PALAEONTOLOGY: Egg-bound in deep-time

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A fossilised egg anchors a new analysis of early egg evolution, suggesting that ancestral amniotes retained eggs for an extended period of development.

Amniotes, the group that includes mammals, reptiles, and birds, arose around 320 million years ago[1] and are characterised, as their name implies, by the common possession of the amniote egg. In the amniote egg, the developing embryo and, often, a nutrient source (yolk) are enclosed in specialised extra-embryonic membranes (the amnion and chorioallantois) that facilitate fetal-maternal interaction and allow the sequestration of potentially toxic nitrogenous waste products. Ultimately, this type of egg allowed vertebrates to fully colonise the land, but there is disagreement as to when it first appeared and the conditions under which it evolved. Writing in Nature Ecology and Evolution, Jiang et al.[2] describe a new fossil egg from the Early Cretaceous (c.120 million years ago [Ma]) of China that sheds light on this important stage of tetrapod evolution.

In what might be called the traditional view, the aquatic or amphibious amniote ancestor began to lay its eggs in damp places on land, possibly to protect them from fish and other aquatic predators. The extra-embryonic membranes, and later a tough outer shell, evolved over time to protect the embryo from desiccation, infection, and predation in the terrestrial environment. The amniote egg was thus 'the key innovation' that permitted full tetrapod colonisation of the land[3].

However, an alternative theory[4] proposed that the amniote egg arose not in association with terrestrial egg-laying (oviparity) but instead to permit extended egg retention (EER), with continuing embryo development, within the maternal uterus. The result was either live birth (viviparity) or the deposition of eggs (oviparity) in which the embryo was already at an advanced stage of development (up to the appearance of the fore- and hindlimb buds or longer). Under this hypothesis, the extra-embryonic membranes evolved to prevent the developing embryos from sticking to the uterine wall (contractile amnion) and to facilitate continued interchange of gases and nutrients between mother and young (chorioallantois)[5].

Among living amniotes, most mammals are viviparous, and squamates (lizards and snakes) practice both viviparity and oviparity, sometimes within a single species depending on environmental conditions. Both groups have EER. By contrast, living archelosaurs (turtles, crocodiles, birds) are universally oviparous, lack EER, and lay eggs in which the embryo is at a very early stage of development[5]. This difference between living amniotes confounds reconstruction of the ancestral condition. This is where fossils can help, on the rare occasions in which young are preserved within, or in close proximity to, an adult body cavity. Thus at least one Palaeozoic amniote (c. 280 Ma) appears to have been viviparous[6], as were marine ichthyosaurs and plesiosaurs[7]. However, uncertainties as to the relationships of the latter groups again complicate ancestral state reconstruction.

In their paper, **Jiang et al.[2]** describe a parchment-shelled egg belonging to a rather obscure group of fossil reptiles. Choristoderes are currently known from freshwater deposits of Middle Jurassic to Miocene age (c. 160-20 Ma) across Europe, North America and Asia[8,9.] Allowing for the vagaries of the fossil record, these reptiles appear to have reached their maximum diversity in the Early

Cretaceous lakes and swamps of eastern Asia (Mongolia, Japan, China), most notably as components of the spectacular Jehol Biota of North-East China (c.125-120 Ma). These Jehol choristoderes display the full range of known choristodere body types, from short-snouted lizard-like reptiles (*Monjurosuchus, Philydrosaurus*), to small-headed long-necked genera resembling mini-plesiosaurs (*Hyphalosaurus*) and long-snouted crocodile-like forms (*Ikechosaurus*)[9] (Fig. 1). Moreover, the sheer volume of specimens and their exceptional preservation increase the importance of these deposits for choristoderan researchers, providing rare insights into the life appearance and reproductive strategies of these enigmatic reptiles.

Jiang et al.[2] report an exquisitely preserved late embryo of the crocodile-like choristodere *Ikechosaurus* encased in a parchment-shelled egg. The specimen confirms oviparity in derived choristoderes but, as an isolated specimen (i.e. not associated with an adult body cavity), it cannot reveal whether choristoderes had EER, because it could represent a parchment-shelled non-EER egg incubated to pre-hatchling stage in a damp terrestrial environment. However, we already know that choristoderes had EER, based on specimens of other Jehol choristoderes (*Monjurosuchus, Philydrosaurus*, *Hyphalosaurus*) found with advanced embryos indicative of viviparity or late-stage oviparity[10,11]. The problem, as with ichthyosaurs and plesiosaurs, is that the affinities of choristoderes have remained poorly understood – with several different hypotheses of relationship proposed (Fig. 1).

This is where the new egg fossil becomes significant. Analysis of the late embryo suggests a pattern of skeletal ossification (in the skull roof, vertebrae, and pelvis) characteristic of living archosaurs rather than squamates. This finding supports placement of choristoderes within the mixed bag of reptiles (archosauromorphs) on the stem of Archosauria (crocodiles, birds, dinosaurs, pterosaurs) (position 1 in Fig. 1). This could mean that EER was originally present in the ancestry of archosaurs, and thus potentially in the ancestry of archelosaurs.

Incorporating this new information into an ancestral character state analysis, **Jiang et al.**[2] find support for EER as an ancestral amniote trait and, by implication, for the evolution of the amniote egg within the maternal uterus rather than on land. The subsequent addition, in different lineages, of leathery or rigid eggshells would have improved egg survival and enabled tetrapods to colonise a wider range of terrestrial environments[5].

Jiang et al.'s[2] paper makes a valuable contribution to ongoing discussion on the evolution of the amniote egg[5]. Nonetheless, further choristodere fossils, especially of older/ancestral taxa, would help to confirm their archosauromorph affinities. Similarly, the recognition of viviparity/late stage embryos (and thus EER) within fossils of other archosauromorph or stem-turtle lineages would provide a critical test of the proposal that EER was originally present on the archosaur or archelosaur stem, as opposed to being acquired *de novo* in choristoderes. Given the low preservation potential of eggs and embryos, however, more answers as to the plasticity of reproductive modes may come from research on living reptiles – especially squamates and their relatives which show a range of strategies from oviparity to live-bearing, and from soft-shelled eggs to those with fully calcified shells[5].

Fig. 1. Choristoderes and their relationships.
A. Silhouettes showing body outlines of different choristoderes in the Jehol Biota;
B. Simplified tree of relationships for Amniota showing different proposed relationships for choristoderes: (1) as archosauromorphs, the group encompassing archosaurs and those more closely related to archosaurs than to other reptiles (e.g. Jiang et al.[2]); (2) as the sister group to turtles and

archosauromorphs (e.g. Lu et al.[11]); (3) as lepidosauromorphs (e.g. Muller[12]); (4) on the diapsid stem (e.g. Evans & Hecht[8]). Key: Green – EER groups; Red – non-EER groups.

Competing interests statement
The author declares no competing interests

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