anatomy that have been purported to give rise to, or coevolve with, birdlike patterns of flow. These features are: (i) a bronchus that has grown deep into the lung as a mesobronchium, (ii) partitioning of the respiratory system into a mechanical part that functions in ventilation and a gas-exchanging region, (iii) intercameral perforations, and (iv) separation of the heart into right and left sides (1, 21). Crocodylians and monitors are also derived in having evolved mechanisms to supplement costal ventilation while exercising (18, 22, 23). Thus, unidirectional flow in these lineages may be one of many derived traits underpinning exceptionally high rates of oxygen consumption during activity.

It is also possible, however, that this pattern of flow evolved before the split of Diapsida into the Lepidosauromorpha (tuatara, lizards, snakes) and Archosauromorpha (crocodylians and birds) in an ectothermic ancestor lacking expanded aerobic capacities and living as long ago as the Permian Period. Unidirectional flow has been purported to serve ectotherms by harnessing the heart as a pump for air during periods of breath-holding (apnea) (12). Light can be shed on this pattern of evolution with observations of more squamates (snakes, lizards), which are the most diverse and largest (~9,000 species) group of living reptiles (24).

Significance

The avian respiratory system appears strikingly distinct from all other animals. Purported key innovations underpinning avian patterns of airflow are an enclosed intrapulmonary bronchus, intercameral perforations, heterogeneous parenchyma; these traits allegedly coevolved with separation of the cardiac ventricle into right and left sides and are presumed to have been favored by selection because they facilitate high activity metabolisms. In contradistinction to these prevailing theories, here we show that unidirectional flow is present in the lungs of the green iguana, an ectothermic animal with low aerobic capacity, no intrapulmonary bronchus, and no intercameral perforations. This discovery indicates a transformation in our understanding of the evolution of the vertebrate respiratory system is needed.

Author contributions: C.F. designed research; R.L.C., B.A.C., E.R.S., and C.F. performed research; R.L.C., B.A.C., and C.F. analyzed data; B.A.C. and C.F. contributed new reagents/analytic tools; and B.A.C. and C.F. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹To whom correspondence should be addressed. Email: cg.frmr@gmail.com.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1405088111/-DCSupplemental.

To test the hypothesis that unidirectional flow is present in squamates other than varanid lizards; to better understand anatomical features that give rise to these patterns of flow; and to gain insight into the underlying value of this pattern of flow, green iguanas (*Iguana iguana*) were studied. Green iguanas differ from monitors because they are herbivores and because they have structurally simple lungs that lack an enclosed intrapulmonary bronchus. Iguanas lack septation of the cardiac ventricle and have poor locomotor stamina. The poor stamina is due in part from an impairment during running in their blood and air circulatory systems (19, 25, 26).

Results

Iguana lungs are simple, large, paired organs that occupy much of the thoracoabdominal cavity; each lung is partitioned by a septum into a small cranial chamber and a much larger caudal chamber (Fig. 1A). The primary bronchus enters into the cranial chamber of the lung from a ventromedial aspect along an oblique trajectory, coursing laterally, caudally, and dorsally. The hilus is located approximately one-third the length of the cranial chamber from its apex and near the most cranial section of the caudal chamber (Fig. 1). The dorsal half of the primary bronchus terminates at the hilus, whereas the ventral half, still containing partial rings of cartilage, flattens and continues distally to form part of the septum separating the chambers (Fig. 1B). A single oval ostium in the floor of the flattened bronchus connects the chambers (Fig. 1C). Otherwise, the septation between the cranial and caudal chamber is airtight (no interchamber perforations are present; $n = 5$). Partial septa line portions of the walls of the lungs forming pockets. These pockets are especially numerous where the heart and lungs are contiguous and along the septum separating the two chambers (Fig. 1A).

In both chambers unidirectional flow occurred in vivo ($n = 5$). Throughout the respiratory cycle, insufflated, aerosolized lipids flowed craniad along portions of the walls of the lungs in both chambers whereas caudally directed flow was primarily confined to the inspiratory phase and was mostly located in high-speed streams emanating in the cranial chamber from the bronchus and in the caudal chamber from the ostium (Fig. 2 and [Movies S1](#) and [S2](#)). Unidirectional flow could not have been generated by the activity of sphincters or other muscular valves because it was also observed in excised lungs under the following two conditions. First, flow was visualized in excised lungs that had been filled with water containing pollen, microspheres, or citrus pulp ($n = 5$). The water was injected and withdrawn through the trachea and the movement of particles within the lungs imaged under a dissecting scope (Fig. 3 and [Movie S3](#)). Second, recordings from heated thermistor flow meters ($n = 10$) that were implanted in the walls of the lungs in situ and in excised lungs (separate experiments) confirmed the pattern of airflow observed in vivo (Fig. 4).

Simulated patterns of flow corroborated the data collected in vivo and in excised lungs and provided insight into the detailed patterns of flow (Figs. 2–5 and [Movies S4](#) and [S5](#)). During inspiration, both the simulations and our visualization of smoke in air and particles in water showed that fluid formed high-speed streams (or jets) as it entered both the cranial and caudal chambers as the lungs expanded. These streams entered the chambers medially and flowed laterocaudad. During exhalation, fluid was pushed out of each chamber with relatively slow speed and, in the caudal chamber, moved primarily craniad along the long axis of the body. Therefore, in the region of the inspired, high-speed fluid stream, the flow was tidal. However, in regions that lie outside the fluid stream, fluid flowed primarily craniad during inspiration and expiration (Figs. 2–4 and [Movies S1–S3](#) and [S5](#)).

Discussion

The pattern of flow in the simple lungs of the green iguana is strikingly similar in many ways to the pattern of flow seen in the

more complex lungs of birds, crocodylians, and varanid lizards. By a simple streaming of the fluid, tidal flow occurs in the iguana

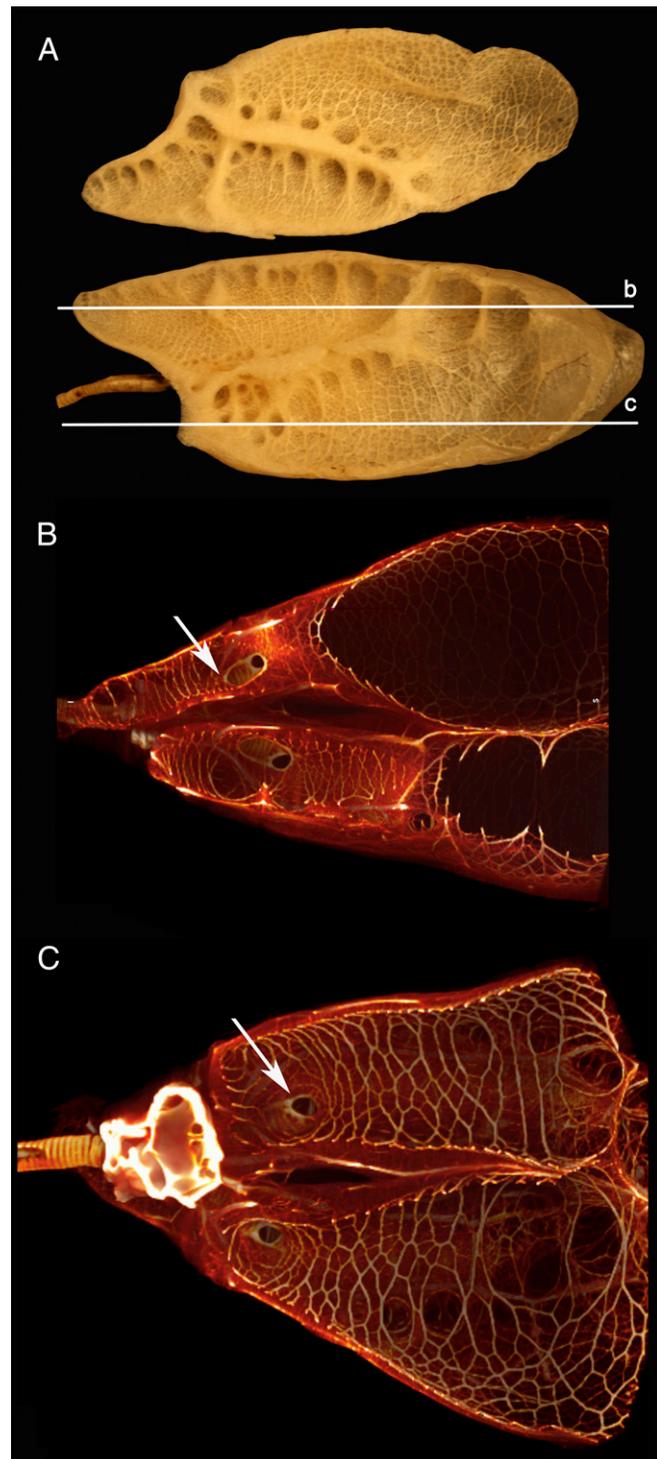


Fig. 1. Anatomy of the respiratory system of the green iguana. (A) Dried left lung transected in the parasagittal plane (cutaway section inverted). White lines (b and c) mark approximate levels of transection shown in volume-rendered μ CT data illustrated in B and C. (B) Dorsal view of both lungs transected in the coronal plane. Arrow points to the location where the primary bronchus enters the cranial chamber of the right lung. (C) Ventral view of the three chambered heart and lungs transected in the coronal plane. Arrow marks the ostium between the chambers of the left lung. Craniad, left.

discretization schemes. No-slip boundary conditions were applied on the walls of the lung and a custom mesh motion boundary condition was developed to expand and contract the lung at a rate of 15 breaths per minute with a tidal volume of 11 mL, which was based on resting data for green iguanas (25). A physiologically realistic motion of the lung walls was prescribed to match in vivo observations of resting breathing. In particular, the caudal chamber was made to expand and contract more than the cranial chamber and the motion of both chambers was prescribed to be greater laterally than medially. A transient simulation of five breathing cycles was carried out to obtain a time-periodic steady state solution. The computation was performed on 96 processors of a high-performance parallel computer cluster at Penn State University. Airflow patterns in the lung were extracted directly from the numerical solutions using the open-source visualization software ParaView (www.paraview.org).

To ensure that the computational solution is independent of the mesh resolution and time step size, a second simulation was carried out using a significantly finer mesh and a much smaller time step. In particular, the finer mesh contained 3.9 million computational elements, more than twice the number of elements in the coarser mesh, and the simulation was performed on 192 processors using a time step size of 0.0005 s, an order of magnitude smaller than that used in the coarser simulation (0.005 s). In summary, the overall flow patterns in the lung for the coarse and fine mesh simulations are extremely similar (Fig. 7), thereby confirming that the reported results are mesh- and time step-independent.

ACKNOWLEDGMENTS. We thank James Butler for insightful conversations and inspiration. This work was funded by the National Science Foundation (IOS 0818973 and IOS 1055080, to C.G.F.).

1. Wolf S (1933) Zur Kenntnis von Bau und Funktion der Reptilienlunge. *Zool. Jahrb. Abt. Anat. Ontol.* 57:139–190.
2. Maina JN (2000) What it takes to fly: The structural and functional respiratory refinements in birds and bats. *J Exp Biol* 203(Pt 20):3045–3064.
3. Duncker HR (1971) The lung air sac system of birds. A contribution to the functional anatomy of the respiratory apparatus. *Ergebn Anat EntwGesch* 45:1–171.
4. Hazelhoff EH (1951) Structure and function of the lung of birds. *Poult Sci* 30:3–10.
5. Butler JP, Banzett RB, Fredberg JJ (1988) Inspiratory valving in avian bronchi: Aerodynamic considerations. *Respir Physiol* 72(2):241–255.
6. Wang N, Banzett RB, Butler JP, Fredberg JJ (1988) Bird lung models show that convective inertia effects inspiratory aerodynamic valving. *Respir Physiol* 73(1):111–124.
7. Wang N, Banzett RB, Nations CS, Jenkins FA, Jr (1992) An aerodynamic valve in the avian primary bronchus. *J Exp Zool* 262(4):441–445.
8. Banzett RB, et al. (1987) Inspiratory aerodynamic valving in goose lungs depends on gas density and velocity. *Respir Physiol* 70(3):287–300.
9. Brackenbury JH (1971) Airflow dynamics in the avian lung as determined by direct and indirect methods. *Respir Physiol* 13(3):319–329.
10. Dotterweich H (1930) Versuch über den Weg der Atemluft in der Vogellunge. *Z Vgl Physiol* 11:271–284.
11. Perry SF (1992) Gas exchange strategies of reptiles and the origin of the avian lung. *Physiological adaptations in vertebrates; respiration, circulation, and metabolism*, eds Wood S, Weber R, Hargens A, Millard R (Marcel Dekker, Inc., New York), Vol 56, pp 149–167.
12. Farmer CG (2010) The provenance of alveolar and parabronchial lungs: Insights from paleoecology and the discovery of cardiogenic, unidirectional airflow in the American alligator (*Alligator mississippiensis*). *Physiol Biochem Zool* 83(4):561–575.
13. Farmer CG, Sanders K (2010) Unidirectional airflow in the lungs of alligators. *Science* 327(5963):338–340.
14. Schachner ER, Cieri RL, Butler JP, Farmer CG (2014) Unidirectional pulmonary airflow patterns in the savannah monitor lizard. *Nature* 506(7488):367–370.
15. Sweet SS, Pianka ER (2007) Monitors, mammals, and Wallace's Line. Third Multidisciplinary World Conference on Monitor Lizards, Alexander Koenig Museum, Bonn, Germany. *Mertensiella* 16:79–99.
16. Thompson GG, Withers PC (1997) Standard and maximal metabolic rates of goannas (Squamata:Varanidae). *Physiol Zool* 70(3):307–323.
17. Farmer CG, Carrier DR (2000) Ventilation and gas exchange during treadmill locomotion in the American alligator (*Alligator mississippiensis*). *J Exp Biol* 203(Pt 11):1671–1678.
18. Farmer CG, Carrier DR (2000) Pelvic aspiration in the American alligator (*Alligator mississippiensis*). *J Exp Biol* 203(Pt 11):1679–1687.
19. Carrier DR (1987) The evolution of locomotor stamina in tetrapods: Circumventing a mechanical constraint. *Paleobiology* 13:326–341.
20. Seymour RS, Bennett-Stamper CL, Johnston SD, Carrier DR, Grigg GC (2004) Evidence for endothermic ancestors of crocodiles at the stem of archosaur evolution. *Physiol Biochem Zool* 77(6):1051–1067.
21. Huxley TH (1882) On the respiratory organs of *Apteryx*. *Proc Zool Soc Lond* 1882:560–569.
22. Owerkowicz T, Farmer CG, Hicks JW, Brainerd EL (1999) Contribution of the gular pump to ventilation. *Science* 284:1661–1663.
23. Gans C, Clark B (1976) Studies on ventilation of *Caiman crocodilus* (Crocodylia: Reptilia). *Respir Physiol* 26(3):285–301.
24. Wiens JJ, et al. (2012) Resolving the phylogeny of lizards and snakes (Squamata) with extensive sampling of genes and species. *Biol Lett* 8(6):1043–1046.
25. Wang T, Carrier DR, Hicks JW (1997) Ventilation and gas exchange in lizards during treadmill exercise. *J Exp Biol* 200(Pt 20):2629–2639.
26. Farmer CG, Hicks JW (2000) Circulatory impairment induced by exercise in the lizard *Iguana iguana*. *J Exp Biol* 203(Pt 17):2691–2697.
27. Hicks JW (1998) Cardiac shunting in reptiles: Mechanisms, regulation and physiological function. *Biology of the Reptilia*, eds Gans C, Gaunt AS (Society for the Study of Amphibians and Reptiles, Ithaca, NY), Vol G, pp 425–483.
28. Hopkins SR, Hicks JW, Cooper TK, Powell FL (1995) Ventilation and pulmonary gas exchange during exercise in the savannah monitor lizard (*Varanus exanthematicus*). *J Exp Biol* 198(Pt 8):1783–1789.