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REPRODUCTIVE TRAITS OF NON-AVIAN THEROPODS

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Abstract: The reproductive biology of non-avian theropods, primarily maniraptorans, is revisited using recent fossil discoveries in the framework of the extant phylogenetic bracket. It is apparent that non-avian theropods shared some reproductive characters with crocodilians, some with birds, but also possessed their own unique features. Whereas the non-avian theropod reproductive system retained primitive archosaurian traits (two functional oviducts, hyper-ellipsoidal eggs), it also possessed derived bird-like characteristics (released one ovum per oviduct, complex eggshell ultrastructures, asymmetrical eggs). Behaviourally, non-avian theropods were more derived than crocodilians in some respects: they laid orderly clutches and the adults were in direct contact with the eggs during the incubation period. However, non-avian theropods and enantiornithine birds, like crocodilians, did not rotate their eggs during the incubation period, which suggests that such a behaviour evolved in birds more derived than enantiornithines.

Key words: Reproduction, non-avian theropod, oviducts, egg, eggshell

INTRODUCTION

Until recently, little was known about the reproductive biology of non-avian theropods because actual theropod eggs had been mistakenly attributed to ornithischian dinosaurs (Sochava, 1972; Horner and Weishampel, 1988, 1996). Now, eggs intimately associated with either adult or embryonic remains are known for allosauroids (Mateus *et al*., 1998), oviraptorosaurs (Norell *et al*., 1994), dromaeosaurids (Makovicky and Grellet-Tinner, 2000), and troodontids (Varricchio *et al*., 2002). New discoveries and re-assessments of previous discoveries over the past few years have given insight into the reproductive characteristics of specific clades of non-avian theropods. Such discoveries are primarily of maniraptoran theropods, and include oviraptorid and troodontid embryos preserved within eggs (Norell *et al*., 1994; Horner and Weishampel, 1996), adult oviraptorids sitting atop their clutches (Norell *et al*., 1995; Dong and Currie, 1996), and a gravid oviraptorosaur with eggs in its pelvis (Sato *et al*., 2005). These latest finds have shed light on reproductive system function, embryonic development, and nesting behaviours in extinct theropods (for review see Horner, 2000; Chiappe, 2004).

This paper reviews recently discovered aspects of the reproductive biology of non-avian theropods, primarily maniraptorans. Comparison with extant animals (crocodilians and birds) that phylogenetically bracket extinct Dinosauria (Witmer, 1995) can reveal evolutionary trends in reproductive characters that are rarely preserved in the fossil record. Hypotheses on reproductive aspects (e.g., ovulation and egg formation) of extinct dinosaurs using the extant phylogenetic bracket (EPB) approach can be confirmed or falsified in light of recent fossil discoveries. The conclusions demonstrate that non-avian theropods retained some primitive reproductive characteristics of archosaurs, shared some derived characteristics with birds, and evolved unique features unparalleled among other archosaurs.

REPRODUCTIVE TRAITS

OVARIAN FUNCTION

Whereas crocodilians have two functional oviducts, birds generally possess only a single functional oviduct. Consequently, according to the EPB approach, the nature of ovarian function is equivocal in extinct dinosaurs. It has been suspected, however, that some non-avian theropods possessed two functional oviducts because the eggs in clutches attributed to troodontids and oviraptorosaurs appear to have been laid in pairs (Dong and Currie, 1996; Varricchio *et al*., 1997). The recent discovery of an oviraptorosaur containing two adjacent eggs within its pelvis confirmed that these dinosaurs retained the primitive archosaurian condition, like crocodilians, of having two functional oviducts (Fig. 1, Sato *et al*., 2005). Ovarian function in primitive extinct birds is unknown, but some extant birds have two functional oviducts in which the left and right oviducts alternate the release of a single ovum during the egg-laying period (Jones *et al*., 1993).

OVULATORY PATTERN

Crocodilians ovulate all ova of a single clutch simultaneously, whereas birds ovulate a single ovum at a time (for review see Palmer and Guillette, 1992). Consequently, ovulatory pattern was unknown for non-avian dinosaurs because EPB could not resolve between these two conditions. However, the discovery of a gravid oviraptorosaur, containing only two eggs, revealed that these non-avian theropods ovulated a single ovum per oviduct at a time (Fig. 1, Sato *et al*., 2005). This evidence thus suggests that the derived ovulation condition of birds evolved among non-avian theropods.

EGG FORMATION

The egg is composed of four distinct components: the yolk, the albumen, the shell membranes, and the eggshell. Crocodilians and birds possess an "assembly line" reproductive system where the various egg components are formed in different anatomical regions as the ovum travels through the oviduct (for review see Palmer and Guillette, 1992). Albumen is the first egg component added to the ovum, followed sequentially by the fibrous shell membranes and the calcareous portion of the shell. In crocodilians, the albumen is secreted in the tube, the shell membranes in the anterior uterus, and the bulk of the calcareous portion of the shell is formed in the posterior uterine region (Palmer and Guillette, 1992). In birds, the albumen is secreted in the magnum, the shell membranes are produced in the distal isthmus (red region),

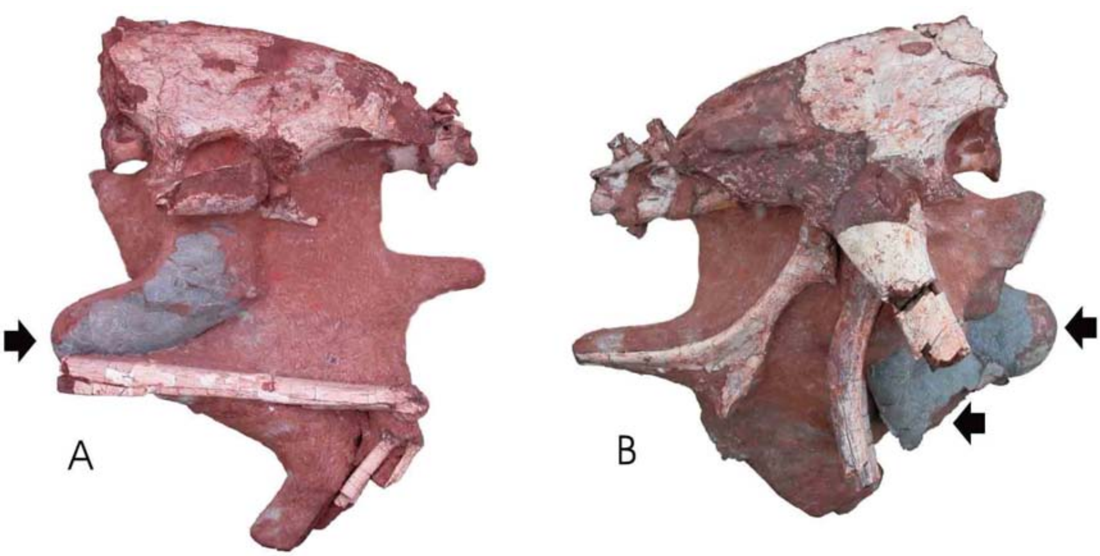

Fig. 1. Oviraptorosaur pelvis containing eggs from Sato *et al*. (2005). A. Left, and B. Right lateral views. Eggs denoted by arrows.

and the calcareous portion is formed in the shell gland (for review see Board and Sparks, 1991). Non-avian theropods are inferred to have possessed an "assembly line" reproductive system like that of crocodiles and birds where various egg components formed in different parts of the oviduct (Palmer and Guillette, 1992).

EGG SHAPE

Egg shape is determined in the isthmus of birds or in the uterus of crocodilians, prior to deposition of the calcareous portion of the eggshell. Crocodilians have eggs that are hyper-ellipsoidal in shape and symmetrical about the equator, whereas bird eggs are usually ellipsoidal and asymmetrical (more pointed at one pole). The asymmetry in bird eggs is thought to result either from the differential contraction of the muscle coat surrounding the isthmus of the oviduct or from differential length or tone of muscle coat fibres (for review see Smart, 1991).

Asymmetric hyper-ellipsoidal eggs, which combine the morphology of crocodilian eggs and the asymmetry of bird eggs, are found among basal ratites (kiwi), basal galliformes (megapodes), enantiornithines (*Gobipteryx*), oviraptorosaurs, and troodontids. Given the phylogenetic distribution of egg shape characters, the hyper-ellipsoidal shape appears to be a primitive feature of archosaurs that was retained in non-avian theropods and primitive birds, whereas egg asymmetry evolved initially among non-avian theropods, possibly maniraptorans. The fact that some non-avian theropods had asymmetrical eggs supports the idea that these non-avian theropods possessed a muscle coat around the isthmus of the oviduct similar to that of birds.

EGGSHELL FORMATION

During egg genesis, the fibrous shell membranes develop around the albumen and serve, in conjunction with oviductal tissues, as a medium for the formation of the calcareous portion of the eggshell. These interactions produce a variety of calcareous ultrastructures in the eggshell (Fig. 2), some of which are inferred to be homologous among crocodilians, non-avian dinosaurs, and birds (Mikhailov, 1992). Among these ultrastructures, the squamatic zone (layer characterized by "scaly" ultrastructure) and the zone of wedges (layer characterized by tabular ultrastructure) represent characters of the eggshell that are shared by theropods (Mikhailov, 1992; Zelenitsky, 2004), but are absent in the eggs of other archosaurs. Because the eggshell ultrastructures are the result of complex cellular and glandular processes within the oviduct, the nature of the shell-forming region and the shelling process in non-avian theropods is inferred to have been most similar to that of birds.

EGGSHELL ORNAMENTATION

Eggshell ornamentation is absent in most crocodilians and birds, as well as in the extinct birds *Lithornis* and *Gobipteryx* (Mikhailov, 1991; Grellet-Tinner and Dyke, 2005). However, ornamentation occurs in some basal extant crocodilians (Alligatoridae) and birds (Galliformes and Ratitae) (Mikhailov, 1997; Zelenitsky, 2004). When present ornamentation forms in the outermost zone(s) of the eggshell.

Eggshell ornamentation has been documented in hadrosaurs and some non-avian theropods, but the evolution of this feature among extinct dinosaurs is difficult to trace because the parentage of most dinosaur eggs is unknown. Like other archosaurs, non-avian theropods form a heterogeneous group in the distribution of eggshell ornamentation. Among maniraptorans, the eggs of troodontids lack ornamentation, whereas oviraptorosaur and presumed dromaeosaurid eggs possess a well-developed ornamentation (Norell *et al.*, 1994; Makovicky and Grellet-Tinner, 2000). Given the random distribution of eggshell ornamentation within Archosauria and the non-homologous nature of the eggshell layers in which it develops, this feature is inferred to have evolved independently several times in this clade (Zelenitsky, 2004).

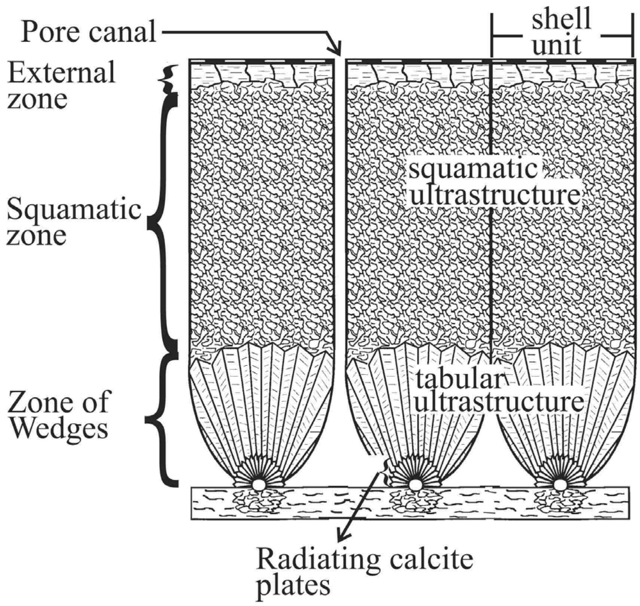

Fig. 2. Schematic of eggshell structure in radial view (after Mikhailov, 1991). Calcareous portion of eggshell is made up of vertically-oriented shell units and of horizontal layers (zones) of ultrastructure.

Although eggshell ornamentation has been inferred to facilitate gas exchange during underground incubation (Seymour, 1979), it is lacking in many underground nesters and it is present in eggshell of some brooding birds. The function of ornamentation within non-avian dinosaurs and extant archosaurs remains a mystery.

OVIPOSITION

Crocodilians deposit all eggs of a clutch in a single event, whereas birds lay one egg at a time. Based on the paired arrangement of eggs in clutches ascribed to troodontids and oviraptorosaurs, it had been inferred that these dinosaurs were laying only two eggs at a time (Varricchio *et al.*, 1997). This hypothesis was later confirmed by the presence of two *in situ* eggs in the pelvic region of an oviraptorosaur (Sato *et al.*, 2005).

NESTING AND INCUBATION

The eggs of crocodilians are laid in a random manner in holes dug in the ground or in mounds constructed by the adults. Incubation does not require parental involvement (for review see Coombs, 1989), although adults are known to sometimes lay their lower throat or thorax on the nest during nest guarding (Cott, 1961). Birds usually lay eggs single-tiered in circular, open nests, which are incubated by an adult sitting directly on the eggs.

Known theropod nesting traces consist of a shallow depression in the sediment with a peripheral rim (Varricchio *et al.*, 1997). Adult theropods have been found sitting directly atop their eggs, although the extent to which body heat or nesting material was used for incubation of eggs remains unknown. It appears that adult contact with the nest is a primitive behaviour for archosaurs, and adult contact with the eggs is a derived behaviour for non-avian theropods (maniraptorans) and birds.

Egg clutches of non-avian theropods are usually circular to subcircular in plan view, with the eggs arranged in a single layer or in multiple tiers (Mikhailov, 1991). Egg orientation and clutch geometry vary, presumably reflecting, at least in part, differing loads exerted on the eggs by the overlying adults adopting different positions. The clutches of troodontids are laid with the long axis of the eggs oriented subvertically to vertically and their pointed poles buried in the sediment (Fig. 3A; Varricchio *et al*., 2002). A troodontid hindlimb found preserved in contact with eggs has led paleontologists to infer that troodontids actively brooded their eggs like birds (Varricchio *et al*., 2002). The vertical orientation of the eggs would have likely permitted the eggs to support the weight of an adult sitting directly on them.

The clutches of oviraptorosaurs display the most complex geometry among non-avian theropods in that they are arranged as a spiral ring of two to three tiers of subhorizontally-oriented eggs and their centre is devoid of eggs (Fig. 3B). It is likely that sediment or nesting material located at the centre of the clutch or around its periphery would have helped to stabilize and maintain this multilayered arrangement. At least four such clutches have been discovered with oviraptorosaur adults sitting in the center of a clutch with their arms surrounding the eggs, in a position comparable to that of brooding birds (Clark *et al*., 1999). Because the weight of the brooding oviraptorosaur was born primarily by the egg-free center

A

B

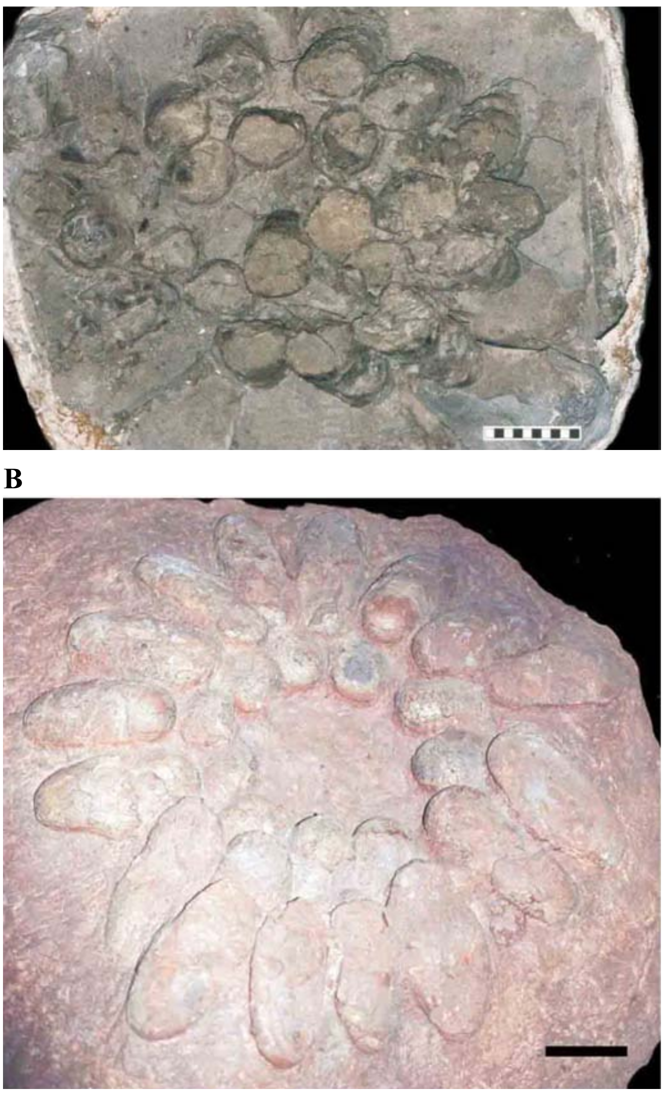

Fig. 3. Maniraptoran egg clutches. A. Troodontid egg clutch from Montana (MOR 963). Tops of eggs missing with long axis of eggs oriented vertically. Scale bar in centimetres. B. Oviraptorosaur egg clutch from China. Eggs were laid sub-horizontally in a multi-layered ring configuration. Scale bar equals 10 centimetres.

of the nest and not by the eggs, the eggs could have been positioned subhorizontally without risk of breakage.

EGG TURNING

Rotation of crocodilian eggs during the early stages of incubation has deleterious effects on the embryos because the embryo must adhere to the inner surface of the eggshell (for review see Deeming, 1991). Egg turning in extant birds, on the other hand, is essential to ensure survival of the embryo (for review see Deeming, 1991). It is suspected that egg turning in birds may have positive effects on the growth of the extra-embryonic membranes, which are vital for nutrition and respiration, or that the adhesion of the extra-embryonic membranes to the shell membrane may have deleterious effects on the embryos. Birds that bury their eggs (e.g, Megapodes, Egyptian plover) or lay their eggs in confined spaces (e.g, kiwi), however, do not rotate them.

It has been suggested that non-avian dinosaurs, like birds, did not rotate their eggs because their eggs were anchored in or partially buried in sediment (Varricchio *et al*., 1997). Interestingly, primitive birds, such as *Gobipteryx*, laid eggs anchored vertically in sediment in a fashion similar to those of troodontids (Sabath, 1991). It is therefore reasonable to assume that primitive birds, like non-avian theropods, did not rotate their eggs, and that egg turning is a character that evolved in birds more derived than enantiornithines.

DISCUSSION AND CONCLUSIONS

Recent fossil discoveries, in conjunction with the extant phylogenetic bracket approach, have greatly improved our knowledge of the non-avian theropod reproductive system and of the evolution of avian reproductive biology. From these sources of information, it has become apparent that non-avian theropods shared some reproductive characteristics with birds, some with crocodilians, but also possessed their own set of unique features.

With regards to the reproductive system, non-avian theropods retained the primitive archosaurian features of having two functional oviducts and in producing hyper-ellipsoidal eggs, the latter of which is also present in primitive birds. However, the non-avian theropod reproductive system had evolved a derived bird-like physiology in that it released one ovum per oviduct, formed complex eggshell ultrastructures, produced asymmetrical eggs, and oviposited eggs of at intervals rather than *en masse*.

Behaviourally, non-avian theropods were unique or shared characteristics with extant archosaurs. Because non-avian theropods did not lay their eggs randomly *en masse*, the paired eggs (one per functional oviduct) could be laid over a period of time in an arrangement conducive for an overlying adult. Like birds, adult non-avian theropods were in direct contact with their eggs, although the extent to which body heat or nesting material was used for incubation is unknown. Some non-avian theropods anchored their eggs in sediment or layered their eggs, so, like crocodilians, they probably did not rotate them. Because the eggs of primitive birds were also anchored in sediment, egg turning is a feature that likely evolved among birds more derived than enantiornithes.

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수각류의 생식 특징

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요 약: 수각류, 주로 Maniraptorans의 생식에 관한 사실이 현재 사용되는 계통발생학 분석 틀에서 새로운 화석들을 이용해 새롭게 조명을 받고 있다. 조류를 제외한 수각류들이 악어와 조류의 생식 특징을 공유한다는 것은 분명하지만 이들은 이들만의 독특한 특징도 갖고 있다. 조류를 제외한 수각류의 생식기관은 원시적인 지배파충류의 속성 (두개의 난관과 길쭉한 알)을 유지하지만 또한 조류의 특징 (난관 한 개의 한 개의 알을 낳으며 복잡한 알껍데기 미세구조, 비대칭의 알)을 갖고 있다. 생태학적으로 어떤 면에서는 조류를 제외한 수각류는 악어보다 더 진화되었다. 즉, 질서정연한 둥지에 알을 낳고 어미는 부화기간 동안 알을 직접 품는다. 그러나 조류를 제외한 수각류와 enantionithine 조류는 악어처럼 부화기간 동안 알을 돌리지 않는데 이러한 특별한 행동은 더 진화된 조류에서 진화된 것임을 암시한다.

주요어: 생식, 조류를 제외한 수각류, 난관, 알, 알껍데기

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