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The Evolution of Unidirectional Pulmonary Airflow

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Conventional wisdom holds that the avian respiratory system is unique because air flows in the same direction through most of the gas-exchange tubules during both phases of ventilation. However, recent studies showing that unidirectional airflow also exists in crocodylians and lizards raise questions about the true phylogenetic distribution of unidirectional airflow, the selective drivers of the trait, the date of origin, and the functional consequences of this phenomenon. These discoveries suggest unidirectional flow was present in the common diapsid ancestor and are inconsistent with the traditional paradigm that unidirectional flow is an adaptation for supporting high rates of gas exchange. Instead, these discoveries suggest it may serve functions such as decreasing the work of breathing, decreasing evaporative respiratory water loss, reducing rates of heat loss, and facilitating crypsis. The divergence in the design of the respiratory system between unidirectionally ventilated lungs and tidally ventilated lungs, such as those found in mammals, is very old, with a minimum date for the divergence in the Permian Period. From this foundation, the avian and mammalian lineages evolved very different respiratory systems. I suggest the difference in design is due to the same selective pressure, expanded aerobic capacity, acting under different environmental conditions. High levels of atmospheric oxygen of the Permian Period relaxed selection for a thin blood-gas barrier and may have resulted in the homogeneous, broncho-alveolar design, whereas the reduced oxygen of the Mesozoic selected for a heterogeneous lung with an extremely thin blood-gas barrier. These differences in lung design may explain the puzzling pattern of ecomorphological diversification of Mesozoic mammals: all were small animals that did not occupy niches requiring a great aerobic capacity. The broncho-alveolar lung and the hypoxia of the Mesozoic may have restricted these mammals from exploiting niches of large body size, where cursorial locomotion can be advantageous, as well as other niches requiring great aerobic capacities, such as those using flapping flight. Furthermore, hypoxia may have exerted positive selection for a parasagittal posture, the diaphragm, and reduced erythrocyte size, innovations that enabled increased rates of ventilation and more rapid rates of diffusion in the lung.

The evolution of the bird lung has long fascinated biologists for several reasons. First, its high capacity for gas exchange is important for sustaining flapping flight, an innovation that allowed birds to interact with their environments in a way that opened ecological opportunity and enabled them

to undergo an extensive adaptive radiation. Second, the avian respiratory system consists of unique structures: avascular air sacs that effect ventilation and highly vascular lungs with counter-intuitive patterns of airflow through most of the bronchial structures. In birds, the primary

bronchus courses the length of the lung, giving rise to secondary bronchi that subdivide into tubules, the parabronchi, which anastomose to complete a circuit. The avian conducting airways are reminiscent of the arteries, capillaries, and veins of the blood circulatory system, and, like the flow of blood, gases move in a consistent direction through most of these airways during both phases of ventilation. In contrast, mammalian conducting airways arborize, with the tips ending in cul-de-sacs (FIGURE 1). Early studies and speculations about patterns of airflow in the avian lung attributed the unidirectional flow to physical valves (86), but a lack of evidence for either sphincters or valve leaflets and the fact that the valves remain effective in dead animals led to the conclusion that aerodynamic valves cause unidirectional flow (Refs. 24, 35; reviewed in Ref. 16). Although physical models proved topography alone can cause unidirectional flow (24, 35), these models are simple compared with avian anatomy, and many questions about the mechanisms underpinning this phenomenon remain unanswered (46). However, the preponderance of the data indicates convective inertia plays a key role (1, 2, 10-12, 14, 16, 47, 49).

The physiological benefit to gas exchange of unidirectional flow has also long been a topic of interest to biologists but is poorly understood in air breathers. With unidirectional flow, countercurrent gas exchange is possible, such as that found in the gills of fish. Countercurrent exchange can produce a reversal of the gradient in partial pressures of respiratory and blood gases such that the tension of oxygen in expired respiratory medium is less than in blood leaving the respiratory organ, and the tension of carbon dioxide in the expired respiratory medium is greater than in blood leaving the respiratory organ. Thus countercurrent mechanisms are more efficient at extracting oxygen and excreting carbon dioxide than is the ventilated pool mechanism of mammals (FIGURE 1). This seems clearly advantageous when animals breathe respiratory media with low oxygen content (e.g., water), but the advantages are less clear when animals breathe air, since extraction of oxygen in air-breathers is normally quite low anyway. The issue of physiological benefit is further clouded by the fact that birds have a cross-current design rather than a countercurrent design (68) and by the fact that both mammals and birds can show a reversal in the gradient between end-tidal carbon dioxide gas and arterial blood (22, 56, 67, 68). In mammals, reversals are particularly prevalent with rebreathing and at the end of apnea, and have been proposed to arise for the following reasons: a mismatch between arterial samples and end-tidal samples, slow equilibration of $\text{CO}_2/\text{HCO}_3^-/\text{H}^+$ in blood, the Haldane effect, membrane charges, and

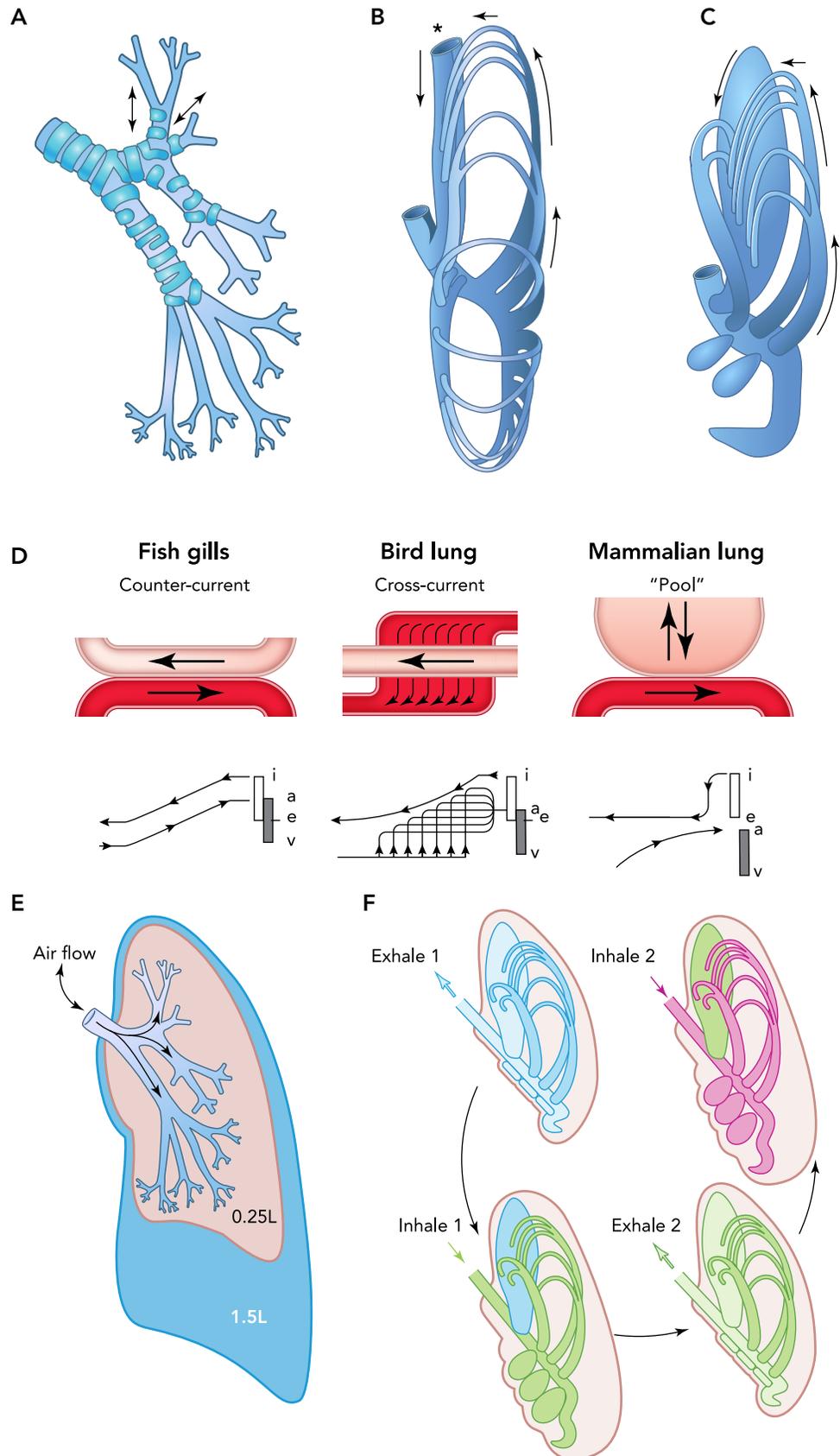
the addition of CO_2 from the esophagus to the end-tidal gas (23, 67). Although a reversal in the gradient for PO_2 is theoretically possible in birds, in practice it is rarely reported. It appears to be more pronounced when the fraction of oxygen in the inspired air ($F_{\text{I}\text{O}_2}$) is lower than normal and $F_{\text{I}\text{CO}_2}$ is raised (69). As previously mentioned, a reversal in the gradient would, theoretically, allow birds to extract more oxygen from the gases they breathe than mammals can and is tied to the idea that bird lungs are more efficient than mammalian lungs (FIGURE 1).

Early work on rates of extraction seemed to confirm the predicted benefits of unidirectional flow. For example, Bernstein and Schmidt-Nielsen measured extraction of oxygen in the fish crow (*Corvus ossifragus*) of 31%, concluded this value was twice as high as what would be expected for a mammal of the same size, and attributed the greater extraction to the unidirectional flow system (7). However, extraction is a function of minute ventilation with respect to gas exchange, and it is clear that, at low minute ventilation, the broncho-alveolar lung is capable of great extraction. For example, the bottlenose porpoise (*Tursiops truncatus*) extracts up to 85% of the oxygen from a breath (62). Furthermore, other mammals have high extraction; for example, extraction in bandicoots (*Isodon obesulus*) and humans is 26% and 30%, respectively (43). Also, many birds have rather low extractions despite their unidirectional flow. For example, in the evening grosbeak (*Coccothraustes vespertinus*) extraction is ~15% (5). Extraction can also vary with ambient temperature when endotherms pant to thermoregulate (51). Thus, although the crosscurrent lung is theoretically capable of greater extraction than the ventilated pool of the bronchial alveolar lung, data from a range of birds and mammals indicate that there is a surprisingly wide range of extractions measured in both lineages, perhaps obscuring the functional underpinning of these differences in lung design. However, this may not be true when $P_{\text{I}\text{O}_2}$ is reduced (e.g., at high altitude) or when $F_{\text{I}\text{O}_2}$ is reduced (e.g., animals living in hypoxic burrows or during periods of the Earth's history when ambient O_2 levels were low).

Importantly, mode of locomotion is inextricably intertwined with the evolution of the capacity of the lung for gas exchange. This capacity can be quantified morphologically by measuring the ratio of the diffusive surface area of the lung and the harmonic mean thickness of the blood-gas barrier, the anatomical diffusion factor (ADF). Birds and mammals using the same mode of locomotion have very similar ADF (31, 48, 60). The power required for locomotor modes such as swimming and running is less than for modes such as flapping flight. Thus swimming and running birds have low

ADF compared with flying mammals, and swimming birds have low ADF compared with running mammals (31, 60), indicating that mode of

locomotion, and not phylogeny or lung design, is the primary selective driver for ADF. Importantly, ADF is not expanded in these groups by the same



mechanisms. For a given body mass and mode of locomotion, the avian harmonic mean thickness of the blood-gas barrier is thinner than that of mammals, whereas in mammals ADF increases are largely due to increases in surface area (48).

This raises a cardinal question that has received surprisingly little attention (85): As birds and mammals experienced directional selection for greater aerobic capacities, why did the heart and other organ systems of the oxygen cascade converge on similar forms but the respiratory systems diverge? It has been proposed that this is not a useful question (85). I disagree. Evolutionary scenarios can lead us to see animals in a new light and to ask questions about extant animals that would otherwise be ignored.

It is possible to address historical questions by integrating data from functional studies with historical data. Incorporating the fossil record provides a dimension of time to the analysis and is paramount to understanding the history of a lineage. Although the respiratory systems cannot be directly studied in the fossil remains of avian and mammalian ancestors, the fossil record provides key information on the modes of locomotion and therefore anatomical diffusive capacities. The fossil record also provides information on ecological niches, diets, community structures, and environmental conditions. Furthermore, the physical laws governing gas exchange are constant through time and have imposed consistent constraints on lineages. For example, studies of animals that live at altitude or in hypoxic burrows have revealed that, when ambient oxygen is low, the diffusive steps of the oxygen cascade are more likely to limit gas transport through the entire oxygen cascade than the conductive steps and that this is due to the fact that rates of diffusion are proportional to gradients in partial pressure of gases (84). While atmospheric levels of oxygen have varied throughout the course of the Earth's history, this fundamental relationship between gradients of partial pressures and rates of diffusion has not changed. Thus integrating knowledge of these physical laws with knowledge obtained from the fossil record, such as

modes of locomotion and environmental conditions, with knowledge of phyletic relationships, and structure-function relationships of the respiratory system of extant lineages enables retrodiction of past events. These threads can be woven together to create a picture of the past, which in turn can serve to direct future investigations toward fruitful lines of inquiry.

Birds are members of a larger clade of organisms, archosaurs, which include many extinct lines, such as pterosaurs and non-avian dinosaurs, and also the living crocodilians and their forebearers. Therefore, comparisons of anatomy and patterns of airflow in the respiratory systems of birds and crocodilians can provide insight into the anatomy and patterns of airflow of their common ancestor and of the extinct lineages bracketed by these living taxa. Additionally, studies of the fossil record can place minimum dates for the origin of the characters.

Crocodilians

Crocodilians are the sole surviving members of a prominent and morphologically diverse clade of vertebrates that thrived during the Triassic Period, some 250 million years ago (15), known as the crurotarsans or pseudosuchia. Members of the crurotarsi occupied a range of guilds. For example, there were herbivorous aetosaurs, giant terrestrial carnivores such as the rauisuchids, and lightly built and gracile lineages that resembled today's ostriches (e.g., *Effigia*). Although the crurotarsans were successful and abundant during this period, by the end of the Triassic, the lineage had died out with the exception of a few crocodylomorph lines. At this time, the avemetatarsalian (ornithodiran) archosaurs, which include the pterosaurs and dinosaurs, came to dominate terrestrial ecosystems. Some of the early crocodylomorphs, (e.g., sphenosuchians) were small terrestrial animals, ranging from the size of a domestic cat to that of a wolf, with the following cursorial skeletal features: reduced number of digits, parasagittal limb postures, gracile limbs with elongated distal elements,

FIGURE 1. Schematic of anatomy, airflow, and patterns of gas exchange in several vertebrates

Dorsal view of the right lung of mammal (A; see also Ref. 55), bird (B; see also Ref. 10), and alligator (C). Airflow is tidal in mammals but unidirectional in both birds and crocodilians. D: mechanisms of gas exchange (see also Ref. 66), showing that a reversal in the gradient for diffusion is possible in cross- and countercurrent exchangers, i.e., partial pressure of oxygen in inspired and expired air, respectively; a, v, partial pressure in systemic arterial and venous blood, respectively. E: schematic of human lung illustrating mixing of inspired air (average volume 0.25 liter) with residual gas (1.5 liter) (see also Ref. 85). F: hypothesized movement of bolus of gas during a bout of breathing in a diving alligator. At the end of the first exhalation, the residual volume is ~22 ml/kg. Some of this air has done gas exchange and resides in the cervical ventral bronchus (light blue), whereas some of it resides in the dorsobronchi (dark blue). During the first inhalation, a breath of air of ~22 ml/kg (green) travels to the ventrocaudal lung and into the dorsobronchi while the residual gas flows into the cervical ventral bronchus (dark blue). During the second exhalation, part of the bolus (dark green) passes through the dorsobronchi and parabronchi, and moves into the cervical ventral bronchus (light green). On the second inhalation, the remainder of the bolus enters the cervical ventral bronchus (dark green), and a new bolus of air (pink) enters the ventrocaudal chambers and the dorsobronchi, completing the cycle.

digitigrade foot posture (57). Studies of extant lineages with these features have shown that they are indicative of a mode of locomotion requiring great aerobic stamina (17). By the early Jurassic, most crocodylomorphs had evolved a less energetically demanding marine or semiaquatic lifestyle, and acquired traits that suited these niches, such as relatively large and heavy bodies, a dorsoventrally flattened skull, and powerful tails. The crown group, which includes all extant species of crocodylians, arose by the end of the Cretaceous Period. Therefore, extant crocodylians are expected to contain a mosaic of characters, some of them suited to their current lifestyles as ectothermic, semiaquatic, piscivores or sit and wait predators, and some features inherited from their terrestrial forebearers.

Extant crocodylians superficially appear strikingly different from their sister taxon, the endothermic and lightly built birds, and yet their cardiopulmonary systems share many features (28, 64). For example, the cardiac ventricle is fully subdivided and composed of much compact myocardium with a rich coronary circulation (26). Their respiratory systems also share many features, which was noted by Thomas Huxley in 1882 (39). Huxley homologized the dilation of the crocodylian intrapulmonary bronchus into an oval sac-like cavity with the mesobronchium and posterior air-sacs of birds and specific conducting airways of the crocodylian with avian entobronchi (ventrobronchi) and ectobronchi (dorsobronchi). He furthermore stated, "The orifices with which the surfaces of all these canals, except the anterior half of the mesobronchium, are thickly set, lead into depressions, which are often so deep as to become cylindrical passages, simulating the parabronchia of birds."

This similarity in structure is mirrored by similarity in function. Measurements and visualizations of flow, both in vivo and in excised preparations, show that American alligators (*Alligator mississippiensis*) have bird-like unidirectional flow through most of their conducting airways (FIGURE 1) (28, 30). In the airways that lie dorsally and laterally, the flow is craniad, and, in the airways that occupy the ventrocranial region of the lung, the flow is caudad (FIGURE 1). This pattern of flow occurs in a range of species: the black caiman (*Melanosuchus niger*), the spectacled caiman (*Caiman crocodilus*), the dwarf crocodile (*Osteolaemus tetraspis*), the saltwater crocodile (*Crocodylus porosus*), the Nile crocodile (*Crocodylus niloticus*), and Morelet's crocodile (*Crocodylus moreletii*) (28). This similarity in structure and function among extant crocodylians with birds strongly suggests the common ancestral archosaur had similar patterns of flow, which raises some interesting questions for the initial selective advantage of this character.

While conventional wisdom has held that unidirectional flow is unique to birds and that it may be an adaptation for flight or endothermy, the discovery of unidirectional flow in crocodylians suggests that neither of these functions underpinned its evolution and that unidirectional flow evolved much earlier than birds. Alternative hypotheses for the functional benefits of this flow should be sought. The following two hypotheses, which are not mutually exclusive, may explain its functional significance: 1) unidirectional flow enables natural selection to take advantage of the heart as a pump for air; 2) unidirectional flow facilitates washout, which could be of benefit to a low-energy lifestyle by reducing the cost of breathing, reducing respiratory evaporative water loss, reducing heat loss from the enthalpy of vaporization, and improving crypsis. All are testable hypotheses.

Cardiogenic Unidirectional Airflow

Ectotherms spend most of their lives holding their breath (33), and therefore gas exchange during apnea is as important as gas exchange during bouts of ventilation. Apnea is also important in diving and torpid birds and mammals. During apnea in many animals, the lungs serve for gas exchange and as a storage depot for oxygen. Studies of mammals have shown that movement of O₂ from dead space into the alveoli during apnea is due to both diffusion and bulk convection that is powered by the beating heart (9, 25, 32, 82). The convection is caused by cardiogenic mixing and is important because it breaks up the stratification of gases that inhibits diffusion (32). The increase in the rate at which oxygen moves from the dead spaces into the alveoli is approximately fivefold over the rate obtained strictly from chemical diffusion (25). In humans, the situation in which cardiogenic gas exchange between the airways and the alveoli is most important is during hypoventilation (for example in patients with emphysema); at these times, cardiogenic airflow probably plays a major role in maintaining arterial oxygenation (82). However, the prime value to gas exchange of cardiogenic flow may be during the prolonged apneas of torpid mammals, in which cardiogenic flow and an open glottis extend apnea significantly (72, 74).

Cardiogenic flow may be important to reptiles as well (34). Because the gas-exchange parenchyma is generally concentrated in small areas of large lungs, unidirectional flow may move gases out of the non-vascularized regions and into the regions containing gas-exchange parenchyma. FIGURE 2 illustrates unidirectional cardiogenic flow measured with a dual heated thermistor flow meter that was placed in the most proximal secondary bronchus of an American alligator, a bronchus that

occupies most of the ventrocranial region of the lungs. The flow during apnea is significant. For example, in 2 min of apnea, the flow through the meter was equivalent to the flow measured during a resting tidal volume (22 ml/kg) (27).

It should be possible to test the hypothesis that cardiogenic flow is important to gas exchange using methodologies similar to those employed by Engel et al. (25) to assess gas mixing due to cardiogenic flow in dogs. Briefly, a bolus of tracer gas was sampled strategically in the bronchial tree in living, anesthetized dogs during a breath-hold. The rate of spread of the gas by both cardiogenic mixing and diffusion was compared with chemical diffusion alone by injecting the same tracer and sampling from the same sites postmortem. Manual oscillations of the heart postmortem enhanced gas mixing. The authors suggest that both Taylor diffusion and convective mixing due to eddy currents and secondary motions are producing the effect. Further analysis of the importance of cardiogenic flow in ectotherms may also be possible using computational fluid dynamic models of pulmonary airflow coupled with XROMM data of the beating heart. These models should reveal the total flow throughout the respiratory system rather than the measurements obtained at a single anatomical point, such as those illustrated in **FIGURE 2**. Efforts to assess the importance of cardiogenic flow to intrapulmonary airflow using the latter approach are currently underway.

Facilitation of Washout

Unidirectional flow is hypothesized to prevent lung gases that are CO₂-enriched and O₂-depleted from mixing with the freshly inspired air, and thereby to decrease the number of breaths needed to flush the respiratory system, and to keep the tension of oxygen high in the gases that are in contact with the blood capillaries. In birds, insufflation of the marker gas argon during resting breathing in ducks (*Anas platyrhyncho*) showed that freshly inspired gas flowed into the posterior air sacs and then moved through the lungs and into the anterior sacs on the subsequent exhalation and inhalation (13). Argon was exhaled out of the anterior air sacs and trachea on the exhalation of the second cycle. Thus the avian respiratory system operates as a two-cycle pump (13). Unidirectional flow could have similar benefit in nonavian reptiles. Minimizing the number of breaths needed for washout could facilitate crypsis, reduce the overall energetic cost of ventilation, lower rates of respiratory evaporative water loss, and lower rates of cooling due to the heat of vaporization. Unidirectional flow may furthermore be important to ectotherms because episodic ventilation decreases the efficiency of CO₂ excretion, independent of

changes in cardiac output (52). During periods of apnea, CO₂ is primarily stored in the tissues and must be rapidly delivered to the lung during short periods of ventilation. Unidirectional flow could prevent this flood of CO₂ from diluting the freshly inhaled air. This effect would be especially important under conditions of low environmental oxygen.

Data on ventilation during diving from American alligators are consistent with this hypothesis. Like other ectothermic amniotes, alligators are intermittent breathers: bouts of breathing are interspersed with apnea, in which PO₂ steadily declines (36). A study of voluntary diving found that alligators spend ~8% of their time breathing and that their tidal volume was 22 ml/kg. Observations of bouts of ventilation ($N = 193$) showed the number of breaths in a ventilatory bout ranged from 1 to 22, with a mode of 2 being three times more common than any other breathing pattern (36). Crocodylians have large lungs (~109 ml/kg), but only a small volume (4 ml/kg) consists of gas-exchange parenchyma; most gas (63 ml/kg) resides in the intrapulmonary bronchus, and only a minor volume (42 ml/kg) resides in the parenchymal air spaces (59). However, when diving crocodylians do not fill the lungs to the full 109 ml/kg, they inspire a volume of ~40 ml/kg (87). Alligators begin a bout of ventilation with a slow exhalation that is composed of air from the ventrocranial chambers [cervical ventral bronchi (CVB)] and that has passed through the

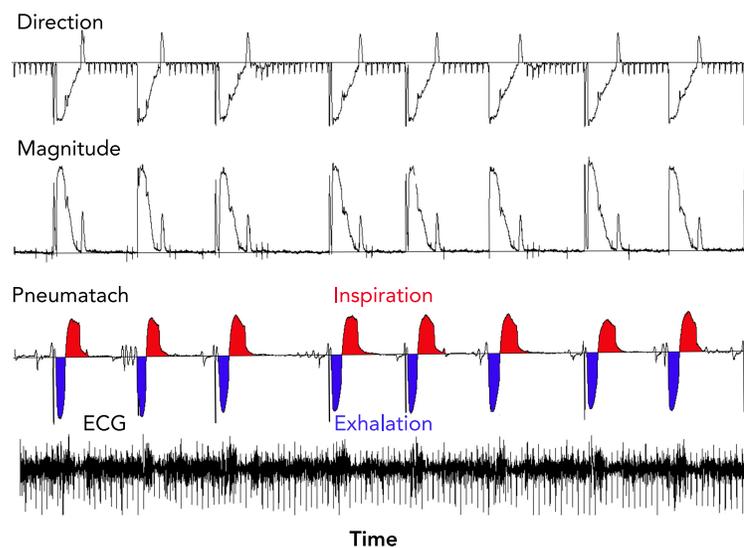


FIGURE 2. Cardiogenic unidirectional flow in an American alligator The top two traces show direction and magnitude, respectively, of flow through a heated thermistor flowmeter that was implanted in the cervical ventrobronchus. The third trace shows ventilation at the nares, measured with a pneumotach; inspiration is a positive trace. The bottom trace is the electrocardiogram. With each contraction of the heart, a pulse of air moves in a unidirectional pattern.

parabronchi and undergone gas exchange. The fresh air of the next inspiration is directed toward the ventrocaudal, sacular regions and the dorso-bronchi (30). On the exhalation of the second cycle, the gases leaving the lung are from the CVB and are composed of stale air, whereas the fresh gases of the sacular locules move through the dorso-bronchi and parabronchi and into the CVB. On the subsequent inspiration, a new bolus of fresh air is brought into the caudal and dorsolateral regions of the lungs, bypassing the ostium to the CVB. Thus, because of unidirectional flow, alligators are theoretically able to flush the entire respiratory system with a two-cycle pump, as is found in birds (FIGURE 1). An observation that supports this hypothesis is that diving alligators are three times more likely to take two breaths in a bout of ventilation than any other combination (36).

In contrast to the two-cycle pump of birds and crocodilians, in mammals, in which gas exchange is best described by a ventilated pool model (FIGURE 1), the P_{O_2} is described by the alveolar gas equation:

$$PA_{O_2} = PI_{O_2} - \frac{PA_{CO_2}}{R} + F$$

where PA_{O_2} and PA_{CO_2} are the alveolar partial pressures of oxygen and carbon dioxide respectively, PI_{O_2} is the inspired partial pressure of oxygen; R is the respiratory exchange ratio, and F is a correction factor that is very small and can be ignored here (83). For a mammal at sea level, PI_{O_2} is given by the fraction of oxygen in dry ambient air multiplied by the difference between the barometric pressure and the partial pressure of water vapor.

$$PI_{O_2} = 0.2094(760 - 47)$$

One can see the how a tidal pool model for gas exchange is a problem under conditions of hypoxia, such as a mammal would encounter in a hypoxic burrow. If the fraction of oxygen in the inspired air is 15%, the respiratory exchange ratio is 0.8, and alveolar ventilation is occurring at a rate that keeps lung P_{CO_2} at ~40 Torr, then alveolar P_{O_2} will be:

$$PA_{O_2} = 0.15(760 - 47) - \frac{40}{0.8} = 57 \text{ Torr}$$

To bring alveolar P_{O_2} up to normal values (100 Torr), the animal must increase rates of ventilation sufficient to lower lung P_{CO_2} to 5.6 Torr.

$$PA_{O_2} = 0.15(760 - 47) - \frac{5.6}{0.8} = 99.95 \text{ Torr}$$

Assuming a constant rate of CO_2 production and using the alveolar ventilation equation, this

requires an increase in ventilation of about sevenfold. Thus there will also be about a sevenfold increase in rates of water and heat loss and in the work of breathing. Importantly, dilution of inspired oxygen by excreted CO_2 should be a lesser problem in a lung with unidirectional flow.

Tests of the importance of unidirectional flow to water and heat conservation and to washout of CO_2 could be done by surgically modifying the respiratory system of crocodilians to convert the unidirectional flow to tidal flow, as was done in birds (68). Once modified, rates of heat loss, water loss, and CO_2 excretion could be compared between unidirectionally ventilated and tidally ventilated lungs with differing levels of inspired oxygen tension.

Lepidosaur

If unidirectional flow is a benefit to low-energy lifestyles, then it is predicted to be present in other ectothermic, lung-breathing vertebrates. Lepidosaur are a clade comprised of the tuatara (*Sphenodon*) and squamates (snakes and lizards). *Sphenodon* has a simple lung consisting of a single chamber, whereas the complexity of the lung varies significantly in squamates (53, 54, 76). Consistent with these hypotheses, unidirectional flow has now been observed in several species of lizards with highly distinct lifestyles and lung anatomy. Monitor lizards are largely convergent with small, predatory mammals (73) and have exceptional capacities, compared with other lizards, for sustaining vigorous exercise (75), whereas green iguanas are herbivores with poor locomotor stamina that is caused by an impairment in the air and blood circulatory systems (18, 29, 79). Patterns of airflow in iguanas are perhaps the more surprising and more informative. Whereas the lungs of monitors consist of numerous chambers and contain some of the features purported to be key to unidirectional flow, such as an enclosed intrapulmonary bronchus intercameral perforations (86), the lungs of green iguanas contain only two chambers, no intercameral perforations, and no enclosed intrapulmonary bronchus (FIGURE 3). Both visualization of flow and computational fluid dynamics simulations provide insight into how this simple anatomy can give rise to unidirectional flow. As with birds and crocodilians, unidirectional flow is cranial along many of the walls, and convective inertia is a key factor driving these patterns of flow (19).

These studies in lizards are clearly too few to draw sweeping generalizations regarding the origin of the trait: more studies are needed. Furthermore, information is needed regarding patterns of airflow in fish, amphibians, and chelonians. Chelonians

are particularly interesting, both because their lungs have a distinct branching pattern compared with lizards and archosaurs and because of their phylogenetic relationship to archosaurs and lizards (21).

Evolutionary Implications

An overarching question driving my research into patterns of airflow in reptiles has been to elucidate why, as the lineages that gave rise to birds and mammals experienced directional selection for greater aerobic capacities, most of the oxygen cascade converged on similar form, but the respiratory systems diverged in form (27). To answer this question requires that the history of these lineages be considered with respect to modes of locomotion, ecological niches, and environmental selective drivers. Divergent evolution can occur when the same selective pressure operates under different environmental conditions. I believe differing ambient oxygen may explain the divergence of these lung structures (27), as well as the trend for innovations that expanded capacities for high minute ventilation, such as a more parasagittal posture and the mammalian (synapsid) diaphragm. The extent to which these innovations were successful influenced the types of ecomorphological niches that could be occupied, and intrinsic constraints on the broncho-alveolar lung prevented Mesozoic mammals from using locomotor modes that require a great aerobic capacity, thereby relegating them to aquatic, semiaquatic, and terrestrial, small body size niches.

Under high levels of atmospheric oxygen (~30–35%), the Permian ancestors of mammals (non-mammalian therapsids) were evolving features suggesting they were trending toward lifestyles that required expanded aerobic capacities. They had a more parasagittal posture, which enables more effective running and breathing, and more cursorial limbs (4, 6, 8, 17, 63, 78). Trackway data corroborate these anatomical data and indicate a gradual shift to a more upright posture began in late Permian synapsids and accelerated in many Triassic lineages (42), when oxygen levels appear to have dropped to ~12–15% (3). When faced with hypoxia, tetrapods generally raise lung P_{O_2} by increasing rates of ventilation. However, no amount of ventilation can compensate for this magnitude of decrease in atmospheric oxygen. Alveolar P_{O_2} would have dropped from 164 Torr with atmospheric oxygen of 30% and $P_{CO_2} = 40$, to 57 Torr with O_2 at 15%. An infinite rate of minute ventilation that reduced lung P_{CO_2} to 0 Torr would still only raise lung P_{O_2} to 107 Torr. These numbers underscore how severely hypoxic the Triassic period would have been to animals adapted to the

high levels of oxygen that were present in the Permian.

Falling levels of ambient oxygen are therefore expected to have affected the evolution of the respiratory system of active animals in two ways. They would have exerted selection for 1) innovations that enable elevated rates of minute ventilation, such as a diaphragm and a parasagittal posture; and 2) innovations that reduce the barrier to diffusion from the lung gases into the erythrocyte, such as a heterogeneous lung with very thin blood-gas barriers and small erythrocytes.

Ambient oxygen has been proposed to have affected the path mammals and birds have taken to

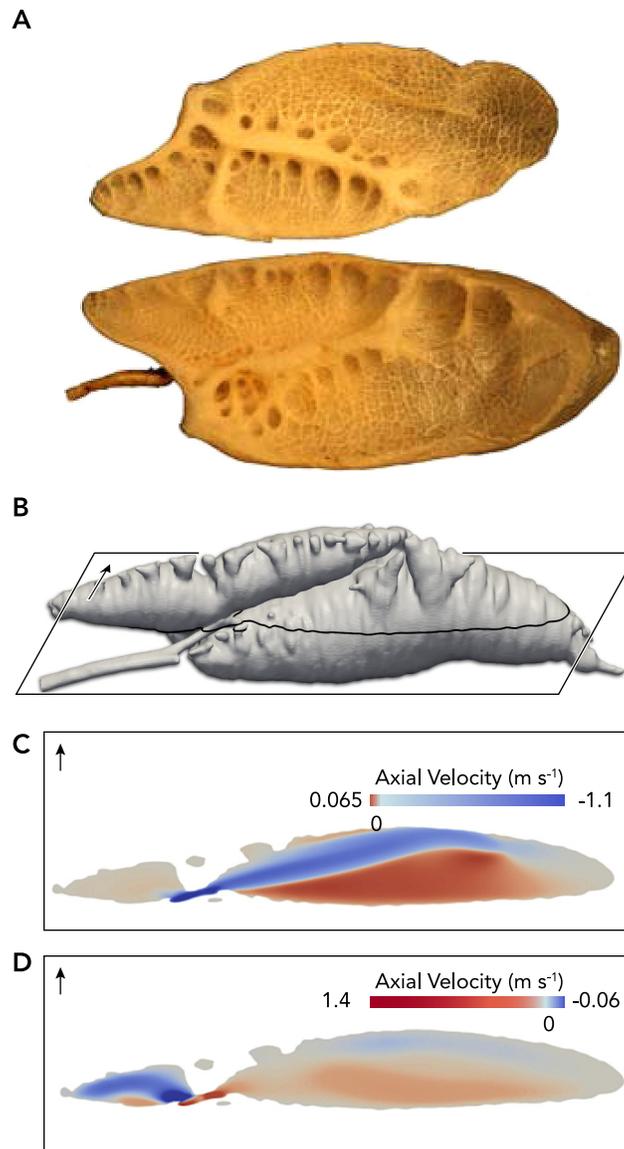


FIGURE 3. Unidirectional flow in the lung of a green iguana

A: dried left lung cut in the parasagittal plane with the lateral portion inverted (top). B: medial view of finite volume mesh created for a computational fluid dynamics model of a right lung. The mesh was constructed from CT data. The coronal plane of section is projected in C and D. C: simulation of flow on inspiration. D: simulation of flow on exhalation. Cool colors indicate flow caudad; warm colors indicate flow craniad (see also Ref. 19).

expand ADF (27). Elevated oxygen of the Permian is expected to have relaxed selection for a thin blood-gas barrier and intensified selection for increased surface area as a means to expand ADF. Even in extant groups, we see that mammalian ADF is expanded over that of non-avian reptiles primarily by an increase in surface area: the harmonic mean thickness of the blood-gas barrier of $\sim 0.5 \mu\text{m}$ is quite similar among both groups (58). The reasons the alveolar lung requires thick barriers have been discussed elsewhere (50, 85) and will only be mentioned here. Selection against a thin blood-gas barrier in mammals arises from the fact that the capillaries undergo mechanical stresses with ventilation, whereas these stresses occur to a lesser degree in the heterogeneous respiratory system of birds, where some structures specialize for ventilation and others for gas exchange (50, 81). In contrast, the low ambient oxygen of the Triassic would have focused selection on thinning the blood-gas barrier as a means to expand ADF (23), and this is best accomplished in a heterogeneous lung. The hypothesis that selection on the thickness of the blood-gas barrier will be influenced by ambient oxygen tension is supported by studies of extant vertebrates. Although both conductive and diffusive steps of O_2 transport determine maximal rates of oxygen consumption ($\dot{V}\text{O}_{2 \text{ max}}$), the relative importance of the diffusive steps increases as the fraction of inspired oxygen decreases (77). West and Wagner (84) analyzed a number of factors limiting $\dot{V}\text{O}_{2 \text{ max}}$ at high altitude, including the P_{50} of the O_2 dissociation curve, total alveolar ventilation, cardiac output, pulmonary capillary transit time, diffusing capacity, and barometric pressure, and showed that $\dot{V}\text{O}_{2 \text{ max}}$ is highly sensitive to membrane diffusing capacity (84). When alveolar Po_2 is inadequate, hemoglobin does not fully saturate before a red cell transits the pulmonary capillary. Importantly, the sigmoidal shape of the hemoglobin dissociation curve produces a nonlinear effect regarding the importance of the relationship between alveolar Po_2 and blood-gas barrier thickness on oxygen uptake. Therefore, under low ambient oxygen, a thin blood-gas barrier will have a disproportionately great advantage for oxygen uptake over a thicker blood-gas barrier.

This evolutionary scenario has potential to explain the divergent evolution of lung structures. It may also help shed light on a long-standing mystery regarding the small body size of Mesozoic mammals. Body size in the mammalian lineage has been extensively studied in terms of its large-scale evolutionary patterns (37, 44, 70). During the Permian Period, synapsids dominated terrestrial ecosystems in the sense that they are the most common fossils found and also the largest animals in their assemblages (Ref. 38 and references there-

in; Ref. 63). After the mass extinction at the end of the Permian, which coincides with the drop in oxygen as well as other major ecosystem changes, large synapsids died out over the Triassic Period (40). A few large-body-size niches were occupied by dicynodonts such as *Placerias* during the Triassic, but by the beginning of the Jurassic the only niches occupied by mammalian synapsids are those of very small body size (FIGURE 4). Most Mesozoic mammals weighed $< 1 \text{ kg}$ (FIGURE 4). Large-body-size niches were dominated by Archosaurs for the next 150 million years (45). The role of oxygen in this faunal replacement has been debated (see discussion in Refs. 71, 80).

Conventional explanations for this pattern of ecomorphological diversification have overlooked the relationship between body size, lung design, locomotor mode, and energetic costs of transport. Cost of transport is greater for small terrestrial animals than large animals (41, 61, 65). For this reason, as well as other biomechanical considerations, there is a range of body sizes, from ~ 5 to 200 kg and with an optimum of $\sim 50 \text{ kg}$, in which adaptations for cursorial locomotion are common (20). Cursorial and saltatorial adaptations are found outside this range (e.g., giraffes and kangaroo rats), but these ecomorphs are rare. In extant assemblages, terrestrial vertebrates that are smaller than $\sim 1 \text{ kg}$ generally do not show selection for expanded aerobic capacities, and these animals tend to occupy low energy niches (scansorial, fossorial, semiaquatic). Importantly, this is not a constraint on body size per se, as evidenced by bats. Rather, it is a manifestation of the fact that cost of transport increases with decreasing body mass, making it harder for small terrestrial mammals to outrun larger ones. This observation is important because patterns of locomotion transcend all taxa and therefore, "... locomotion is among the most fundamental influences constraining animal evolution" (Ref. 65, p. 14). The interaction between mode of locomotion, lung design, and environmental oxygen may well explain the puzzling pattern of faunal turnover in the Permian and Triassic. Perhaps the question that should be posed is not why were Mesozoic mammals so small and Mesozoic archosaurs so big but the more inclusive question of why didn't Mesozoic mammals use modes of locomotion that require great aerobic capacities (e.g., flapping flight, cursorial running) while these modes were widespread in archosaurs? The modern analogs of Mesozoic mammals occupy ecomorphological niches with low demands on aerobic capacities. For example, a modern analog of *Morganucodon* and *Yanoconodon* is the short-tailed opossum; modern analogs of *Haldanodon*

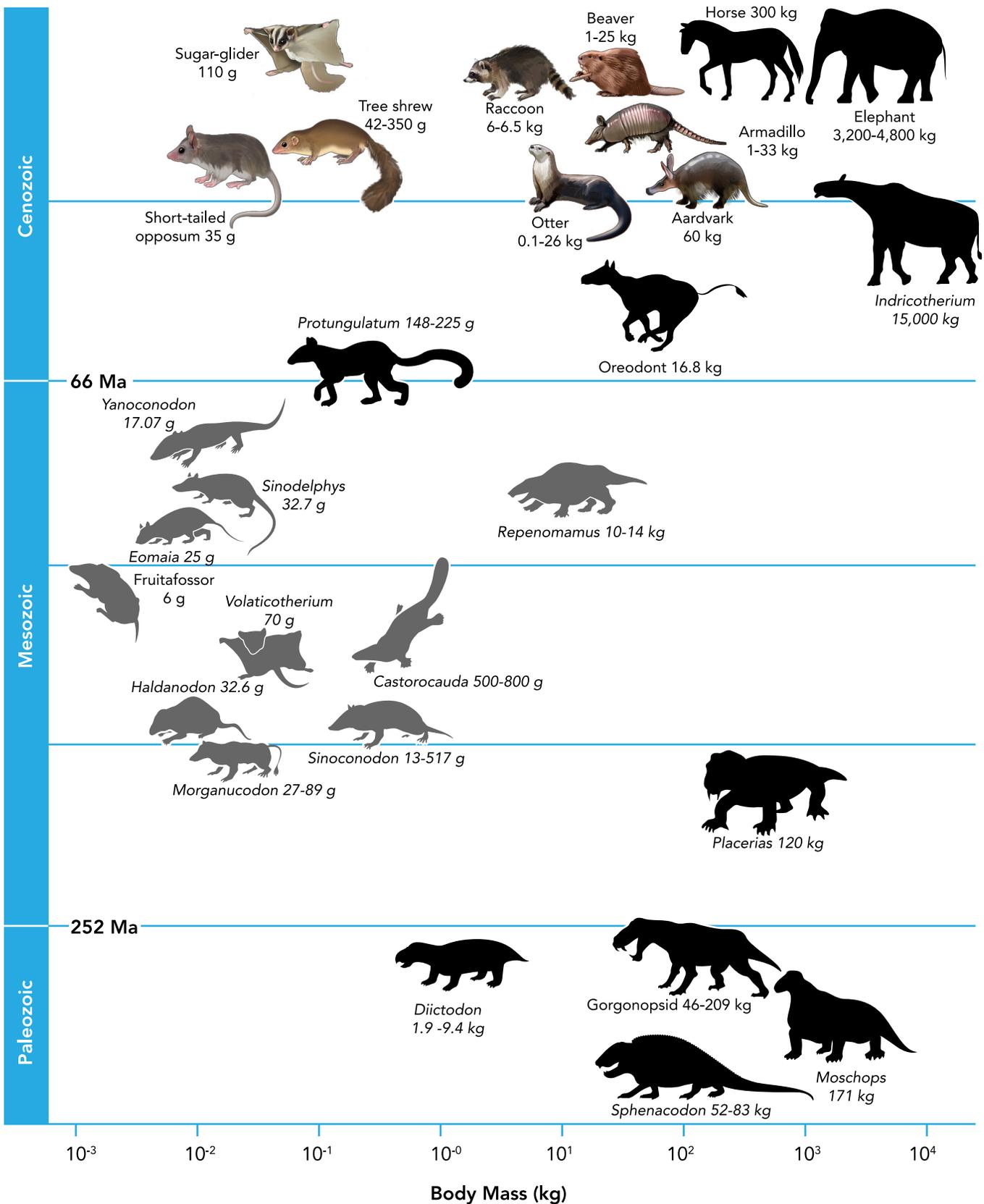


FIGURE 4. Body size and ecomorphological disparity in selected mammals and non-mammalian synapsids
 Modern analogs of Mesozoic mammals (light gray silhouette) are illustrated in color. Although Mesozoic mammals occupied a variety of niches, these were mainly limited to small body sizes, and none show adaptations for sustaining vigorous exercise. In contrast, Cenozoic mammals include many species that are adapted for sustaining vigorous exercise. Images are modified from Ref. 45 or obtained from PhyloPic.org. Body size estimates are based on regressions following Ref. 18a or obtained from the following sources: <http://genomics.senescence.info/> and Refs. 8, 18a, 21a, 31a, 34a, 36a, 40a, 40b, 45, 45a, 45b, 52a, 61a, 62a, 63a, 70, 71a, 85a.

and *Castorocauda*, beavers, otters, and semi-aquatic moles; *Sinoconodon* and *Repenomamus*, the raccoon; *Fruitafossor*, the armadillo and Aardvark; *Sinodelphys*, and *Eomaia*, the tree shrew; *Volaticotherium*, the sugar glider (45). I propose the broncho-alveolar lung, with its requirement for a thick blood-gas barrier and therefore its intrinsic limitations in low-oxygen environments, was a historical constraint that restricted Mesozoic mammals to these ecomorphological niches. Therefore, unidirectional flow in the ancestors of diapsids may have been a pre-adaptation, enabling the evolution of a heterogeneous respiratory system with thin barriers to diffusion, which expanded the lung's capacity for oxygen uptake under hypoxia, and allowed archosaurs to be successful as highly active animals in a hypoxic world.

Summary

The discovery of unidirectional pulmonary airflow outside of birds indicates a transformation in our understanding of this trait is necessary. A number of tenets of the conventional wisdom need to be reevaluated regarding the functional underpinning of the trait, the selective drivers, the date of origin, and the mechanisms by which this flow arises. Conventional wisdom has held that unidirectional flow is an adaptation for efficiency of gas exchange and linked it to the high metabolic demands of flapping flight and endothermy. Clearly, this cannot be the case, given that lizards and crocodylians do not fly and are ectotherms. Furthermore, unidirectional flow did not arise with birds but appears to have evolved very early, perhaps with the evolution of the Diapsida, or perhaps it is even older. Studies on patterns of airflow in the lungs of fishes, amphibians, and turtles are needed to clarify the date of origin of the trait and the functional significance of this flow, and to provide a more complete understanding of the evolution of the vertebrate lung. Initial selective pressures for unidirectional flow could have been heat conservation, water conservation, a reduced cost of breathing, and improved crypsis.

Although not evolving with birds, unidirectional flow may have been an exaptation in birds and other archosaurs, which facilitated high rates of gas exchange under low ambient oxygen, because unidirectional flow allowed selection for consolidation of respiratory parenchyma into regions of the lung undergoing minimal mechanical stresses and thereby was permissive to the evolution of a thinner blood-gas barrier than is possible in the broncho-alveolar lung. A thin blood-gas barrier has a greater impact on arterial saturation under conditions of low oxygen, when binding occurs in end-capillary blood on the steep part of the

sigmoidally shaped oxygen-hemoglobin dissociation curve than when ambient oxygen levels are high and end-capillary blood is saturated. Thus this lung may have allowed birds to evolve energetically costly forms of locomotion (flapping flight) during the hypoxia of Mesozoic Era. This relationship between lung design, costs of transport, and environmental oxygen may explain the small body size of Mesozoic mammals: the broncho-alveolar lung with its thick blood-gas barrier made mammals poorly suited to niches that required great aerobic stamina. ■

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