Lungs of the first amniotes: why simple if they can be complex?

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We show—in contrast to the traditional textbook contention—that the first amniote lungs were complex, multichambered organs and that the single-chambered lungs of lizards and snakes represent a secondarily simplified rather than the plesiomorphic condition. We combine comparative anatomical and embryological data and show that shared structural principles of multichamberedness are recognizable in amniotes including all lepidosaurian taxa. Sequential intrapulmonary branching observed during early organogenesis becomes obscured during subsequent growth, resulting in a secondarily simplified, functionally single-chambered lung in lepidosaurian adults. Simplification of pulmonary structure maximized the size of the smallest air spaces and eliminated biophysically compelling surface tension problems that were associated with miniaturization evident among stem lepidosaurmorphs. The remaining amniotes, however, retained the multichambered lungs, which allowed both large surface area and high pulmonary compliance, thus initially providing a strong selective advantage for efficient respiration in terrestrial environments. Branched, multichambered lungs instead of simple, sac-like organs were part and parcel of the respiratory apparatus of the first amniotes and pivotal for their success on dry land, with the sky literally as the limit.

1. Introduction

Adaptations in the respiratory system were required to ensure success in colonizing dry land and in the evolution of high aerobic performance and active flight [1]. While particularly in tetrapods the main organs for aerobic gas exchange are lungs, their evolutionary origin can be traced back to fish-like ancestors [1]. In amniotes, the primarily fully terrestrial vertebrates, lungs are the principle sites for air breathing and their anatomy exhibits tremendous structural diversity. It ranges from simple, single-chambered organs to highly complex, multichambered and branched lungs, as well as transitional types covering almost everything in between [2,3]. Although aspiration breathing is an essential character of the amniote condition [2], it is commonly assumed, based on the presence of single-chambered lungs in the majority of lepidosaurs, that the rather simple organs observed in extant amphibians also represent the ancestral amniote gas exchanger [4–7]. This combination of an effective breathing mechanism with simple lungs represents a paradox for basal amniotes. It further suggests that the complex, multichambered lungs of mammals, crocodiles/birds, turtles and monitor lizards each evolved independently as an exit from this paradox [1,2]. The present study addresses the question whether the latter scenario can be supported or if a common Bauplan of amniote lungs other than that of a simple ‘sac’ exists.

2. Material and methods

We studied the lungs of 187 specimens, representing 73 species from 42 different ‘families’, covering all major tetrapod lineages. Lungs were excised, dehydrated
and dried or injected with silicone elastomer for macroscopic examination. A tuatara lung was scanned with a \textit{v|x|s} \mu CT device (phoenix/X-ray) at the Steinmann-Institut, Universität Bonn (Germany) with a resolution of 69.57 \textmu m per voxel and visualized using VGStudio MAX v. 2.0 (Volume Graphics). Embryos of the Madagascar ground gecko, \textit{Paroedura}

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**Figure 1.** Pulmonary diversity in tetrapods. (a) Even the largest amphibians possess single-chambered lungs with an apical entrance. (b) Amniotes exhibit a subapical bronchial entrance and a variety of internal structuralization. Large septa can separate chambers, which, in turn, are partially subdivided by smaller septa (Se), exactly followed by branches of the pulmonary artery (Pa). (c) The subapical bronchial entrance (*) is seen even in the vestigial left lung of cobras. (d,e) There, only the right lung (Rlu) develops to a functional organ. These lungs are remarkably tube-like and exhibit tissue for gas exchange (Get) only immediately posterior to the bronchial entrance (Be), which more or less continuously follows the trachea (Tr), while the more distal portion is characterized as a sac-like region (sac, only partially shown). Despite this fundamentally single-chambered appearance, not only is the bronchial entrance subapical, but also the pulmonary artery (Pa) exhibits the strict hierarchical branching pattern typical of amniote lungs. (f–h) The tuatara exhibits all these traits. (i) Embryonic lungs of Madagascar ground geckos show a dorsal bud that makes the bronchial entrance subapical. (j) Later in ontogeny, additional buds form an almost multichambered lung. (k–m) In the expansion phase, the lung dilates, obscuring the initial multichamberedness.
The lungs of lepidosaurs have in common with the more complex amniote lungs a subapical bronchial entrance, near the point where the pulmonary artery and their lungs were removed. In amphibians, the entrance to the lung is apical (figure 1a), as is also the case for the pulmonary artery (electronic supplementary material, figure S1a). In multichambered lungs, the pulmonary artery follows the major branches of airways (figure 1b). In multichambered lungs, the pulmonary artery follows the major branches of airways during early organogenesis, appears not yet to have been reconstructed (figure 1b). In this ‘branching phase’, the most anterior element becomes enlarged and extends craniad, causing the bronchial entrance to become subapical (figure 1i). During the ensuing ‘expansion phase’, the central pulmonary lumen becomes dilated, obscuring the sequential structure, which can be identified in the adult only by small septa, each with individual vascular supply (figure 1k–m; electronic supplementary material, figure S2). The early stages are strikingly similar to the sequentially branched structure of developing mammalian [14], chelonian [15], crocodylian [16] and avian lungs [17] (electronic supplementary material, figure S1c) and are also virtually identical with the Bauplan of monitor lungs (electronic supplementary material, figure S3). Interestingly, the vestigial left lung of cobras exhibits a remarkable similarity to the gecko’s lungs during early ontogeny, including the subapical bronchial entrance (figure 1c). Such a subapical bronchial entrance, as well as sequential internal subdivision during early organogenesis, appears not yet to have been observed in amphibians. Furthermore, no blood vessel pattern

Figure 2. Pulmonary evolution in amniotes. In amphibians, lungs are single-chambered and initially only complement gills and skin (a). In amniotes, the lungs are the principle site for gas exchange; multichamberedness is shared by all amniotes and was a key for conquering dry land (b). In mammals, pronounced intrapulmonary branching led to the bronchoalveolar lung (c); turtle lungs approximate the plesiomorphic amniote condition (d). Archosaurs (crocodiles, birds) retained a multichambered lung, and birds evolved air sacs and the parabronchial lung (e). The ancestors of lepidosaurs underwent miniaturization, preventing them from retaining a multichambered lung. Multichamberedness is still ontogenetically visible, despite the adult single-chamberedness (f).
that betrays a transient subapical bronchial entrance exists in either embryonic or adult lungs of the species studied (electronic supplementary material, figure S1a). Such a rather simple internal Bauplan even applies to the largest extant amphibians: e.g. the Japanese giant salamander (Andrias japonicus) (figure 1a).

We suggest that a common genetic programme for intrapulmonary branching, acting during early ontogenetic stages, is shared at least by all amniotes and represents a fertile ground for further research. Ancestral state reconstructions support the plesiomorphy of the amniote pattern described here (see electronic supplementary material, figures S4 and S5). Thus, the lepidosaurs must have undergone a secondary simplification of their pulmonary Bauplan rather than inheriting an undivided and unbranched lung structure from their ancestors.

As multichambered lungs can provide greater surface areas and higher pulmonary compliances than single-chambered lungs of equivalent volume, they are more cost-effective to ventilate [18,19]. Thus, an evolutionary scenario whereby single-chambered lungs represent a more highly derived condition raises the question whether this simplification presented some selective advantage, or multichambered lungs presented a disadvantage. A strong disadvantage would have been present if lepidosaurs evolved from miniscule ancestors. In fact, the Mesozoic fossil record of stem lepidosauromorphs shows that species with a body cavity length of less than 3 cm, comparable with the smallest extant lizards, actually existed [20,21]. A respective miniaturization of a multichambered lung would result in extremely tiny air spaces. As surface tension increases dramatically with a decrease in diameter, the result would be low-compliance lungs that are extremely difficult to inflate without an auxiliary breathing structure [18,19]. Mammals and birds of such small size (alveolar diameter approx. 50 m and air capillaries of 3 m, respectively) compensate for these biophysical constraints through a variety of auxiliary structures, such as the diaphragm and the air sacs, respectively [1]. In newborn, extremely tiny marsupials, the lungs are very immature and require substantial postnatal development to become functional, while oxygen is supplied via cutaneous gas exchange [22].

The majority of lepidosaurs, however, evolved a functionally single-chambered lung, whereby the ancestral branched nature is evident only during early development, when the lungs are still fluid-filled and non-functional.

In this context, it is of particular interest that varanoid lizards are highly derived squamates [13] and that they exhibit very complex and multichambered lungs [3]. We hypothesize that basal representatives of the varanoid lineage exploited the ‘multichamberedness’ of lung Anlagen retained from the ancestral amniote condition to ‘reinvent’ a multichambered lung (see electronic supplementary material, figure S3).

In summary, single-chambered lungs of lizards and snakes reveal the same basic ontogenetic amniote pattern observed in more complex, multichambered ones. In spite of their extreme complexity, mammalian lungs display an underlying developmental sequence that can be explained by only three different types of branching [14]. Findings from turtles [23] and archosaurs [10,17], where the pulmonary Bauplan follows a similar strict hierarchical ontogenetic sequence, as well as the data for lepidosaurs presented here, indicate that deep homologous genetic processes are involved in these diverse lineages. Taken together, the evidence integrated here is consistent with a scenario for amniote lung evolution (figure 2) that starts with complexity as a key to terrestrialization.

Acknowledgements. Specimens for analysis were made available by Wolfgang Böhme, Guarino Colli, Frank Glav, Michael Franzen, Warren Fitch, Luc Vives and Thomas Ziegler. Irina Rulf helped with μCT work and Michael H. Hofmann provided laboratory space in Bonn. Dalton de Souza Amorim, Eduardo Andrade Botelho de Almeida, Flavio Alcino Bockmann are thanked for advice concerning the ancestral state reconstructions. M.L. and S.F.P. conceived the study and wrote the paper; M.L. and K.G. collected data; M.L. and T.K. analysed data; all authors contributed to and approved the final version of the paper.

Funding statement. M.L. was supported in part by a doctoral fellowship from the Universitat Bonn; S.F.P. by a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior fellowship awarded to T.K. (Edital CGCI 014/2008). A Fundação de Amparo à Pesquisa do Estado de São Paulo grant to T.K. financed work in Brazil (2006/60140-4). A fellowship from the Deutscher Akademischer Austauschdienst financed the stay of K.G. in Brazil.

Conflict of interest. The authors declare no conflict of interest.

References


