Archaeology and phylogeny to ascertain the evolution of amniotic egg and viviparity – Review

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Abstract

Which came first, the chicken or the egg? That is not a pun, joking or irrelevant question. Two hypotheses have tried to answer it. The first, and accepted for years, proposes that the evolutionary process went from oviparity, through an Embryonic Retention (ER) phase, to viviparity. In other words, the simpler egg came first, which hatched into the more complex chicken. That is known as the terrestrial model. Indeed, fishes typically lay eggs, albeit some sharks are viviparous. Even some living (extant) mammals are oviparous, (monotremes like platypus and echidnas). But new evidence has challenged such traditional view, proposing that it was the other way round. Thus, first came the chicken, which eventually laid the egg. That is the Extended Embryo-Retention (EER) model. In the first evolutionary step of this scenario, ancient reptiles, birds and mammals gave birth to offspring. Subsequently, some of them developed oviparity. Indeed, several archaeological and phylogenetic discoveries have shown that first, during the ER period, thin layers of tissues (extraembryonic membranes) evolved for a better development of the embryo, given rise to the amniotes. It is known that living reptiles (like crocodiles and turtles) and birds typically show non-EER oviparity. But curiously, most lizards, snakes and mammals exhibit oviparity with EER or viviparity. While the debate continues, both archaeology and molecular biology will contribute to clarify this interesting topic.

Key words: Dendrogram, embryogenesis, ontogeny, mother, fetus, interactions, antagonistic, *Ikechosaurus*.

Resumen

¿Qué fue primero, el huevo o la gallina? No se trata de un juego de palabras, una broma o una pregunta irrelevante. Dos hipótesis han intentado responderlo. La primera, y aceptada desde hace años, propone que el proceso evolutivo pasó de la oviparidad, a través de una fase de Retención Embrionaria (RE), a la viviparidad. En otras palabras, primero surgió el huevo más simple, que eclosionó en el pollo más complejo. Es lo que se conoce como modelo terrestre. De hecho, los peces suelen poner huevos, aunque algunos tiburones son vivíparos. Incluso algunos mamíferos vivos (extantes) son ovíparos (monotremas como los ornitorrincos y los equidnas). Pero nuevas pruebas han puesto en entredicho esta visión tradicional, proponiendo que fue al revés. Así, primero fue la gallina, que acabó poniendo el huevo. Este es el modelo de la Retención Embrionaria Extendida (REE). En el primer paso evolutivo de este escenario, los antiguos reptiles, aves y mamíferos eran vivíparos. Posteriormente, algunos de ellos desarrollaron la oviparidad. De hecho, varios descubrimientos arqueológicos y filogenéticos han demostrado que primero, durante el periodo RE, desarrollaron finas capas de tejidos (membranas extraembrionarias) para un mejor desarrollo del embrión, dando lugar a los amniotas. Se sabe que los reptiles vivos (como cocodrilos y tortugas) y las aves muestran típicamente oviparidad sin REE. Pero curiosamente, la mayoría de los lagartos, serpientes y mamíferos presentan oviparidad con REE o viviparidad. Mientras continúa el debate, tanto la arqueología como la biología molecular contribuirán a aclarar este interesante tema.

Palabras clave: Dendrograma, embriogénesis, ontogenia, madre, feto, interacciones, antagónico, *Ikechosaurus*.

Introduction

Traditionally, archaeology and molecular biology pertained to completely separated scientific areas. Fortunately, such scenario changed with the development of in vitro amplification systems and massively-parallel nucleic-acid sequencing platforms. Other areas contributing to such breakthrough were metabolomics, proteomics and stable-isotope research. All that has opened the door to new exciting studies, allowing to decipher previously intractable enigmas. That applies not only to extant (currently living), but even to extinct species. Thus, the marriage between archaeology and molecular biology has taken place, and is yielding surprising results, as we have previously shown (Dorado et al, 2007-2022).

In this scenario, there is a long-lasting evolutionary question in science, which has been graphically summarized by the following question: *which came first, the chicken or the egg?* That it is not a futile question, but a significant one. It should be clarified that this enigma relates to early amniotes. They are a group of vertebrates that undergo embryonic or fetal development within an amnion, which is a protective membrane (whether inside an egg or not). In other words, did early reptiles, birds and mammals laid eggs, or did they give birth to their offsprings first, further evolving the capacity to produce eggs later on? Also important is to note that the word egg in this context does not mean just the female sexual cell (ovule; complementary to the male spermatozoid), but an elaborated structure with complex fetal membranes (also known as amniotic egg). Indeed, such remarkable development significantly contributed to the evolutionary success of reptiles, birds, and mammals. Fishes and amphibians are not amniotes. Sharks usually lay eggs, but some are viviparous. Whale sharks are even more curious, using both strategies: they first generate eggs, that develop inside the body of the mother, and then give birth to offsprings.

There are two hypotheses trying to answer the question about the chicken and the egg. Thus, the conventional terrestrial model involves non-Extended Embryo-Retention (non-EER) oviparity (laying hard-shelled or soft-shelled eggs), that further evolved into EER with oviparity, and eventually viviparity (giving birth to live young). That could be driven by adaptation to the dry and hot terrestrial environments, as well as to overcome antagonistic interactions between fetus and mother during EER (Romer, 1957). But other hypothesis, supported by recent archaeological discoveries, has challenged such former assumption, with the proposal of the EER with viviparity model (Hubrecht, 1910; Mossman, 1987; Lombardi, 1994; Laurin and Reisz, 1997; Laurin, 2005; Jiang et al, 2023). In such a model, initially, all amniotic animals tended to viviparity. Therefore, it was the chicken before the egg (Jiang et al, 2023).

The beauty of science is that any hypothesis can become an accepted theory, but such status only holds until a new challenging hypothesis arises, with stronger scientific support. That may be the case of the chicken/egg dilemma. In this scenario, archaeological and phylogenetic analyses have allowed to reach a surprising conclusion, showing again the power of the scientific method. The two hypotheses are summarized below (Fig. 1).

Terrestrial model: first came the egg and then the chicken

When the chicken/egg enigma was proposed, it was assumed that the obvious answer was that first came the egg, and then the chicken (Romer, 1957). Indeed, many species phylogenetically previous to amniotes lay eggs and are not viviparous. Such model was further reinforced with the surprising fact that some living mammals known as monotremes (indigenous to Australia and New Guinea) are not viviparous, but lay eggs. Currently living monotremes are the platypus (*Ornithorhynchus anatinus*) and four species of echidnas: Western long-beaked echidna (*Zaglossus bruijni*), Sir David's long-beaked echidna (*Z. attenboroughi*), Eastern long-beaked echidna (*Z. bartoni*) with four subspecies, and short-beaked echidna (*Tachyglossus aculeatus*).

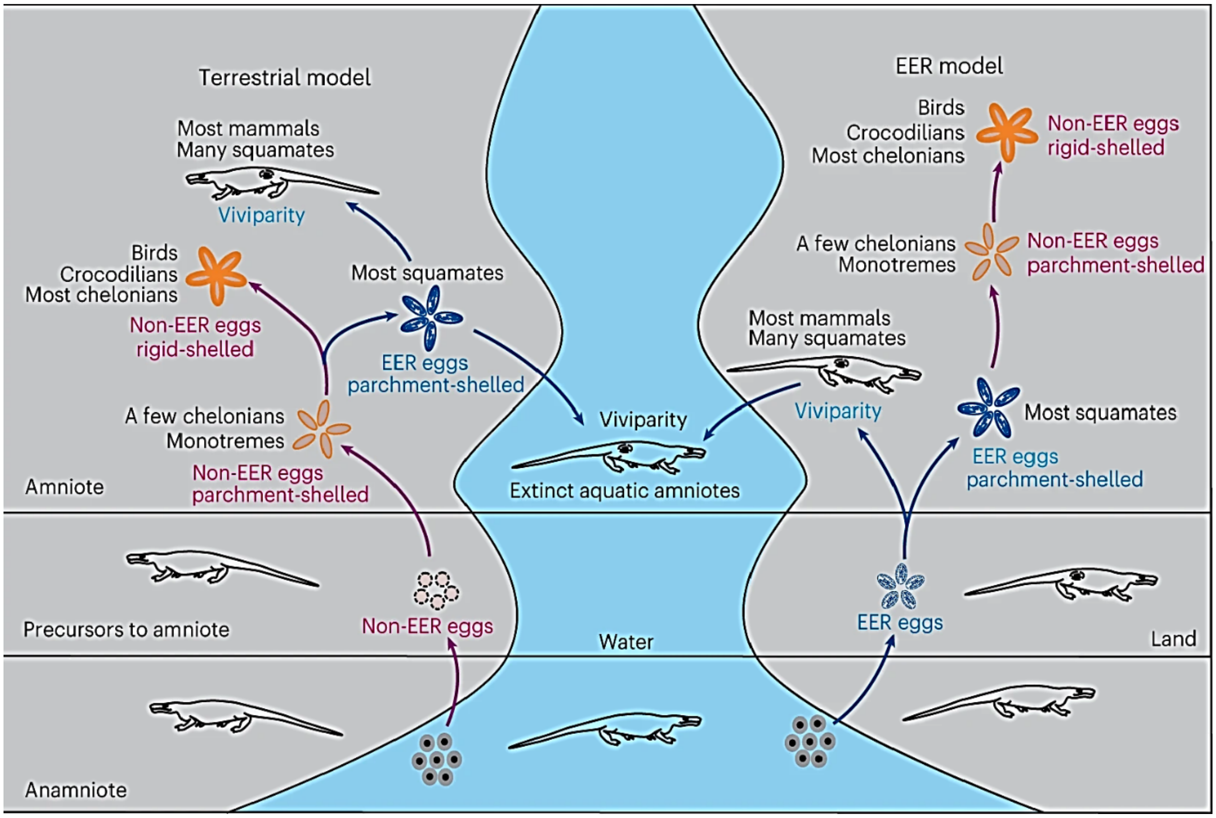

Figure 1. Two hypotheses about the evolution of the amniotic egg. The terrestrial model (left) proposes an original non-EER oviparity (purple) and further evolution towards oviparity with EER and viviparity (blue) evolving multiple times. By contrast, the EER model (right), describes the evolution of primitive EER across oviparity to viviparity (blue), with non-EER oviparity (purple) evolving multiple times. © Springer Nature (Jiang et al, 2023).

On the other hand, marsupials (native from Australasia and America) are viviparous, but do not have placenta. Therefore, they give birth to much undeveloped offsprings than placental mammals. That could be interpreted as an evolutionary step forward from oviparous mammals, but not yet as evolved as placental ones. Interestingly, surprisingly, and even shockingly, the placenta evolved from retroviral infection (Mi et al, 2000).

EER model: first came the chicken and then the egg

The previous terrestrial model has been widely accepted until recently, when archaeological and phylogenetic studies have revealed surprising results. The fossil record had previously shown that choristoderes were morphologically diverse, including lizard- or crocodile-like reptiles, as well as long-necked morphologies. They lived from the Middle Jurassic-Triassic to the Miocene periods [168 to 20, and even 11.6 million years ago (Mya)]. They were considered viviparous. Yet, an oviparous choristodere specimen (*Ikechosaurus* sp.) from the Lower Cretaceous period (125 to 120 Mya) has been recently found in China, surprisingly containing an articulated embryo inside a parchment-shelled egg (Jiang et al, 2023).

Such remarkable discovery, together with other evidence, has been recently analyzed. That has generated a dendrogram (phylogenetic tree) of oviparity, EER and viviparity for extant and extinct amniotes. Surprisingly, it shows that the original reproduction of basal archosauromorphs was EER (including viviparity). In other words, this model proposes that the extraembryonic membranes arose to control fetal-maternal interactions during extended EER. Thus, early reptiles, birds and mammals would have given birth to their young and then, some of them, developed the ability to lay eggs. Curiously, currently living crocodiles, turtles and birds typically exhibit non-EER oviparity (laying eggs at an early developmental stage), but most lizards, snakes and mammals exhibit oviparity with EER or viviparity (Jiang et al, 2023) (Fig. 2). Even fishes like some sharks are viviparous, although they are not amniotes, as indicated before.

These findings reveal signs of embryonic retention capacity and viviparity in ancestors of all major evolutionary branches of both Amniota (lizards and relatives) and Archosauria (crocodiles, dinosaurs, and birds) clades. This way, the mother retains the young for a variable period of time, until conditions are best for survival. Therefore, instead of the hard-shelled egg being an original evolutionary success, this research points out that it was actually the EER that triggered the change.

Before the amniotes, the first tetrapods that evolved limbs from fish fins typically had amphibious habits. They lived in or near water for feeding and reproduction, like modern amphibians such as frogs, toads, and salamanders. However, this changed with the arrival of the amniotes, 320 million years ago. They emerged from the water because they developed a waterproof skin and other ways to control water loss. Among them, the amniotic egg was of paramount importance, becoming a great success. It was a kind of “private pond”, in which the developing reptile was protected from desiccation in dry and hot climates. That allowed the amniote to move away from the water’s edge, conquer and dominate terrestrial ecosystems.

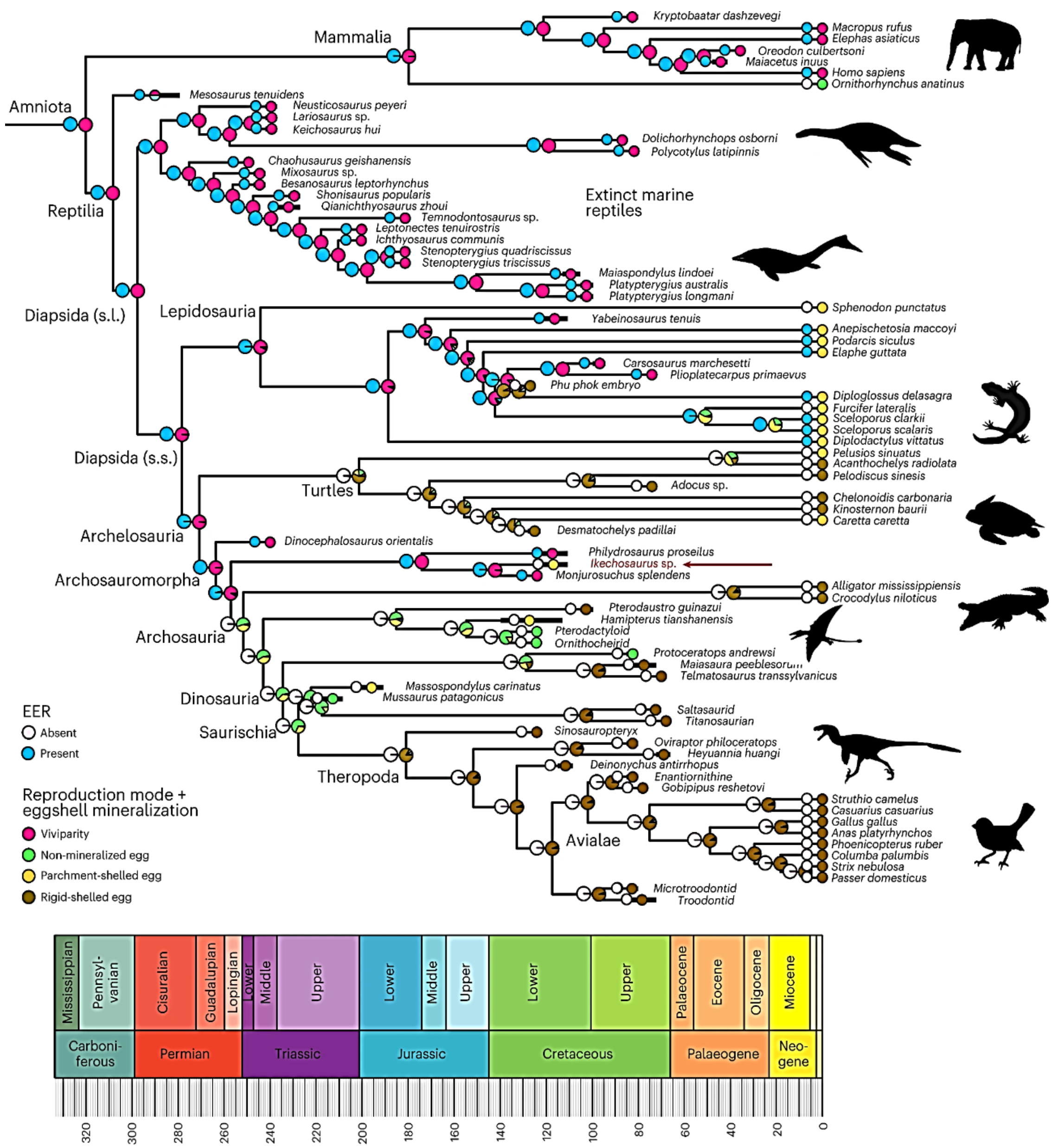

Figure 2. Phylogeny of oviparity, EER and viviparity in amniotes. The dendrogram includes 80 species (51 extinct and 29 extant), in relation to reproduction modes, eggshell mineralization, non-EER, EER and viviparity. © Springer Nature (Jiang et al, 2023).

Curiously, fossil evidence has also shown a preference for hatchlings, through viviparity. Indeed, many species were life-bearers, including Mesozoic marine reptiles, such as ichthyosaurs and plesiosaurs. The remarkable *Ikechosaurus* evidence described above underlines the recurrent interaction between oviparity and viviparity, that is found in several groups: not only in lizards. Interestingly, the practice of EER is widespread among present-day vertebrates. That is particularly relevant among lizards and snakes.

Offsprings are released both within and without eggs. There appears to have ecological advantages to practicing EER, allowing mothers to release their young when temperatures are warm enough and food supplies are plentiful.

Concluding remarks and future prospects

The recent proposal in this topic that viviparity arose first, and then came oviparity in amniotes, is certainly provocative. That is particularly significant, taking into account the previous opposite assumption, that has been accepted for decades. The new discoveries reinforce the idea of reproductive flexibility in the animal kingdom, even at the beginning, and underscore how nature's survival strategies can be much more diverse and adaptive than previously assumed. Indeed, the complex journey of life on Earth continues to reveal surprises. On the other hand, although ontogeny does not recapitulate phylogeny in all instances, sometimes resembles it. Embryogenesis, ontogeny, phylogeny, and molecular biology will further allow to better dissect this interesting evolutionary topic. That will strengthen the recent relationships between archaeology and such disciplines. The future is certainly promising in this interesting scientific topic.

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References

Dorado G, Gálvez S, Rosales TE, Vásquez VF, Hernández P (2021a): Analyzing modern biomolecules: the revolution of nucleic-acid sequencing – Review. *Biomolecules* (section Molecular Genetics) 11: 1111 (18 pp).

Dorado G, Jiménez I, Rey I, Sánchez-Cañete FJS, Luque F, Morales A, Gálvez M, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2013): Genomics and proteomics in bioarchaeology - Review. *Archaeobios* 7: 47-63.

Dorado G, Luque F, Esteban FJ, Pascual P, Jiménez I, Sánchez-Cañete FJS, Raya P, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2021b): Molecular biology to infer phenotypes of forensic and ancient remains in bioarchaeology – Review. *Archaeobios* 15: 49-64.

Dorado G, Luque F, Esteban FJ, Pascual P, Jiménez I, Sánchez-Cañete FJS, Raya P, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2022): Involvement of nucleic-acid methylation on biology and evolution: from first hominids to modern humans – Review. *Archaeobios* 17: 104-116.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Pérez-Jiménez M, Raya P, Gálvez M, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2015): Second-generation nucleic-acid sequencing and bioarchaeology - Review. *Archaeobios* 9: 216-230.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Pérez-Jiménez M, Raya P, Sáiz J, Sánchez A, Martín J, Rosales TE, Vásquez VF, Hernández P (2016): Sequencing ancient RNA in bioarchaeology - Review. *Archaeobios* 10: 103-111.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Raya P, Sáiz J, Sánchez A, Rosales TE, Vásquez VF (2017): Clustered Regularly-Interspaced Short-Palindromic Repeats (CRISPR) in bioarchaeology - Review. *Archaeobios* 11: 179-188.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Raya P, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2018): Evolution from first hominids to modern humans: philosophy, bioarchaeology and biology - Review. *Archaeobios* 12: 69-82

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Raya P, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2019): Bioarchaeology to bring back scents from extinct plants - Review. *Archaeobios* 13: 66-75.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Raya P, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2020): Implications of non-coding RNA on biology and evolution: from first hominids to modern humans - Review. *Archaeobios* 14: 107-118.

Dorado G, Rey I, Rosales TE, Sánchez-Cañete FJS, Luque F, Jiménez I, Gálvez M, Sáiz J, Sánchez A, Vásquez VF (2009): Ancient DNA to decipher the domestication of dog - Review. *Archaeobios* 3: 127-132.

Dorado G, Rey I, Rosales TE, Sánchez-Cañete FJS, Luque F, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Hernández P, Vásquez VF (2010): Biological mass extinctions on planet Earth - Review. *Archaeobios* 4: 53-64.

Dorado G, Rosales TE, Luque F, Sánchez-Cañete FJS, Rey I, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Vásquez VF, Hernández P (2011): Ancient nucleic acids from maize - Review. *Archaeobios* 5: 21-28.

Dorado G, Rosales TE, Luque F, Sánchez-Cañete FJS, Rey I, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Vásquez VF, Hernández P (2012): Isotopes in bioarchaeology - Review. *Archaeobios* 6: 79-91.

Dorado G, Sánchez-Cañete FJS, Pascual P, Jiménez I, Luque F, Pérez-Jiménez M, Raya P, Gálvez M, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2014): Starch genomics and bioarchaeology - Review. *Archaeobios* 8: 41-50.

Dorado G, Vásquez V, Rey I, Luque F, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Hernández P (2008): Sequencing ancient and modern genomes - Review. *Archaeobios* 2: 75-80.

Dorado G, Vásquez V, Rey I, Vega JL (2007): Archaeology meets Molecular Biology - Review. *Archaeobios* 1: 1-2.

Hubrecht AA W (1910): Memoirs: the foetal membranes of the vertebrates. *Journal of Cell Science* 2: 177-188.

Jiang B, He Y, Elsler A, Wang S, Keating JN, Song J, Kearns SL, Benton MJ (2023): Extended embryo retention and viviparity in the first amniotes. *Nature Ecology & Evolution* 7: 1131-1140.

Laurin M, Reisz RR (1997): A new perspective on tetrapod phylogeny. In: Sumida S, Martin KLM (eds): "*Amniote Origins*". Academic Press (Cambridge, MA, USA): 9-59.

Laurin, M (2005): Embryo retention, character optimization, and the origin of the extra-embryonic membranes of the amniotic egg. *Journal of Natural History* 39: 3151-3161.

Lombardi J (1994): Embryo retention and the origin of the amniote condition. *Journal of Morphology* 220: 368.

Mi S, Lee X, Li X, Veldman GM, Finnerty H, Racie L, LaVallie E, Tang XY, Edouard P, Howes S, Keith JC Jr, McCoy JM (2000): Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* 403: 785-789.

Mossman HW (1987): *Vertebrate Fetal Membranes*. Rutgers University Press (New Brunswick, NJ, USA).

Romer AS (1957): Origin of the amniote egg. *The Scientific Monthly* 85: 57-63.