

TITANOSAUR REPRODUCTIVE BIOLOGY: COMPARISON OF THE AUCA
MAHUEVO TITANOSAUR NESTING LOCALITY (ARGENTINA), TO THE
PINYES *MEGALOOOLITHUS* NESTING LOCALITY (SPAIN)

by

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ABSTRACT

Taphonomic and microstructural studies at the Late Cretaceous Auca Mahuevo titanosaur nesting site (Argentina) reveal significant differences in reproductive attributes compared to alleged sauropods producing *Megaloolithus* eggs at the Pinyes locality (Spain). Auca Mahuevo clutches contain 15-40 *M. patagonicus* eggs; many of the 12-14 cm eggs contain titanosaur remains. Six clutches include both normal and abnormal eggs exhibiting three types of abnormal morphology: Type I displays two normal, superimposed eggshells, while Type II and III exhibit a normal inner eggshell, with one and three overlying eggshells, respectively. Previous studies that endeavor to link egg abnormalities to dinosaur extinction lack taphonomic and rigorous statistical methods.

The Pinyes locality occurs in the Tremp Formation, exposed in the Spanish Pyrenees. The overbank deposits contain clutches with 4-12 eggs; none of the 16-24 cm *M. Siruguei* eggs contain embryos. Although often assigned to sauropod dinosaurs, *M. siruguei* differs from *M. patagonicus* in clutch size, egg volume, shell thickness, pore density, and incubation mode; thus, taxonomic assignment to sauropods seems questionable. The water vapor conductance rates (\dot{G}_{H_2O}) of the Auca Mahuevo and Pinyes eggs are 341 and 3979 mg H_2O /(dayTorr), respectively. These values support previous interpretations of egg burial for Pinyes clutches and open incubation (substantiated by trace fossil nests) for the much larger Auca Mahuevo titanosaur clutches. In addition, the potential \dot{G}_{H_2O} of the titanosaur egg resembles that of some Late Cretaceous theropod eggs that are partially buried in sediment. The \dot{G}_{H_2O} of Auca Mahuevo egg is 2.7 times greater than an avian egg of comparable size and the microenvironment of the nest remains unclear. Comparison of the fossil eggs to those of modern reptiles is difficult, due to the paucity of studies and broader range of values reported for reptile eggs.

Detailed sedimentological studies are essential in order to distinguish biological features from those resulting from taphonomic or geologic phenomena. The taphonomic context and spatial association of fossil eggs provide an essential framework for comparisons of the reproductive biology of different dinosaur species, time periods, and paleogeographic regions.

CHAPTER 1

INTRODUCTION

Sauropods are a group of quadrupedal herbivores that lived from the Late Triassic to latest Cretaceous time (Wilson and Rogers, 2005). Titanosaurs represent the largest clade within Sauropoda and account for approximately one-third of sauropod diversity (McIntosh, 1990; Wilson and Sereno, 1998; Powell 2003; Upchurch et al., 2004). As suggested by the name, some members of the titanosaur clade attained huge size (45m and 100 metric tons), but many were of more moderate proportions. European dwarf forms (5.25 m, 600kg) allegedly represent island populations (Sander et al., 2004, 2006).

Distinctive “wide-gauge” track ways distinguish titanosaurs from other sauropod dinosaurs and these ichnofossils date to the Middle Jurassic, while the oldest osteological remains come from the Upper Jurassic Tendaguru beds of Tanzania (Wilson and Sereno, 1998; Upchurch et al., 2004). Although titanosaurs are primarily known from the Cretaceous of South America, they also inhabited India, Africa, Madagascar, North America, Asia, and Europe (McIntosh, 1990; Wilson and Sereno, 1998; Powell, 2003; Paik et al., 2004). From their middle to late Jurassic origins, titanosaur sauropods achieved global distribution and persisted until the peak of continental isolation at the end of the Cretaceous Period (Wilson and Sereno, 1998).

The titanosaur clade has long been associated with a type of eggshell structure referred to as *Megaloolithus* in the current fossil egg classification system (Mikhailov, 1997). Megaloolithid nesting horizons occur world wide, with the oldest and most

extensively documented localities occurring in southern France, northern Spain, India and South America (Sander per. comm.; Mohabey, 2005). Upper Cretaceous rocks of Korea also yield important megaloolithid localities (Paik et al., 2004). *Megaloolithus* eggshell fragments are reported from Africa, although complete eggs from these sites are unknown (Garcia, et al., 2003; Gottfried et al., 2004). Sauropod anatomy, physiology, and reproductive behaviors such as egg incubation strategy, site fidelity, and colonial nesting are often inferred from *Megaloolithus* nesting localities. Assignment of these eggs to sauropods, however, typically relies on the large egg size or the presence of sauropod bones within the same stratum or formation (Erben et al., 1979; Seymour, 1979; Powell, 1987; Mohabey, 1990, 1996; Calvo et al., 1997; Garcia et al., 2003). However, these *Megaloolithus* eggs lack embryonic remains necessary for definitive taxonomic referral to sauropod dinosaurs.

In 1998, an extensive dinosaur nesting horizon discovered in Neuquén Province in northern Patagonia produced an unprecedented number eggs containing diagnostic embryonic remains, allowing the first definitive assignment of *Megaloolithus* eggs to titanosaur sauropod dinosaurs (Chiappe et al., 1998, 1999, 2001; Salgado et al., 2005). Detailed taphonomic studies of the nesting horizons during five field seasons produced a wealth of information on titanosaur reproductive biology, including nest structure, egg microstructural characteristics, and reproductive behaviors (Chiappe et al., 1998, 1999; 2005; Grellet-Tinner, 2005; Grellet-Tinner and Chiappe, 2004; Jackson et al., 2004; Grellet-Tinner et al., 2006). Recent review of the literature reveals that significant differences may exist in titanosaur nesting biology at the Auca Mahuevo site, compared

to other localities. For example, titanosaur egg clutches in Argentina are much larger in the number of eggs per clutch, the eggshell porosity appears lower, and the type of nest construction differs from that documented from *Megaloolithus* sites in Europe, India, and Asia (Erben et al., 1979; Seymour, 1979; Vianey-Liaud et al., 1987; Powell, 1987; Mohabey, 1984, 1990, 1996; Mohabey et al., 1993; Calvo et al., 1997; Sanz et al., 1995; Sanz and Moratalla, 1997; Chiappe et al., 1998; 1999; 2003; 2004). If these differences are biologically significant, rather than resulting from taphonomic processes, they may provide insight regarding the influence of continental fragmentation on titanosaur distribution and paleoecology.

Detailed taphonomic studies conducted at Auca Mahuevo (Chiappe et al., 1999, 2005; Jackson et al., 2004) provide an opportunity to compare and contrast the reproductive biology of these South American titanosaurs to purported titanosaurs of Europe. More specifically, I compare Auca Mahuevo to the Pinyes nesting locality, a recently discovered *Megaloolithus* site in the Upper Cretaceous Tresp Formation of northern Spain. Research in the Tresp basin and contemporaneous fossil egg localities in southern France resulted in the earliest associations of megaloolithid eggs with sauropod dinosaurs (Buffetaut and Le Loewff, 1994). Interpretations of these European localities significantly influence current concepts of sauropod reproductive biology, as well as dinosaurs in general.

To evaluate previous interpretations and hypotheses concerning sauropod reproductive biology and behavior, I employ two distinct but integrated approaches: (1) taphonomic study of the nesting localities and (2) microscopic analysis of eggshell

structure. Microscopic analyses include assessment of (a) pathological conditions of fossil eggs; (b) diagenetic affect on eggshell structure; and (c) water vapor conductance rates of fossil eggs. This research will provide a better understanding of titanosaur reproductive biology and paleoecology, and the results are applicable to other dinosaur species and nesting sites. Finally, comparative studies of dinosaur nesting horizons will hopefully lead to taphonomic models for the formation of fossil egg assemblages, allowing recognition of recurring patterns of preservation in the fossil record.

Dissertation Outline

The dissertation research presented here follows the following format: Chapter 2 provides a brief review of previous work from the Auca Mahuevo locality and describes the first *in situ* titanosaur clutches containing abnormal, pathological eggs. In this study I examine previous interpretations regarding abnormal conditions in “sauropod” eggs and the possible influence of egg pathology on dinosaur extinction. Chapter 3 examines the relationship between taphonomy and interpretations of reproductive biology in extinct taxa. The study documents the first eggshell abnormalities from four taxonomically identified Mesozoic amniotes, and focuses on differentiation of multilayered eggshells that result from a biological process, from “stacked” eggshell resulting from taphonomic phenomena. The study concludes by establishing criteria for recognition of pathological conditions in the rock record. Chapter 4 introduces the Pinyes fossil egg locality in Lleida Province, of northern Spain and provides a detailed description of the sedimentology and taphonomy of the fossil egg locality. Chapter 5 examines hypotheses

regarding embryonic physiology and sauropod egg incubation environment by calculating water vapor conductance rate of an Auca Mahuevo titanosaur egg, with comparison to a *M. siruguei* egg from the Pinyes locality. Chapter 6 compares and contrasts the Auca Mahuevo and Pinyes fossil egg localities and discusses two aspects of this research in greater detail: taxonomic egg identification and pedogenesis and inferences of reproductive behaviors. Finally, the results of the research are summarized, and the directions of future research discussed.

Methods and Materials

Some field and laboratory methods are specific to individual sites or represent an integral part of a particular study. Therefore, I include the description of these techniques in the appropriate chapters. In contrast, all studies include similar microscopic analysis of eggshell structure, using the following specimen preparation technique. Eggshell fragments were cleaned and half of the specimens were prepared as radial or tangential thin section (30 μm thick) by a commercial petrographic laboratory or the Gabriel Laboratory for Cellular and Molecular Paleontology at the Museum of the Rockies, Montana State University. The thin sections were studied by transmitted and polarized light microscopy, using a Nikon Eclipse E600 equipped with a digital camera. The remaining half of each specimen was coated with gold (10 nm), and mounted on aluminum stubs. Specimens were imaged under a J.R. Lee Instrument Personal SEM or a JEOL 6100 SEM with Backscattered Electron Imaging (BEI) capabilities, coupled to a Noran Voyager Energy Dispersive X-ray (EDX) system, located at the Museum of the

Rockies and the Image and Chemical Analysis Laboratory, Montana State University, respectively. Structural attributes (e.g., shell thickness, pore width) were measured with Scion image analysis software available without charge through the National Institute of Health (NIH) web site: <http://rsb.info.nih.gov/nih-image/default.html>.

CHAPTER 2

ABNORMAL TITANOSAUR EGGS: *IN SITU* CLUTCHES FROM THE AUCA MAHUEVO LOCALITYIntroduction

The 1997 discovery of the extensive dinosaur nesting site of Auca Mahuevo in Upper Cretaceous (Campanian) (Dingus et al., 2000) strata in southwestern Argentina (Fig. 2.1A) allowed the first definitive assignment of megaloolithid eggshell structure to

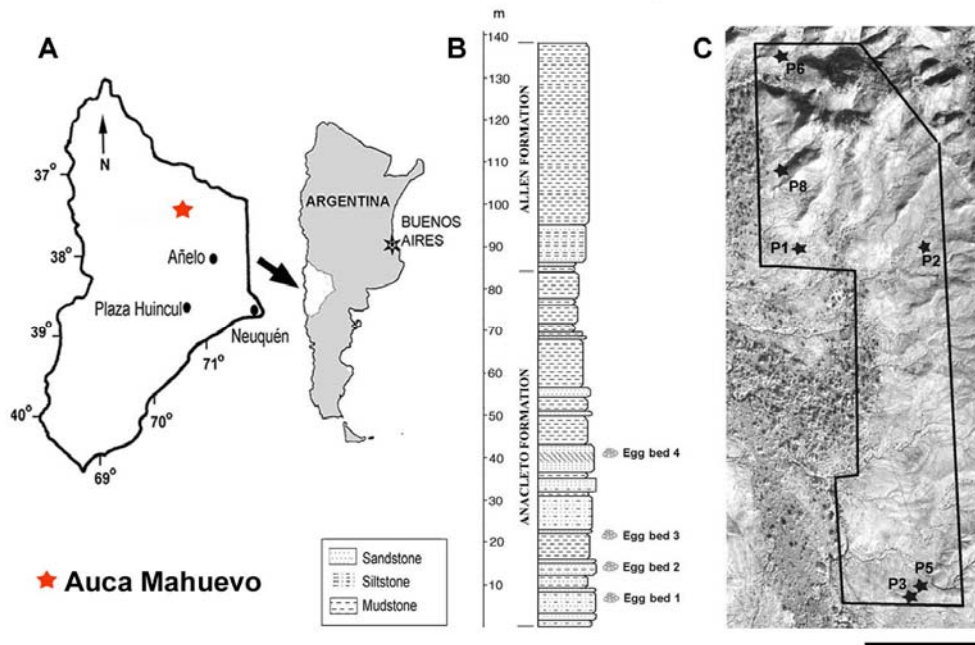


Figure 2.1. The Auca Mahuevo study site. (A) Location map. (B) Detailed stratigraphic column for the Anacleto Formation and overlying unit showing positions of egg beds 1-4. geographic location. (C). Air photograph of the study area showing location of the 6 clutches containing abnormal eggs (P1, P2, P3, P5, P6, P8).

titanosaur sauropod dinosaurs, based on embryonic remains inside the eggs (Chiappe et al., 1998, 1999, 2001). At least four egg-bearing layers (egg beds 1-4, in ascending

stratigraphic order) are identified within the Auca Mahuevo section (Fig. 2.1B) (Dingus et al., 2000; Chiappe et al., 1999). Specimens from all egg-bearing horizons exhibit similar size, shape, microstructure, and surface ornamentation as eggs from egg bed 3 that contain titanosaur osteological remains. Two egg horizons, egg beds 3 and 4, extend laterally for up to eight kilometers (Chiappe et al., 2005). Excavation of a quarry in egg bed 3 showed the arrangement of *in situ* eggs, while mapping of over a hundred clutches at a nearby locality documented clutch distribution (Chiappe et al., 1999). This latter locality, a low-relief erosional surface exposing egg beds 2 and 3 (Fig. 2.1C), also produced the first sauropod egg clutches containing abnormal, multilayered eggs.

Multilayered eggshell occurs occasionally in the hard-shelled aragonite or calcite eggs of some extant amniotes due to prolonged egg retention, often resulting from environmental or physiological stress (Romanoff and Romanoff, 1949; Erben, 1970; Erben et al., 1979; Ewert et al., 1984; Hirsch, 2001; Solomon, 1997; Jackson and Varricchio, 2003). Although pathological conditions are occasionally reported in dinosaur eggshells, most descriptions pertain to isolated fragments rather than eggs from *in situ* clutches (Sochava, 1971; Erben et al., 1979; Kerourio, 1981; Mohabey, 1984; Vianey-Liaud et al., 1994; Zelenitsky and Hills, 1997; Hirsch, 2001), and none are taxonomically identified on the basis of embryonic remains.

Despite the paucity of taxonomically referable *in situ* material, several studies have nonetheless endeavored to link climate change and abnormal fossil eggshell to dinosaur extinction (Erben et al., 1979; Yang et al., 2001; Zhao et al., 2002). Inferences of dinosaur physiology and reproductive anatomy are also based on comparisons of fossil

eggshell abnormalities with those occasionally found in eggs of living taxa (Erben, 1970; Erben et al., 1979; Ewert et al., 1984; Hirsch 1989; Hirsch, et al., 1989; Hirsch, 1994b; Zelenitsky and Hills, 1997; Carpenter, 1999). However, assessment of dinosaur physiology or reproductive anatomy based on multilayered eggshells remains problematic.

I present the first systematic study of eggshell abnormalities from clutches of a known taxon within Dinosauria, titanosaur sauropods from the Auca Mahuevo locality in Argentina. More specifically, my objectives are to determine the incidence of these abnormalities by assessing the abundance of clutches possessing abnormal eggs, the number of abnormal eggs in each of these clutches, and by documenting the microstructural differences of normal and abnormal eggs. I describe the methodology employed in conducting this survey, the taphonomy of clutches containing abnormal eggs, and three distinct forms of eggshell abnormality present in the eggs. Finally, I discuss previous hypotheses that relate multilayered eggshell to the terminal Cretaceous extinction event and interpretations of dinosaur reproductive anatomy and physiology.

Geology

Auca Mahuevo is located 87 km southeast of Rincón de los Sauces City, Neuquén Province, Argentina (Fig. 2.1A-C). The extensive outcrops of Upper Cretaceous rocks at this site include the uppermost units of the Neuquén Group and overlying lowermost units of the Malargüe Group (Fig. 2.1B). Approximately 1,300 meters thick, the Neuquén Group (Cenomanian – lower to middle Campanian age) consists of siliclastic

terrestrial sediments, deposited within braided and meandering fluvial systems. These deposits accumulated during the terminal back-arc stage of the Neuquén basin (Cazau and Uliana, 1973; Gazzera and Spalletti, 1990; Dingus et al., 2000).

Auca Mahuevo occurs within the Anacleto Formation, which is composed of predominantly fluvial sandstone, siltstone, and mudstone (Fig. 2.2). The Anacleto Formation conformably overlies the Bajo de la Carpa Formation, which contains coarser-grained sandstones and conglomerates in the study area (Dingus et al., 2000). A low angle disconformity (Ardolino and Franchi, 1996) occurs at the contact between the



Figure.2.2. The Anacleto Formation and overlying Allen Formation. Arrow marks approximate boundary between the formations (J. Schmitt photograph).

Anacleto Formation and overlying Allen Formation (Malargüe Group) (Fig. 2.2). In the study area, the Anacleto Formation attains a thickness of 85 meters (Fig. 2.1B); reddish and reddish-brown mudstones and interbedded gray-green, thin sandstone bodies characterize the sequence. The reported age of the Anacleto Formation varies from early to middle Campanian (Legarretta and Gulisano, 1989); paleomagnetic data places Auca Mahuevo within a Reverse interval, most likely correlative with C33R, between 83.5 and 79.5 Ma (Dingus et al., 2000).

Field Methods

Site Selection

A randomly selected site (~38,000 m²) was chosen within the larger Auca Mahuevo study area after field reconnaissance (Fig. 2.1C). Criteria used for site selection included the presence of hundreds of exposed *in situ* egg clutches and the ability to assign these clutches to their proper stratigraphic unit (egg bed 2 or 3) (Chiappe et al., 1999; Dingus et al., 2000). Clutches on this relatively flat surface typically contain eroded eggs exposed in plan view (Fig. 2.3A). However, dense concentrations of fragmented eggshells are also present, representing more extensively weathered clutches (Chiappe et al., 1999). The weathered condition of most fossil material within the study area necessitates an explanation of the terms and criteria used in this survey.

Terms and Criteria Used for Investigation

Intact clutches previously quarried from egg bed 3 contain up to 35 eggs; each

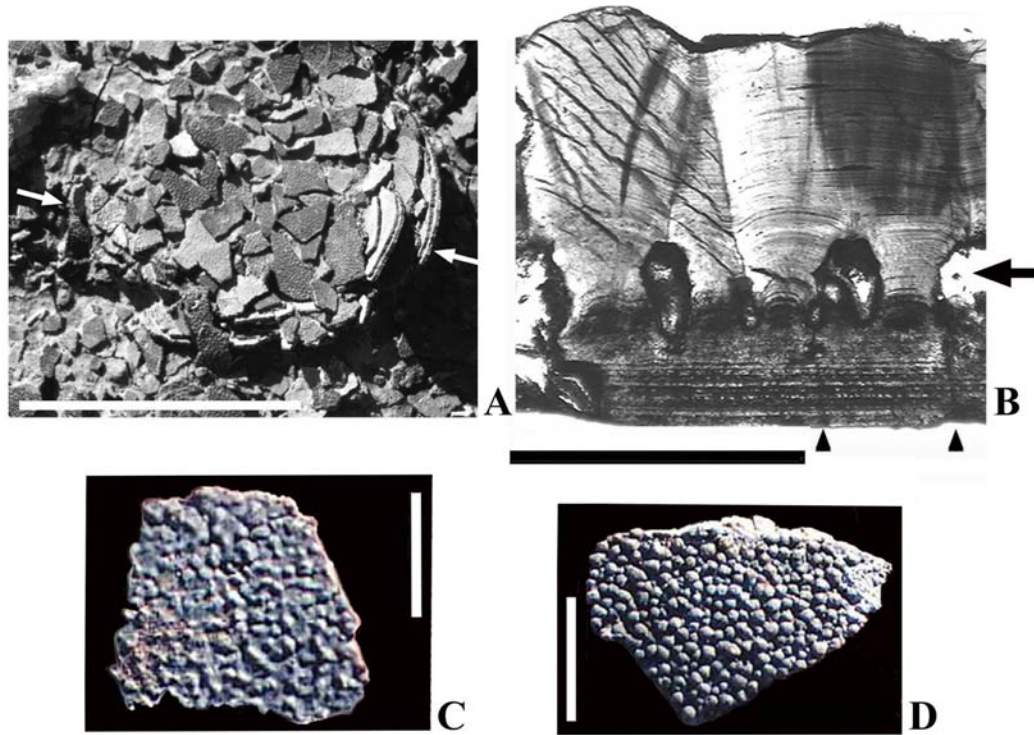


Figure 2.3. Abnormal and normal egg and eggshells. (A) Weathered abnormal, multilayered egg from clutch P1 (MCF-PVPH-251) exposed in cross section. Arrows show double eggshell and maximum egg diameter. Scale bar equals 10 cm. (B) petrographic thin section of eggshell from an egg with normal megaloolithid calcite structure from P-5 (MCF-PVPH-254) showing preserved stratified membrane beneath the interior shell surface. Note membrane thickness and nucleation cites at the outer membrane surface. Triangles denote pores that traverse the membrane; arrow marks eggshell dissolution and replacement by calcite. Scale bar equals 1 mm. (C, D) surface of normal and multilayered eggshell, respectively, from clutch P-5 (MCF-PVPH-254). Note the more rugose surface ornamentation in D. Eggs are adjacent one another and subject to similar weathering. Scale bars equal 1 cm.

clutch covers an area of approximately one square meter (Chiappe et al., 1999). In the study area, differential weathering often exposes eggs in different areas of the same clutch. Therefore, eggs that are less than one meter apart and separated by abundant eggshells are considered to belong to the same clutch. Eggs within these clutches occur in close proximity to one another and the eggshell is oriented vertically in the sediment, preserving a significant portion ($>1/3$) of the original egg perimeter in cross section (Fig.

2.3A). Normal eggs from this locality (some containing sauropod embryonic remains) are approximately 15 cm in diameter, with 1.00 to 1.78 mm-thick megaloolithid eggshell structure (Fig. 2.3B) (Chiappe et al., 1998). The surface ornamentation consists of tubercles (< 1 mm diameter) that occasionally coalesce to form ridges (Figs. 2.2C). An abnormal egg, therefore, is defined as an egg with an unusually thick eggshell (>2.5 mm) comprised of two or more superimposed eggshell layers, with rugose, prominent tubercles that are typically greater than 1 mm in diameter (Figs. 2.3D). Petrographic thin sections and scanning electron microscopy provide verification of initial field identification of abnormal eggs.

The Survey

Systematically spaced, parallel transects approximately one meter apart were conducted within the ~38,000 m² study site. Each clutch was examined for characteristics described above and then marked in order to avoid duplication. Sedimentological and taxonomic attributes were documented for each site that exposed a clutch containing abnormal eggs. These clutches were photographed and eggshell samples from normal and abnormal eggs were removed for laboratory analysis. To calculate the area of the study site, five-meter long cross marks were spray painted on the ground and the area was photographed from a small airplane. Each clutch was located on the aerial photograph and assigned to the appropriate stratigraphic layer (Fig. 2.1B,C). To conserve the fossil material, only one clutch containing abnormal eggs was excavated. This clutch was mapped using a 1m² grid and graph paper, and the trend and plunge of slickenlines were measured with a Brunton compass and noted on the map.

Results

Taphonomy

Exposures of egg bed 3 produced 329 *in situ* egg clutches within the 35,000 m² study area; five of these clutches contain both normal and abnormal eggs. Fourteen clutches, one of which contains a multilayered egg, are present in the more limited exposures of egg bed 2 (3,000 m²). The total surface area of egg beds 2 and 3 includes regions where eggs are absent due to eroded gullies or the eggs are poorly exposed because of vegetation cover. In addition, criteria used to eliminate possibly transported material also contribute to an artificially low clutch density. Furthermore, since other clutches are unexcavated, the total computed number of eggs (normal and abnormal) (Table 2.1) represents a minimum number present and additional eggs may remain undetected in the substrate. Where more than one egg occurs within a clutch, the eggs are distinguished by sequential numbering. The following general description applies to all clutches present within the study area, followed by a more detailed description of a nearly complete clutch (P-6) excavated from egg bed 3.

Table 2.1. Summary of taphonomic and structural information for six clutches.

Clutch	Egg bed	Total No. of Eggs	Abnormal Eggs	Egg Size Range	Surface Morph.	Structural Type	Comments
P-1	2	3	1	13-15 cm	normal	I	—
P-2	3	3	1	13 cm	rugose	I, II, III	—
P-3	3	5	2	12-15 cm	rugose	II, III	P-3-1 & P-3-2 exhibit III & II, respectively
P-5	3	4	1	12-17 cm	rugose	II, III	—
P-6	3	30	3	8-15 cm	rugose	II	present in all 3 eggs
P-8	3	5	2	10-12 cm	rugose	II, III	present in both eggs

General Clutch Description. The weathered clutches from egg beds 2 and 3 preserve normal and abnormal eggs exposed in plan view (Fig. 2.3A) and occur in similar sandy, reddish brown mudstone. The sediments surrounding these clutches exhibit no lithologic evidence of nest structure (Chiappe et al., 2004), and the condition of the eggs prior to burial (intact or hatched) remains uncertain in all clutches except P-6, discussed below. In clutches containing more than one multilayered egg (P-3, P-6, P-8), the eggs are adjacent to each other, rather than separated by normal eggs. A narrow (1-2 cm) blue-green “halo” outlines the exposed eggs, indicating chemical reduction of the sediment immediately surrounding the egg, possibly the result of decomposition of the contents after burial. Occasionally, small (< 3 mm) calcium carbonate nodules are aligned along the egg perimeter, replacing portions of missing eggshell. Clutch P-5 represents the only clutch with associated bone: immediately down slope from a normal egg, an unidentified bone fragment adheres to a small (< 1.5 cm) piece of normal eggshell. No additional bone was present in the vicinity.

Clutch P-6. In the steeper terrain of the southwest portion of the study area, egg bed 3 crops out 3.3 meters above the base of a slope and approximately one meter below a horizon containing small (~ 1.0 cm) caliche nodules. The laterally continuous exposures of egg bed 3 produce dense concentrations of eggshell one to three meters apart along the contour of the hill. Most of these concentrations exhibit eggshells with normal surface ornamentation. However, abundant multilayered eggshells with rugose surface morphology cover the slope immediately below two partially eroded, abnormal eggs (Fig. 2.4A-D). Collection on the slope beneath these two eggs produced 382

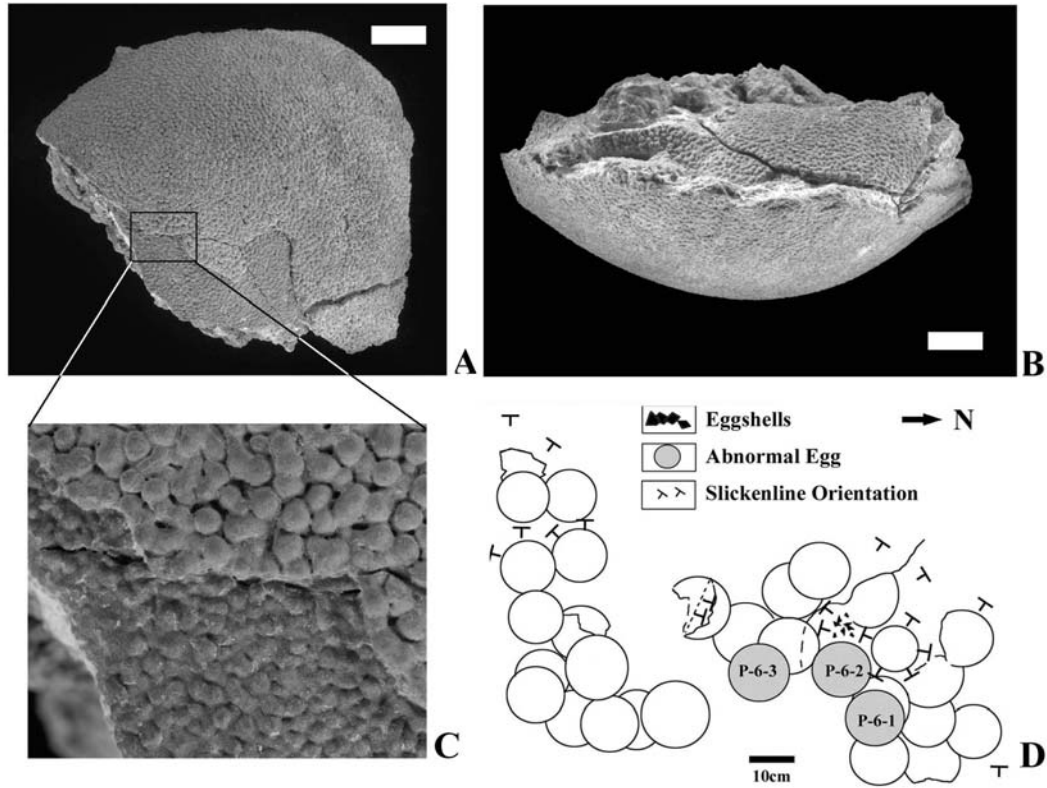


Figure 2.4. An excavated clutch, P6 (MCF-PVPH-514). (A, B) Eggs P-6-2 and P-6-3, respectively, two eroded abnormal eggs that occupied the highest level in the clutch. Scale bars equal 1 cm. (C) Enlargement of double eggshell layers indicated by box in A. Note difference in diameter in tubercles of the inner and outer eggshell layers. (D) field map of the clutch P6 showing location of three multilayered eggs. Dip symbols indicate direction of slickenlines on egg and rock surfaces

fragments of multilayered eggshell and 14 single-layer fragments. One egg (P-6-2) exhibits missing portions of the outer abnormal shell, exposing normal eggshell beneath the abnormal layer (Fig. 2.4A,C). These 14 normal specimens, therefore, most likely represent eggshell that have separated from the multilayered egg during recent weathering.

Excavation of the clutch revealed a total of 30 eggs, 27 normal eggs and 3 multilayered eggs with rugose surface ornamentation (Fig. 2.4D). The eggs are distributed in three levels within the clutch; the three abnormal eggs occur at the highest level, in close proximity to one another (Fig. 2.4D). Both normal and abnormal eggs in P-6 contain concave-down eggshell fragments, indicating that whole eggs were crushed by lithostatic compression. The narrow blue-green “halos” surrounding the eggs resemble those present in the other five clutches and elsewhere in egg bed 3. Diagenetic growth of gypsum crystals within this zone facilitates rapid weathering of the fossil material. Mudstone surrounding and filling the eggs shows relatively abundant parallel striations (slickenlines), with varying orientations (Fig. 2.4D) and one to several centimeters of vertical offset, as determined by field measurements using a Brunton compass and a centimeter ruler, respectively.

Discussion of P-6 Taphonomy. The absence of nearly any normal eggshell on the slope below this clutch suggests that recent erosion was limited to the abnormal eggs occupying the highest level within the clutch. The number (30) and distribution of eggs in P-6 typifies other intact clutches previously excavated from egg bed 3 (Chiappe et al., 1999), and the three egg levels represent the maximum number of superimposed eggs yet documented at this locality (unpublished data). Clutch P-6, therefore, most likely represents a complete, *in situ* clutch. Burial and preservation of the eggs, like other clutches from egg bed 3, resulted from suspension settling of fine-grained material during a flood event (Chiappe et al., 1999).

The highly variable orientation of slickenlines on the surface of the mudstone results from vertisol development within the nesting horizon (Chiappe et al., 1999). In a seasonally wet and dry climate, repeated shrinking and swelling occurs in high clay-content soils. During the rainy season the clay expands and blocks of soil shear off and slide past each other under pressure, producing striated surfaces (Brady and Weil, 2002). The presence of slickensides inside some eggs indicates that vertisol development occurred (or continued) after burial and subsequent infilling of the eggs by sediment. Pedogenesis undoubtedly produced compaction and displacement of some eggs within this clutch. However, movement was typically no more than a few centimeters, based on field measurements of offset rock surfaces, suggesting minimal change in the original egg orientation and distribution.

The level at which the abnormal eggs occur and their close proximity to each other in clutch P-6 is significant. Some extant turtles produce more than one clutch in a season. Occasionally, one or more eggs are retained in the oviduct and additional eggshell layers are deposited over the eggs with shelling of the next clutch. These retained eggs represent the first eggs laid with subsequent oviposition. Ewert et al., (1984) suggested that if dinosaurs produced multiple clutches in a single season, one might expect to find retained eggs at the bottom of the clutch. Although a reasonable hypothesis, the presence of the three abnormal eggs at the highest level in clutch P-6 indicates that they are the last eggs laid by the female sauropod. Although multilayered eggs also occur adjacent to each other in unexcavated clutches (P-3 and P-8), no assessment is possible of the level at which they occur.

Microscopic Analysis of Eggshell

Diagenesis. Scanning electron microscopic (SEM) images and petrographic thin sections of eggshells from the six clutches often show soft tissue preservation in the form of eggshell membrane. Embryonic integument (Chiappe et al., 1998) and eggshell membrane (Grellet-Tinner, 2005) previously reported from other eggs in this study area further support this interpretation. In the six clutches containing abnormal eggs, permineralized eggshell membrane is present at the base of some eggshells (Fig. 2.3B) and occasionally separates the multiple layers in abnormal eggs (Figs. 2.5A,B). In addition, freshly broken eggshell from the multilayered egg in clutch P-5 exhibits an 860 μm -long feature (9 μm thick) that consists of two intertwined strands that appear to flatten before entering an opening in the calcite eggshell (Fig. 2.5C-E). Elemental analysis shows no quantitative difference in composition between the calcite eggshell and the permineralized strand. This structure may represent permineralized protein matrix, an organic latticework on which the calcite mineral is deposited during eggshell formation. However, differentiating fossilized contaminants such as bacteria or fungi from the protein matrix of the original eggshell remains problematic because of their similar morphology (Jackson et al., 2002).

In all specimens, diagenetic dissolution of eggshell calcite took place primarily between individual shell units at the interior eggshell surface and, less frequently, within

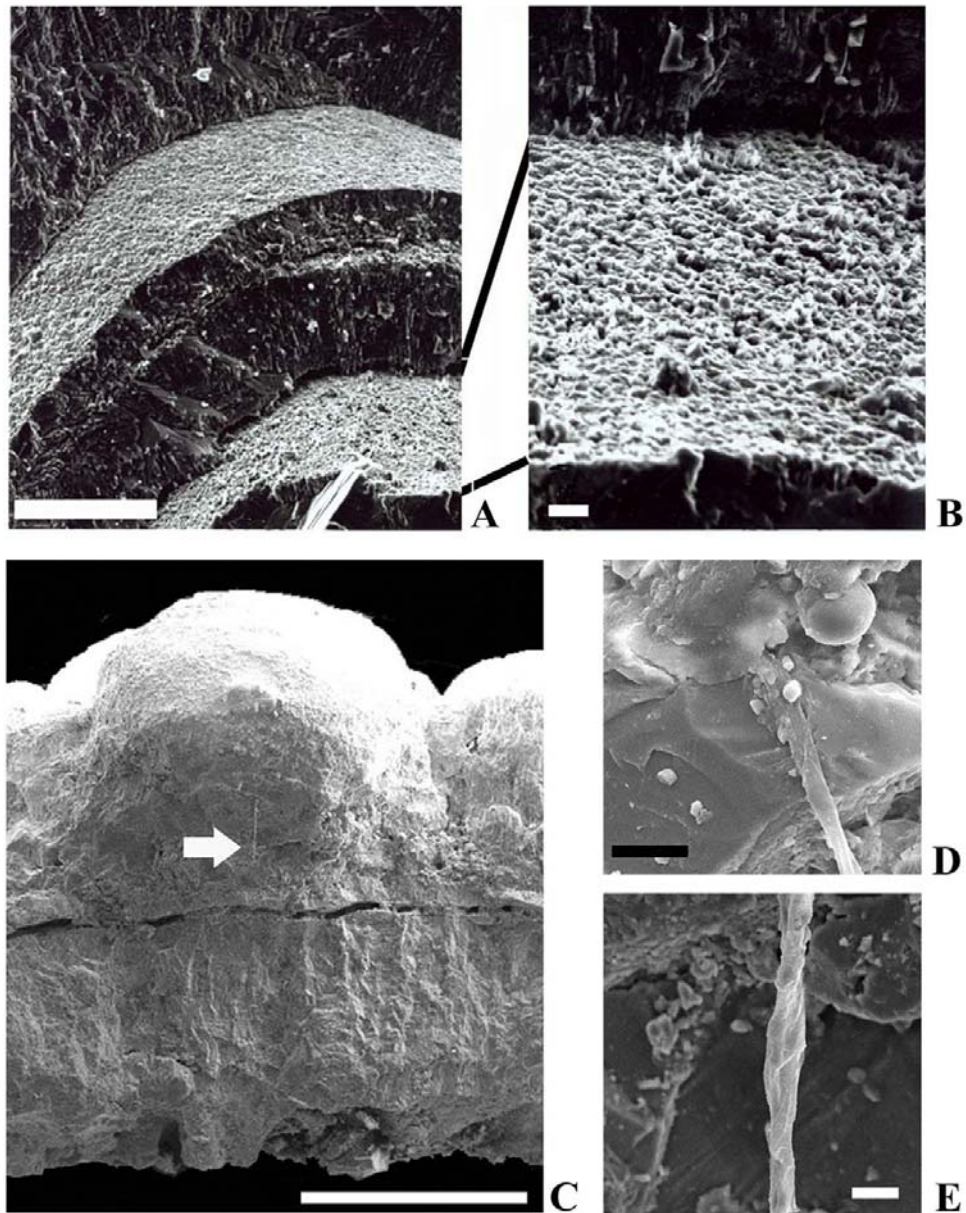


Figure 2.5. Soft tissue preservation. (A, B) SEM image of multiple shell layers and preserved membrane in P-8-2 (MCF-PVPH-255). (C) SEM image of multilayered eggshell (MCF-PVPH-254). Arrow marks the location of permineralized strand shown in D and E. Scale bar equals 1 mm. (D) upper portion of strand shown in C as it enters an opening in the eggshell calcite. Scale bar equals 10 μm . (E) enlargement of fibrous twisted structure shown in C. Scale bar equals 10 μm .

the upper portion of the shell (Figs. 2.5-2.7). Reprecipitation of spary calcite accompanied this diagenetic alteration in most specimens. Authigenic analcime replacement often occurs in association with the protein membrane (Figs. 2.6A-E), suggesting that the membrane was preferentially susceptible to analcime replacement. Identification of the zeolite mineral is based on crystal morphology in thin section and SEM images (Fig. 2.6C) and energy dispersive x-ray (EDX) analysis of individual crystals.

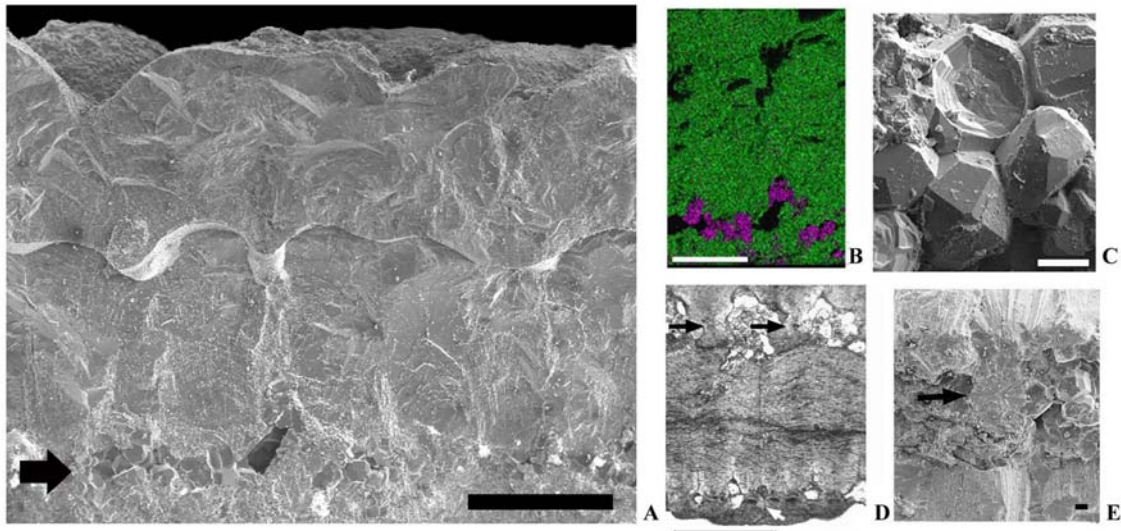


Figure 2.6. Diagenetic eggshell alteration. (A) SEM image of abnormal eggshell from P-8-2 (MCF-PVPH-255). Arrow indicates area of membrane replacement by analcime. Scale bar equals 1 mm. (B) elemental map of inner eggshell of same egg with magenta areas indicating analcime replacement in the membrane area of the inner eggshell. Scale bar equals 1 mm. (C) close up of analcime crystal morphology. Scale bar equals 10µm. (D, E) black arrows point to calcite nucleation sites surrounded by authigenic analcime in laterally adjacent sites in P-1 (MCF-PVPH-251) and between the inner and outer eggshell in P-2 (MCF-PVPH-252), respectively. Note the distance from the surface of the inner eggshell to the nucleation site, denoting approximate thickness of the former membrane.

Eggshell Microstructure. All normal, single shell eggs from the six clutches exhibit megaloolithid eggshell structure (Fig. 2.3B) identical to eggs from egg bed 3 that contain titanosaur sauropod embryos (Chiappe et al., 1998, 2001). Abnormal eggs from

all six clutches display an unusually thick shell comprised of superimposed eggshell layers (Figs. 2.4A-C, 2.5A-C, 2.6A-D, 2.7). The inner eggshell shows similar thickness, calcite microstructure, and surface ornamentation as normal sauropod eggs from this site. However, the additional abnormal layers vary in number and structure (Fig. 2.7). This variation occurs within and among the six clutches and even within a single egg. The outer surface of all multilayered eggs displays rugose ornamentation, except for the abnormal egg in clutch P-1 that exhibits normal ornamentation. Table 2.1 summarizes three types of abnormal morphology (Types I-III) documented in the multilayered eggs and provides additional data from the six clutches. When more than one abnormal egg is present in a clutch (e.g. P-8), the eggs are sequentially numbered: P-8-1, P-8-2.

Type I Morphology. This type of abnormal eggshell morphology exhibits two superimposed eggshell layers, both with normal megaloolithid structure and thickness (Fig. 2.7A; Chiappe et al., 1998). Occasionally, remnants of permineralized membrane that separate the superimposed eggshell layers exhibit nucleation sites comprised of radiating calcite spherulites. Under crossed polars in petrographic thin sections, these spherulites exhibit pseudouniaxial crosses and extinction occurs simultaneously in both eggshell layers when the microscope stage is turned (Fig. 2.7A). Where more extensive diagenetic replacement occurs, these nuclei comprised of radiating crystals “float” within authigenic analcime crystals between the eggshell layers (Fig. 2.6D, E). These calcite

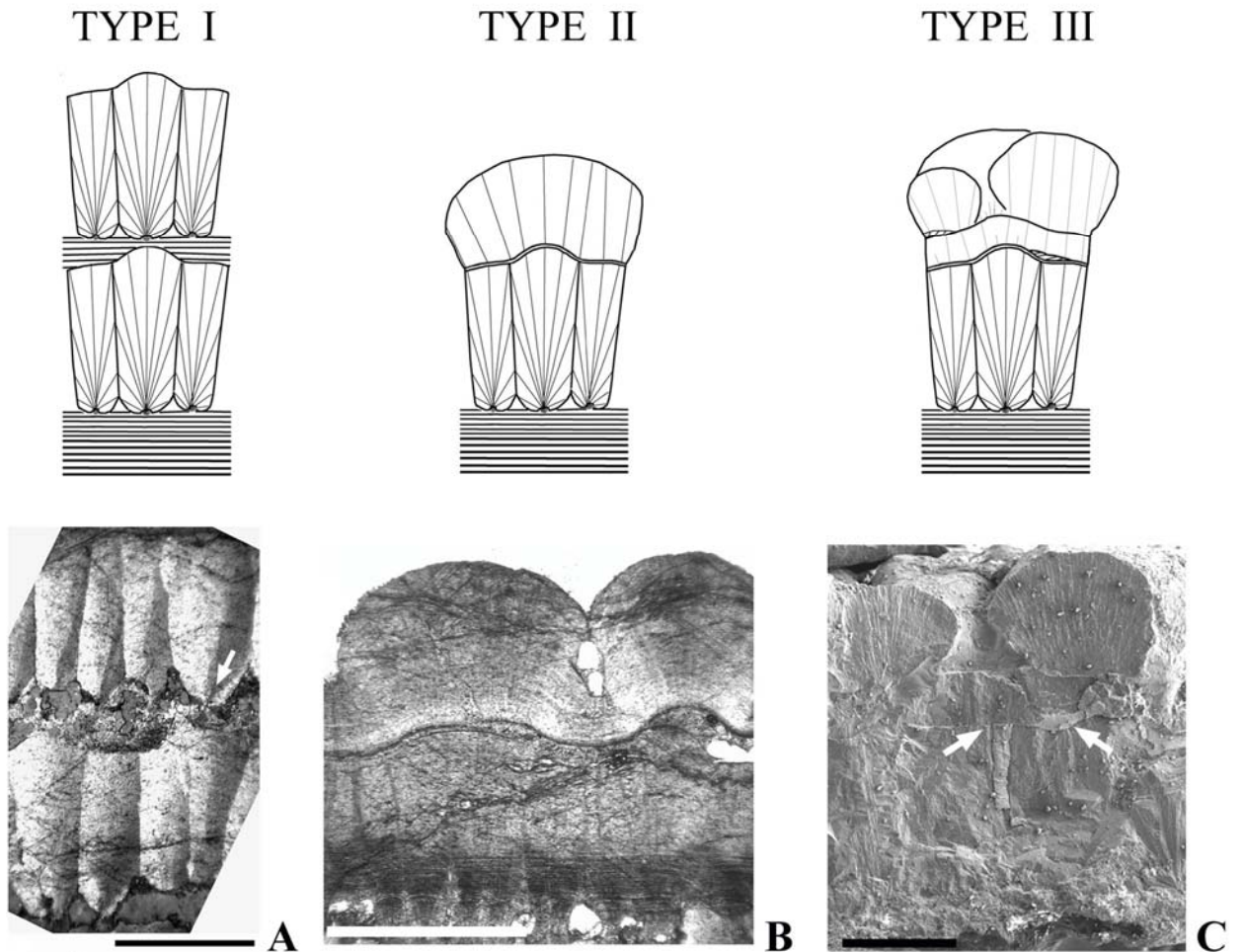


Figure 2.7. Types I-III abnormal morphology in schematic and microscopic images. (A) Type I morphology showing a normal eggshell overlain by a structurally complete eggshell layer. Arrow in thin section indicates pseudouniaxial cross at the nucleation site in P-1 (MCF-PVPH-251). (B) Type II morphology in eggshell thin section from P-5 (MCH-PVPH-254); note abnormal layer closely conforms to the unweathered tuberculate surface ornamentation of the underlying egg. (C) SEM image of Type III morphology in P-3 (MCF-PVPH-253) comprised of three eggshell layers. Left arrow points to pore truncated by the second shell layer; right arrow shows membrane fragment between layers 1 and 2. Scale bars on all images equal 1 mm.

nucleation sites are lateral to one another, at a consistent distance above the unweathered surface of the underlying eggshell (Fig. 2.6D,E).

Type II Morphology. The inner eggshell exhibits normal structure, while the outer eggshell is typically thinner and lacks calcite nuclei (Figs. 2.5C, 2.6A, 2.7B, 2.8A, B). Calcite crystals are “seeded” to the underlying eggshell, with or without visible membrane separation. The base of the abnormal layer closely conforms to the tuberculate surface ornamentation of the underlying egg and the calcite structure of the abnormal eggshell maintains the established crystal orientation. Under crossed nicols in thin sections, a sweeping extinction pattern extends through both the inner and outer eggshell layers simultaneously in some areas, indicating optical continuity between the two eggshells (Fig. 2.8B). However, where multiple shell units of the inner eggshell correspond to a single shell unit in the outer eggshell layer, the extinction pattern may be disrupted.

Type III Morphology. This structure consists of three or more superimposed eggshell layers (Figs. 2.5A,B; 2.7C; 2.8C,D). The innermost eggshell exhibits normal calcite structure and ornamentation. The abnormal layers follow the contour of the underlying shell, displaying a smooth basal contact, and an absence of calcite nucleation sites. In some areas of the abnormal egg in clutch P-2, the second and third abnormal layers display small tubercles on the outer shell surface (Fig. 2.8C, D). Remnants of mesh-like permineralized membrane often separate the calcite layers (Figs. 2.8A, B; 2.5C). Occasionally, the additional shell layers block pores that traverse the inner eggshell, thereby restricting gas exchange to the embryo (Fig. 2.7C). Shell units

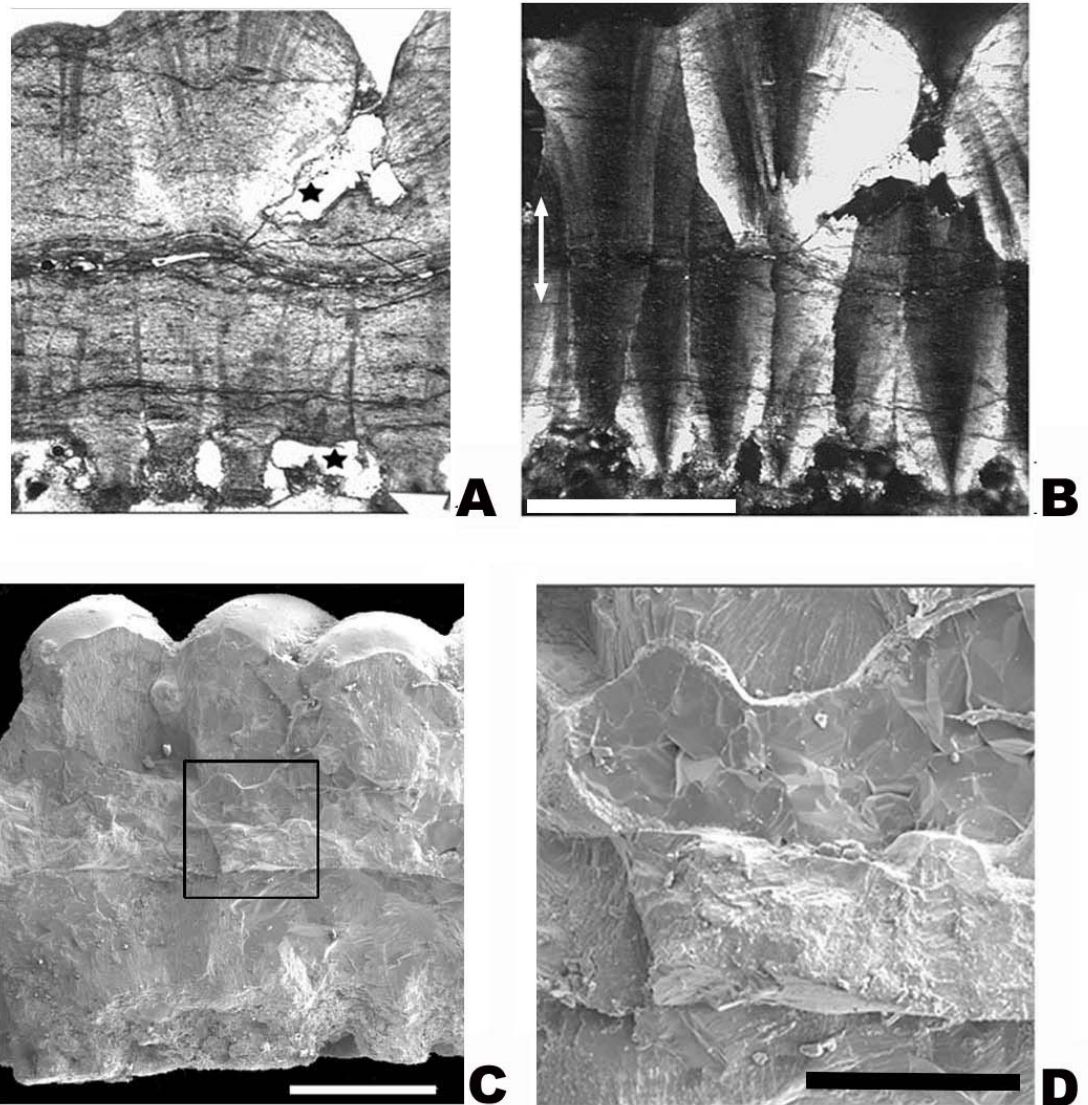


Figure 2.8. Type II and III morphology. (A) Eggshell from an abnormal egg in clutch P-2 (MCF-PVPH-252) showing Type II structure. Star symbols indicate areas of eggshell dissolution and replacement by calcite. (B) Same eggshell under cross polars. Vertical arrow indicates extinction pattern that crosses the inner and outer eggshell in the left $\frac{1}{4}$ of the photograph; upper shell unit in the center of the photograph shows disruption of extinction pattern. (C) Four eggshell layers in the abnormal egg from clutch P-2 (MCF- PVPH-252). (D) Close up of C showing the small tuberculate ornamentation present in layers 2 and 3.

comprising the outermost eggshell terminate in broad (>1.0 mm), rounded tubercles, producing rugose surface ornamentation compared to normal eggs exposed within the same clutch (compare Fig. 2.7C to D).

Discussion

Occurrence of Eggshell Abnormalities

Modern amniotes that lay hard-shelled eggs include some geckos and turtles and all crocodilians and birds. Egg abnormalities in these taxa occasionally result from egg retention, often in response to physiological or environmental “stress”. Such stress may result from absence of appropriate nesting substrate or material (Ewert et al., 1984; Hughes et al., 1986), handling, oviduct obstruction (Asmundson, 1933), disease or injury (Romanoff and Romanoff, 1949), diet (Grau and Kamei, 1949), and high population density (Ferguson, 1982; Solomon, 1997). With prolonged egg retention, additional eggshell layer(s) may be deposited over the first eggshell (Romanoff and Romanoff, 1949; Erben et al., 1979; Ewert et al., 1984; Jackson and Varricchio, 2003).

With the exception of turtles, documentation of abnormal, multilayered eggshell in extant amniotes is very limited. Erben et al. (1979) reported this multilayered condition in an unidentified crocodilian egg; however, the paper lacks citation, photographs, and description of the egg or eggshell microstructure. Some birds occasionally produce abnormal, superimposed eggshell layers that are similar in structure to abnormal dinosaur eggshells (Solomon, 1997; Jackson and Varricchio, 2003). However, reports of multilayered avian eggs are rare. In contrast, multilayered eggs are

relatively common in some hard-shelled turtle eggs, currently reported in at least nine extant species (Cagle and Tihen, 1948; Erben, 1970; Erben et al., 1979; Ewert et al., 1984; Schleich and Kästle, 1988).

Multilayered dinosaur eggshells are reported from Upper Cretaceous rocks of Asia, Europe, and North and South America (Dughi and Sirugue, 1958; Thaler, 1965; Erben, 1970; Sochava, 1971, Mohabey, 1984; Zhao et al., 1991; Vianey-Liaud et al., 1994; Powell, 1987; Zelenitsky and Hills, 1997; Ribeiro, 1999; Zhao et al., 2002).

Reports of intact, multilayered eggs include only three isolated eggs from different localities in Maastrichtian rocks of southern France (K  rourio, 1981) and a single egg from the Jurassic Morrison Formation in Utah (Hirsch et al., 1989). Jackson et al. (2002) reported a multilayered fossil turtle egg from the Judith River Formation of Montana, and Schleich and K  stle (1988) reported multilayered gecko eggs from the Oligocene of Germany. Although present in a variety of fossil eggshell types, the multilayered condition is most frequently reported in the megaloolithid eggshell structure (Hirsch, 2001).

Abnormalities in Titanosaur Eggs

In extant taxa, eggshell abnormalities provide information on the timing of stress (Tyler and Simkiss, 1959; Hughes et al., 1986; Solomon et al., 1987; Solomon, 1997; Jackson and Varricchio, 2003). Fossil eggshells provide similar information: in all abnormal eggs from the six sauropod clutches, the inner eggshell is comparable in thickness, microstructure, and surface ornamentation to normal titanosaur eggs from the Auca Mahuevo locality. Therefore, the adverse stimuli that resulted in egg retention

occurred at or near the end of shell deposition, before oviposition could occur.

In Type I morphology (Fig. 2.7A), secretion of additional membrane followed this egg retention. Where analcime or calcite replaces the membrane between the superimposed eggshell layers, the original membrane thickness may be inferred by measuring the distance from the surface of the underlying eggshell to the calcite nuclei at the base of the overlying, abnormal eggshell layer (Fig. 2.6D,E). From these measurements I conclude that the additional membrane deposited over the retained egg was thinner than that present at the interior shell surface of normal eggs (compare figs. 2.3B to 2.6D,E). In eggshell of extant birds, the organic cores are located within the upper surface of the two-layered membrane structure, adjacent to the shell. Proteins of these organic cores are thought to initiate calcite nucleation and exert critical control over the resulting crystal morphology (Nys, et al., 1999; Dennis et al., 1996). Despite significant reduction in membrane thickness between the superimposed eggshells in P-1, calcite nucleation sites are present at the interior surface of the abnormal layer, thereby producing normal calcite structure in the overlying eggshell.

In Type II and III morphologies, nucleation sites are absent at the base of the abnormal eggshell layers and these additional eggshell layers are structurally incomplete (Figs. 2.6A, 2.7 B,C, 2.8 A-C). The superimposed layers are closer together than in Type I morphology, suggesting further reduction or absence of membrane and elimination of the organic cores necessary for normal eggshell structure. In these specimens, mineral deposition follows the crystallographic “template” provided by inner eggshell. Lateral expansion of the shell unit, unimpeded by competition from crystal growth of an adjacent

nucleation site, produces the broad, bulbous surface ornamentation characteristic of most multilayered eggshells from this locality. In at least one multilayered egg (P-2), the eggshell exhibits highly variable morphology that includes all three types of abnormal morphology (Types I, II, III) within the same abnormal egg.

Comparison to Eggs of Extant Taxa

Morphology similar to Type I occurs in both extant turtle and bird eggs (Ewert et al., 1984; Jackson and Varricchio, 2003). In both living taxa, the abnormal shell layer exhibits organized structure. Nucleation sites at the base of the outer, abnormal layer are often further apart than those at the base of the inner eggshell (Ewert et al., 1984: figs. 8, 9; Jackson and Varricchio, 2003), a condition also observed in the abnormal sauropod egg from clutch P-1 (Fig. 2.6D). In Type I sauropod morphology, however, the outer eggshell is structurally complete and of normal thickness. In contrast, multilayered bird and turtle eggs exhibit a thinner and structurally incomplete outer eggshell, compared to the underlying egg (Erben et al., 1970 fig. 2; Ewert et al., 1984: figs. 9, 10; Jackson and Varricchio, 2003).

Abnormal shell morphologies similar to Types II and III also occur in fossil and extant turtle eggs and the membrane that separates the multiple, mineralized layers varies in thickness (Erben et al., 1979; Ewert et al., 1984: figs. 7, 8; Hirsch, 2001: figs. 12, 13; Jackson et al., 2002: fig. 11). To the best of my knowledge, abnormal morphology similar to Type II and III has not been documented in birds, despite numerous studies that include a variety of abnormal eggshell conditions (Hargitt, 1897; Curtis, 1916;

Asmundson, 1933; Romanoff and Romanoff, 1949; Grau and Kamei, 1949; Sykes, 1955; Tyler and Simkiss, 1959; Von Nathusius translated by Tyler, 1964; Hughes et al., 1986; Solomon et al., 1987; Solomon, 1997; Jackson and Varricchio, 2003).

Previous Hypotheses Based on Abnormal Eggshell

All previous reports of pathological dinosaur eggshells pertain to isolated, taxonomically unidentified eggs (K  rourio, 1981; Hirsch et al., 1989) or eggshell fragments (Dughi and Sirugue, 1958; Thaler, 1965; Erben, 1970; Sochava, 1971; Erben et al., 1979; K  rourio, 1981; Mohabey, 1984; Powell, 1987; Vianey-Liaud et al., 1994; Zelenitsky and Hills, 1997; Ribeiro, 1999; Zhao et al., 1991; Yang et al., 2001; Zhao et al., 2002) rather than in situ egg clutches. Thus, most literature on fossil dinosaur eggshell abnormalities is incomplete in terms of taphonomy, descriptive parameters, and taxonomic identification necessary for interpretation of their occurrence, formation, and phylogenetic significance. Several authors, however, have attempted to correlate eggshell abnormalities with Late Cretaceous climate change, iridium anomalies, and dinosaur extinction (Dughi and Sirugue, 1958; Thaler, 1965; Zhao et al., 1991; Yang et al., 2001; Zhao et al., 2002).

Implications for Cretaceous Extinction Scenarios

Erben et al., (1979) report that pathological conditions occur in 10% of eggshells in Upper Cretaceous rocks from France and the authors suggest a progressive, stratigraphically upward increase in these abnormalities as a result of changing climatic

conditions. Similarly, Zhao et al., (2002) report a 56% and 74% frequency of pathological eggshells in the oospecies *Macroolithus yaotunensis* near the Cretaceous-Tertiary boundary in two locations in the Nanxiong Basin, South China. However, there is no indication in either report that the fossil material represents *in situ* eggs or clutches, nor do the authors discuss the statistical methods used for calculating abnormal eggshell abundance or the alleged increase in eggshell abnormalities over a stratigraphic interval. Furthermore, K  rourio (1981) considered at least one of the eggshell localities examined by Erben to represent material reworked from the underlying strata.

These and other attempts to link abnormal, multilayered eggshell to climate change and dinosaur extinction fail to consider the magnitude of geologic time associated with the Cretaceous-Tertiary extinction event. In extant animals, eggshell abnormalities may result from environmental stress related to overcrowding, drought, or substrate conditions (Ewert et al., 1984; Ferguson, 1982). Although acute stress may affect eggshell structure for a considerable time (Hughes et al., 1986; Solomon, 1997), this time interval (hours to weeks in birds and reptiles) is extremely short when compared to the much longer intervals of geologic time ($\geq 10^2$ - 10^4 years) associated with global climate change or the terminal Cretaceous extinction event (Dingus, 1984).

Without taphonomic context provided by *in situ* dinosaur egg clutches, multilayered eggshell fragments provide little stratigraphic resolution or information about how frequently this abnormal condition occurred in dinosaur populations. For example, sauropod clutches from Auca Mahuevo typically contain up to 35 eggs (Chiappe et al., 1999) and partial fragmentation of only two multilayered eggs within

clutch P-6 resulted in nearly 400 specimens of abnormal eggshell at an early stage of clutch weathering. Subsequent transport and distribution of this material could contribute to the perception of a more frequent occurrence of eggshell abnormalities in an area. The large clutch size and laterally extensive nesting horizons characteristic of titanosaurs at this site and other megaloolithid eggshell sites worldwide undoubtedly contribute to more frequent reports of multilayering in this type of eggshell structure. Although an unprecedented number of clutches are present at the Auca Mahuevo locality, the incidence of clutches containing abnormal eggs (6 of 343 clutches examined) and number of abnormal eggs within these clutches (maximum 3 of 30 in P-6) appears very low.

Inferences of Dinosaur Physiological and Reproductive Anatomy

Aspects of dinosaur physiology and reproductive anatomy have also been inferred by purported similarities or differences in eggshell abnormalities present in fossil and extant eggs (Erben et al., 1979; Hirsch et al., 1989; Hirsch, 1994a; Carpenter et al., 1994; Zelenitsky and Hills, 1997). For example Dughi and Sirugue (1958) interpreted multilayering of eggshell as an interruption in shell deposition during brief cold periods and hypothesized dinosaur ectothermy based on this abnormal eggshell condition. The authors, however, provided no modern analog for this process. Erben et al., (1979) compared abnormalities in dinosaur eggshell to similar multilayered eggshell (ovum in ovo) of living birds. Citing stress-related hormonal changes that can affect domestic fowl, these authors speculated that environmental change and overpopulation might have produced frequent aggressive interactions that upset female estrogen levels, resulting in

abnormal eggshell formation. Clearly, the fossil record is not capable of recording the information necessary to test this anthropocentric speculation. Furthermore, Erben et al., (1979) hypothesized that a genetic mutation spread rapidly through dinosaur populations near the end of the Cretaceous, increasing the frequency of pathological eggs, with a corresponding reduction of offspring. However, it is difficult to envision how a genetic change that leads to embryonic mortality can propagate through a dinosaur population.

Abnormal, multilayered eggshells have also been used to infer dinosaur reproductive anatomy (Hirsch 1989; Hirsch et al., 1989; Hirsch, 1994a; Zelenitsky and Hills, 1997; Carpenter, 1999). For example, multilayered eggshell was thought to occur exclusively in eggs of reptiles and not birds (Hirsch et al., 1989, Zelenitsky and Hills, 1997). However, superimposed eggshells occur in archosaurs (birds, possibly crocodilians) and non-archosaurian reptiles (turtles, geckos), despite significant differences in reproductive anatomy (Erben et al., 1979; Ewert et al., 1984; Schleich and Kästle, 1988; Palmer and Guillette, 1992; Jackson and Varricchio, 2003). The range of eggshell abnormalities present in sauropod eggs from Auca Mahuevo (Fig. 2.7A-C) encompasses that documented in both birds and turtles, suggesting that eggshell abnormalities represent a common response to physiological or environmental stress in amniotes that lay hard-shelled eggs (Jackson and Varricchio, 2003). However, the presence of both multilayered eggs and normal eggs within the same sauropod clutch raises intriguing questions regarding clutch formation and, ultimately, the evolution of reproductive mode within Archosauria.

Conclusions

Exposures of egg bed 3 (~ 35,000 m²) within the study area contain 329 in situ egg clutches, while the more limited exposures of egg bed 2 (~ 3000 m²) contain 14 clutches referable to titanosaur sauropod dinosaurs. Five clutches from egg bed 3 and one clutch from egg bed 2 contain both normal and multilayered eggs within the same clutch. Excavation of clutch P-6 reveals 30 eggs distributed in three levels: 27 normal eggs and three laterally adjacent, multilayered eggs that occur at the highest level in the clutch, representing the last eggs laid by the titanosaur female.

Microscopic examination shows that the structure of the innermost layer in all abnormal eggs is identical to the normal, megaloolithid calcite structure present in other Auca Mahuevo eggs containing diagnosable titanosaur embryonic remains.

Permineralized eggshell membrane, often replaced by authigenic analcime, occurs at the base of some eggshell and/or separates the inner from the outer eggshell layers. Three distinctive types of abnormal morphology characterize the abnormal eggshell layers:

Type I, a structurally complete eggshell with basal nucleation sites and typical megaloolithid structure, thickness, and ornamentation; Type II, a thinner than normal eggshell that lacks calcite nuclei and exhibits rugose ornamentation; and Type III, multiple shell layers that lack nucleation sites and display rugose ornamentation in the outermost eggshell layer. Variation in abnormal morphology occurs among eggs of the same clutches and within a single egg. This morphological variation encompasses abnormal shell morphology documented in both archosaurs (birds) and non-archosaurian reptiles (turtles), despite significant differences in reproductive anatomy that

characterizes these groups. This suggests that abnormal eggshell is a common response to environmental or physiological stress in amniotes that lay hard-shelled eggs.

Previous attempts to relate the presence of pathological eggshell in Upper Cretaceous rocks to environmental change and dinosaur extinction have relied on isolated eggs or multilayered eggshells rather than in situ egg clutches containing abnormal eggs of a known dinosaur taxon. These reports lack the rigorous statistical documentation necessary for supporting the alleged increase of egg abnormalities in the stratigraphic interval that precedes the Cretaceous-Tertiary boundary. Furthermore, although acute stress may affect eggshell structure for a considerable length of time relative to the reproductive cycle in modern amniotes, this brief interval of time is not comparable to the larger magnitude of geologic time involved in global climate change and the Cretaceous extinction event.

CHAPTER 3

RECOGNITION OF VERTEBRATE EGG ABNORMALITIES
IN THE FOSSIL RECORDIntroduction

The study of vertebrate fossil eggs is based primarily on knowledge of egg formation and structure in extant taxa. However, several limitations inhibit the study of fossil material: diagenetic alteration of calcite eggshell microstructure, enlargement and in-filling of eggshell pores by authigenic minerals, preservation bias against soft or pliable eggshell, and the lack of taxonomically referable specimens (Hirsch and Packard, 1987; Hirsch 2001; Jackson et al., 2004). Furthermore, organic components (e.g. membrane, matrix proteins, cuticle, and pore coverings) are rarely preserved in the fossil record and therefore most descriptions document only the inorganic structure of fossil specimens. Despite these limiting factors, eggshell as a genetically-controlled biomineralized tissue can provide important information on dinosaur physiology, phylogenetic relationships, and paleoenvironment (Erben et al., 1979; Mikhailov, 1992; Varricchio et al., 1997, 1999; Chiappe et al., 2001; Grellet-Tinner and Chiappe, 2004; Zelenitsky and Modesto, 2003; Zelenitsky et al., 2002; Varricchio and Jackson, 2004a, 2004b, 2004c; Schweitzer et al., 2005; Grellet-Tinner, 2005; Grellet-Tinner et al., 2006).

Abnormalities that affect eggshell structure are documented from a variety of fossil eggs, and the majority of reports describe a multilayered eggshell condition. As discussed in Chapter 3, comparison of abnormalities in fossil specimens to those reported

in modern eggs has resulted in inferences of dinosaur reproductive anatomy, physiology and extinction (Dughi and Sirugue, 1958; Thaler, 1965; Erben, 1970; Erben et al., 1979; Ewert et al., 1984; Hirsch et al., 1989; Zhao et al., 1991; Hirsch, 1994b; Zelenitsky and Hills 1997; Carpenter 1999; Yang et al., 2001; Zhao et al., 2002)). While many of these scenarios are highly speculative and untestable from the fossil record (Jackson and Varricchio, 2003; Jackson et al., 2004), eggshell abnormalities, nevertheless, provide valuable information regarding dinosaur reproductive biology (Hirsch, 2001).

The study of fossil egg abnormalities, however, requires differentiation between multilayered eggshells that result from a biological process, and “stacked” eggshell resulting from taphonomic phenomena (Hirsch 2001). For example, Elzanowski (1981) describes pathological double eggshells in a specimen containing an unhatched “Gobipteryx” embryo. Sabath (1991), however, considers the multiple layers the product of crushed, overlapping eggshell fragments. In addition, Erben et al. (1979) report an increase in pathological eggshell toward the Cretaceous-Tertiary boundary in southern France and relate this abnormality to environmental degradation and dinosaur extinction. The report, however, includes minimal description of taphonomic attributes. Furthermore, time-averaging of different horizons and diagenetic cementation of crushed eggshells may account for the high percentages of apparent egg “abnormalities” reported by these authors.

Taphonomic context, therefore, represents an integral part of the study of pathological conditions in fossil eggs. Documentation of abnormal features facilitates a better understanding of this condition and allows assessment of the usefulness of egg

abnormalities for inferences of reproductive biology in extinct taxa. Taxonomically referable specimens are particularly important for recognizing phylogenetically-significant patterns in the fossil record and may prove valuable for understanding the evolution of reproductive anatomy in modern amniotes, particularly within Archosauria.

The study of abnormal titanosaur eggs described in the preceding chapter identified three types of abnormal egg morphology and provided a better understanding of abnormal egg formation. In this chapter, I document eggshell abnormalities from three dinosaur clades and the first abnormal Cretaceous turtle egg. I discuss abnormal egg formation and problems inherent in taxonomic referral of fossil specimens, and review features that allow differentiation of eggshell abnormalities from those resulting from diagenetic or pedogenic processes. Finally, I provide criteria for identification of abnormal multilayered eggs and eggshell in the fossil record.

Description

Fossil Turtle Eggshell

The abnormal fossil turtle egg from the Judith River Formation occurred in a weathered clutch containing at least 13 eggs, some of which exhibited normal eggshell (Fig. 3.1) and contained embryonic remains. The 3 x 4 cm abnormal egg is largely intact but compressed. The pale cream-colored, relatively smooth surface exhibits some irregularity as a result of surficial weathering. The thickness of the eggshell varies and the abnormal layer appears unevenly distributed over the egg.

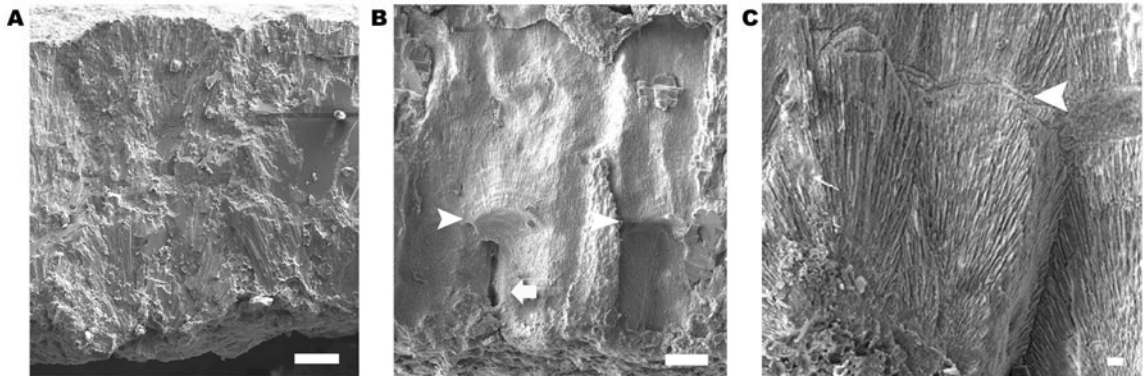


Figure. 3.1. Cretaceous turtle eggshell. (A) SEM of normal eggshell from an egg containing embryonic remains. Note the slightly flared shell unit and domed ornamentation. (B) Two superimposed eggshell layers in abnormal egg. White triangles indicate upper surface of the inner eggshell layer; white arrow shows elongate pore behind the impression. (C) Needle-like aragonite crystals of the inner eggshell layer. Note varying morphology of outer shell units. White triangle points to the contact between inner and outer eggshell layers. Scale bars: A,B = 100 µm; C = 10 µm.

Examination of the 700 µm-thick specimen by scanning electron microscopy (SEM) reveals two superimposed eggshell layers (Fig. 3.1B,C). The 229 µm-thick inner eggshell layer exhibits a single structural layer composed of spherulites of radiating, acicular aragonite crystals. Some nucleation sites at the inner eggshell surface are well preserved, while others display extensive dissolution and reprecipitation of sparry calcite. Radiating from the closely spaced shell-unit nuclei on the shell inner surface, the needle-like crystals expand laterally towards the upper surface of the inner shell, terminating as 100-150 µm-wide domes (Fig. 3.1C). Surface morphology varies, and some shell units are more elongated and pointed. Juxtaposed upon the inner eggshell, the 471 µm-thick, outer eggshell layer lacks nucleation sites and closely conforms to the contour of the underlying egg ornamentation (Fig. 3.1C). Scanning electron microscopy reveals a relatively straight pore, 14 µm in diameter. Whether this elongate pore extends into the

overlying eggshell layer is uncertain since the pore lies behind a shell unit impression (Fig. 3.1B).

Hadrosaur Eggshell

A single eggshell (ES-114) was collected from the middle subfacies at the Willow Creek anticline, Two Medicine Formation (Lorenz and Gavin, 1984). The 1807 μm -thick specimen consists of two superimposed eggshell layers (Fig. 3.2). The inner layer measures 1000 μm in thickness and exhibits identical microstructure as hadrosaur

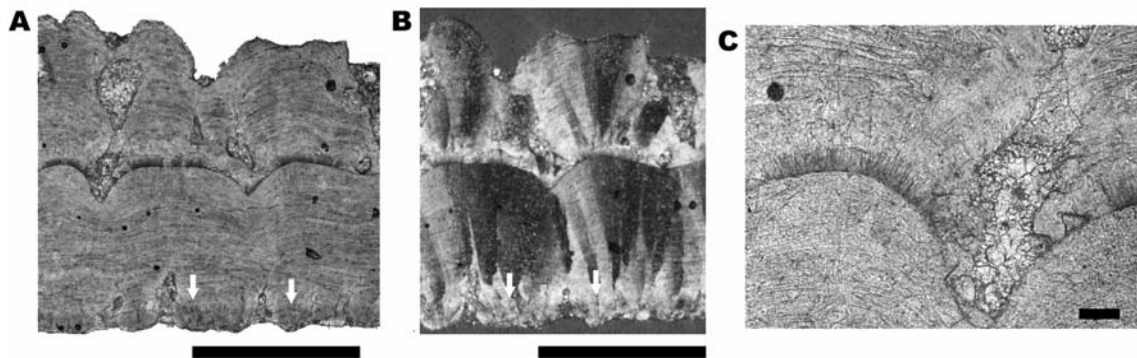


Figure 3.2. Hadrosaur eggshell. (A) Radial thin section of hadrosaur eggshell in PPL. Note radiating spherulites at base of the inner eggshell below white arrows. (B) Same eggshell under crossed polars. (C) Enlargement of spherulites at the base of the inner eggshell layer showing absence of calcite nuclei. Abnormal eggshell layer closely conforms to the surface ornamentation of the underlying eggshell layer. An oblique, sparry calcite-filled pore is visible to upper left of scale bar. Scale bars: A,B. = 1000 μm ; C = 100 μm .

eggs and eggshell associated with hadrosaur hatchlings from the same locality (Hirsch and Quinn, 1990). The basal portion of the eggshell displays closely spaced centers of radiating calcite crystals that extend outward from nucleation sites located at the inner eggshell surface (Fig. 3.2A,B). The visibility of the spherulites decreases abruptly about 170 μm above the nuclei and remains obscured throughout the rest of the eggshell thickness. Horizontal accretion lines are gently undulating, but where associated with

pores the lines dip downward around the structure. These sparry calcite-filled pores are irregular in shape and distribution and oblique to the shell surface. The outer surface of this inner eggshell layer is well preserved, with an undulating structure that forms ridges and valleys. Rotation of the stage under crossed polars reveals a sweeping extinction pattern in the calcite crystals of the spherulitic shell units, suggesting a radial orientation of the crystals.

The basal portion of the 807 μm -thick outer, abnormal eggshell layer closely conforms to the surface ornamentation of the underlying shell (Fig. 3.2A-C). Definitive nucleation sites are difficult to identify at the lower surface of the abnormal shell layer and the radiating crystals extend only 35-50 μm above the contact with the underlying eggshell (i.e. base of the outer eggshell) (Fig. 3.2C). With these exceptions, the outer eggshell exhibits nearly identical structure to the underlying eggshell layer. Although the outer eggshell layer displays extensive damage from erosion or thin section preparation, some complete shell units are preserved. The thickness of this layer falls within the range of variation displayed by the underlying, inner eggshell layer.

Titanosaur Eggshell

The two eggshell fragments were surface collected from egg bed 3 and 4 at the Auca Mahuevo locality in Argentina (Chiappe et al., 1998). These laterally continuous egg-bearing horizons contain abundant egg clutches, a significant number of which contain titanosaur embryonic remains (Chiappe et al., 1998; Salgado et al., 2005). Based on the inner eggshell microstructure, I infer that both fragments described below are also

derived from titanosaur eggs. The abnormal eggshells, however, represent isolated fragments not associated with a specific egg or clutch.

Egg Bed 3 Specimen (PVPH 255). This 3770 μm -thick specimen consists of two superimposed eggshell layers (Fig. 3.3A,B). Along the lower surface of the 1740 μm -

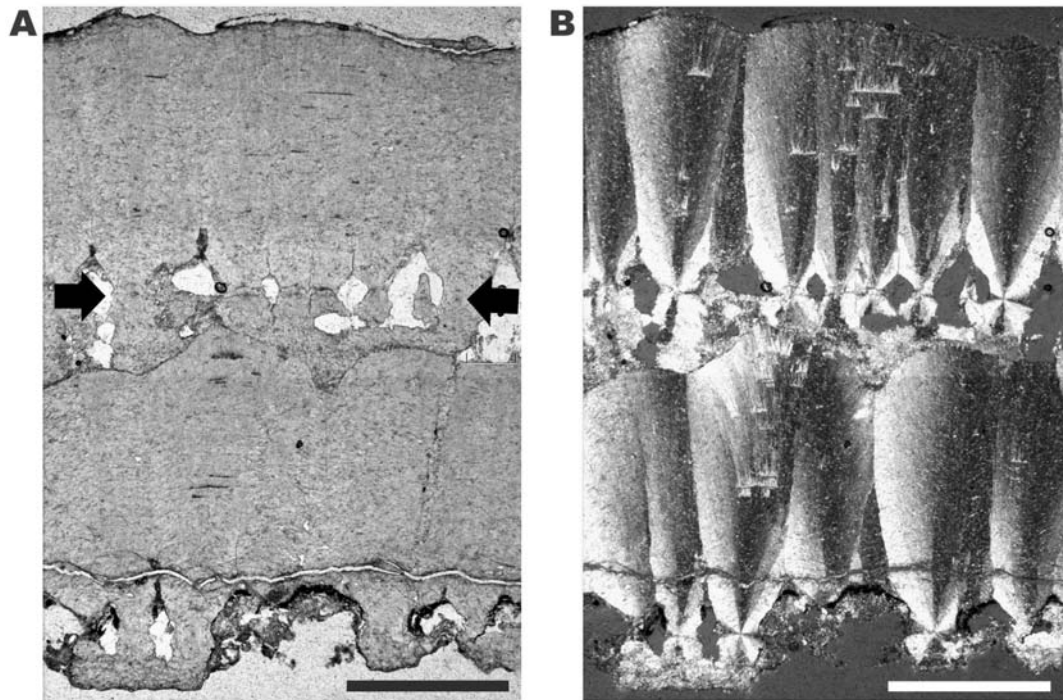


Figure 3.3. Type I Titanosaur eggshell. (A) Thin section of Type I abnormal morphology in titanosaur eggshell (PVPH 255) showing normal microstructure in both eggshell layers. Black arrows indicate level of preserved calcite nucleation sites. (B) Same eggshell under crossed polars. Scale bars equal 1000 μm .

thick inner eggshell layer, calcite spherulites radiate from central nucleation sites.

Diagenetic calcite replaces the original eggshell between the nuclei. The laterally and vertically extended spherulites form shell units with parallel to sub-parallel margins that flare slightly toward the outer shell surface. Relatively flat or tuberculate ornamentation

characterizes the surface of the inner eggshell layer. Remnants of permineralized membrane separate the two eggshell layers in some areas and overlie a pore that traverses the inner eggshell; this pore does not extend into the overlying, abnormal layer. The approximately 350 μm -thick membrane shows significant diagenetic alteration, but exhibits horizontal stratification and preserved nuclei in some areas (Fig. 3.3). The nuclei occur at consistent distances above the inner eggshell surface and are laterally adjacent to one another. The contact between the inner eggshell and the overlying membrane varies from smooth to irregular. Irregular contacts are produced by pressure solution and associated development of micro-stylolites along the eggshell-membrane contact.

The base of the overlying outer eggshell shows evidence of eggshell dissolution and subsequent reprecipitation of sparry calcite. Spherulites radiating from the organic cores within the membrane grow laterally until truncated by crystal growth from the adjacent nucleation site (Fig. 3.3). The shell units are approximately 1660 μm in height, terminating in a flat, undulating, or tuberculate surface. Shell unit margins are parallel to sub-parallel, flaring moderately toward the outer region of the eggshell. The eggshell surface displays similar ornamentation as normal eggs from this site. Unlike Type I morphology documented in the previous study (Fig. 2.7A; Jackson et al., 2004), shell units in the inner and outer eggshell layers are not always vertically superimposed.

Egg bed 4 Specimen (PVPH 256). The 2500 μm -thick eggshell consist of two superimposed eggshell layers: a 1333 and 1166 μm inner and outer eggshell layer,

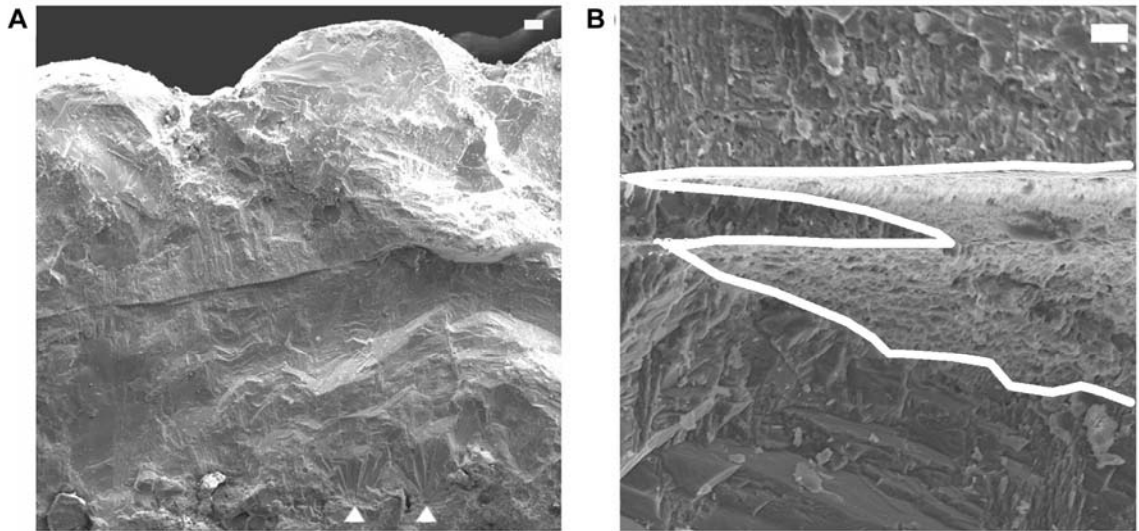


Figure 3.4. Type II Titanosaur eggshell. (A) SEM of Type II abnormal morphology in titanosaur eggshell (PVPH 256) in SEM. White triangles denote shell unit nuclei. Note lack of nucleation sites at the base of outer eggshell layer. Scale bar = 100 μm . (B) Intercalation of eggshell and permineralized membrane between the first and second shell layers of PVPH 256. White line denotes boundary of membrane. Scale bar = 10 μm .

respectively (Fig. 3.4A, B). Radiating calcite spherulites extend outward from nucleation sites to form parallel to sub-parallel shell units that are 350 to 550 μm in diameter and flare slightly toward the upper surface of the inner eggshell layer. Three or more shell units underlie the flat or featureless portion of the egg surface between tubercles (Fig. 3.4A).

Permineralized membrane separates the inner and outer eggshell layers in some areas of the abnormal specimen. This mesh-like structure is occasionally intercalated with narrow, tapered fragments of eggshell that occur between the inner and outer eggshell layers (Fig. 3.4B). Nucleation sites are absent at the base of the outer abnormal eggshell layer, and the interior edge of this abnormal eggshell closely conforms to the surface ornamentation of the underlying eggshell (Fig. 3.4B). Calcite spherulites are

traceable from the inner to the outer shell layer in some areas, and crystal orientation established in the underlying shell units is maintained in the spherulitic units of the overlying, abnormal outer eggshell layer. The unusually broad, 1300 μm -wide tubercle on the shell surface is composed of multiple shell units, thus producing a more rugose ornamentation than that present in normal titanosaur eggs at this site (Grellet-Tinner et al., 2004).

Prismatic Theropod Eggshell

The 1703 μm -thick, multilayered eggshell (ES-127) from the Sevenmile Hill locality, Two Medicine Formation of Montana consists of two superimposed layers (Fig. 3.5A-D). When viewed under plain polarized light (PPL), the specimen displays diagenetic alteration expressed as a mosaic of equant calcite crystals (Fig. 3.5A). The 783 μm -thick inner eggshell layer lacks calcite nuclei due to diagenetic dissolution or erosion. Faint prismatic columns are visible in the inner eggshell under SEM, while thin sections viewed under polarized light microscopy reveal columnar extinction (Fig. 3.5B,D). A smooth contact separates this upper surface of the inner eggshell layer from a thin (~ 50 μm) calcite band composed of two laminae of nearly equal thickness. The inner lamina exhibits blocky prismatic structure that is in optical continuity with the prismatic columns of the underlying eggshell (Fig. 3.5D). This inner lamina, visible under crossed polars in thin section, is

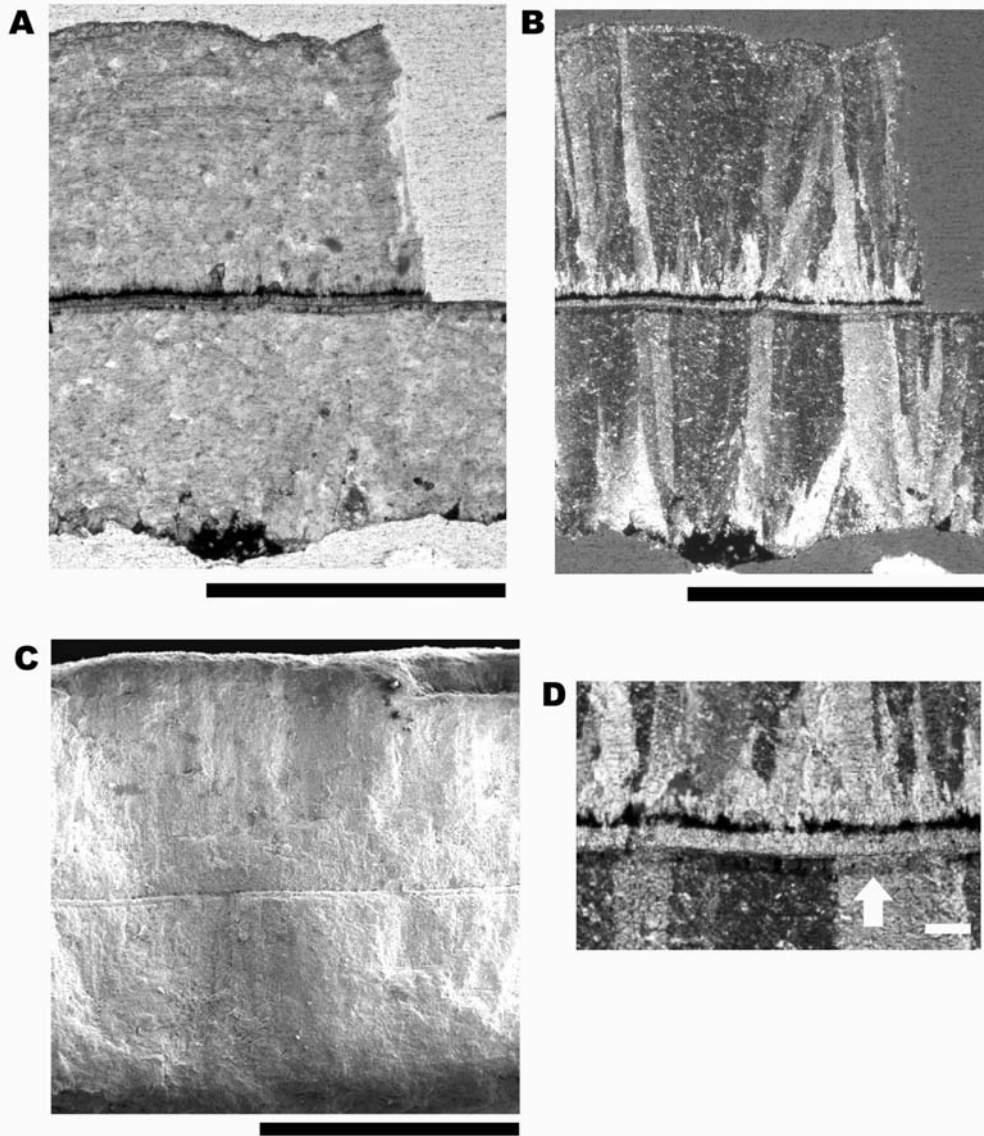


Figure 3.5. Prismatic eggshell. (A) Two superimposed prismatic eggshell layers, both with diagenetic alteration. Two thin laminae (external layer) comprise the outer portion of the inner eggshell. The outer lamina is missing where detachment of inner and outer shells occurred, (B) Same eggshell under crossed polars, showing columnar extinction. (C) SEM image showing two superimposed eggshell layers. Note the missing portion of the external layer in the upper right corner of the outer eggshell layer. (D) Planar contact between inner and outer eggshell layers in plain polarized light. Note the two laminae comprising the external layer below the dark irregular band. White arrow points to base of lower lamina. The inner eggshell lacks of surface ornamentation. Scale bars: A-C = 1000 μm ; D = 50 μm .

difficult to detect in SEM images. The slightly thicker outer lamina exhibits diagenetic recrystallization. The upper surface of this lamina displays no evidence of surface ornamentation and exhibits a smooth, planar contact with the overlying abnormal eggshell layer. However, detachment of the inner from the outer lamina occurs in some areas of the specimen (Fig. 3.5B).

A thin laterally continuous, dark opaque material is present at the base of the overlying, outer abnormal eggshell layer (Fig. 3.5D). The mammillary cones in the basal portion of this 850 μm -thick eggshell layer are approximately 80 to 100 μm in height. Microscopic examination under cross polars reveals a gradual transition from this thin mammillary layer to the overlying prismatic layer of the abnormal eggshell. In SEM images, a thin line separates the upper prismatic columns from a diagenetically altered 50 μm -thick crystalline layer, a feature not detectable in thin section. Detachment of this outer layer in some areas of the eggshell further delineates this contact (Fig. 3.5C). This outer abnormal eggshell layer displays a smooth to irregular surface. A columnar extinction pattern is evident under crossed polars (Fig 3.5B,C).

Discussion

Egg Abnormalities in Extant Taxa

In modern taxa that lay hard-shelled eggs (i.e. some geckos and turtles, and all crocodilians and birds) adverse stimuli from physiological or environmental stress occasionally produces prolonged egg retention by the female (Romanoff and Romanoff 1949; Erben et al., 1979; Ewert et al., 1984; Jackson and Varricchio, 2003). During this

time, additional shell layers may be deposited over the retained egg(s). The resulting, unusually thick eggshell is comprised of one or more superimposed layers that are often separated by protein membrane (Romanoff and Romanoff, 1949; Erben et al., 1979; Ewert et al., 1984; Kohring, 1999; Solomon, 1997; Jackson and Varricchio, 2003).

Multilayered eggs are relatively common in modern turtles, having been reported in at least ten species (Cagle and Tihen, 1948; Erben, 1970; Erben et al., 1979; Ewert et al., 1984; Schleich and Kästle, 1988; Kohring 1999). In contrast, examples of this condition in avian eggs are rare (Romanoff and Romanoff, 1949; Solomon, 1997; Jackson and Varricchio, 2003; Hayes, 2005). Erben et al. (1979) briefly mention multilayering of eggshell in an unidentified crocodilian egg. However, inadequate documentation produces uncertainty concerning this report (Jackson and Varricchio, 2003; Jackson et al., 2004).

Egg Abnormalities in Extinct Taxa

Most studies of fossil eggshell abnormalities pertain to isolated fragments rather than *in situ* eggs or clutches (Dughi and Sirugue, 1958; Thaler, 1965; Erben, 1970; Sochava 1971, Mohabey, 1984; Zhao et al., 1991; Vianey-Liaud et al., 1994; Powell, 1987; Zelenitsky and Hills, 1997; Ribeiro, 1999; Zhao et al., 2002). Until recently, reports of multilayered dinosaur eggs included only three specimens: two eggs from separate localities in Upper Cretaceous rocks of southern France (Kérourio, 1981) and a single egg from the Jurassic Morrison Formation of Utah (Hirsch et al., 1989). However, the Auca Mahuevo locality in Argentina recently yielded six clutches that contain both normal and abnormal titanosaur eggs (Chapter 2; Jackson et al., 2004). Eggshell

abnormalities are also reported in fossil eggs outside of Archosauria: Kohring (1999) describes multilayered turtle eggs from the Miocene of the Czech Republic, and Schleich and Kästle (1988) report multilayered gecko eggs from the Oligocene of Germany.

Reproductive Anatomy

The reproductive anatomy of modern turtles and other non-archosaurian reptiles differ significantly from that of archosaurs such as crocodilians and birds. Turtles, for example, possess two active ovaries and oviducts, with simultaneous ovulation of ova. A single region of the oviduct, the uterus, produces both membrane and eggshell (Aitken and Solomon, 1976). In turtles, the oviductal wall next to each egg secretes the eggshell membrane first, then the aragonite mineral (Ewert et al., 1984). Simultaneous shelling of several eggs occurs in the oviduct, and the entire clutch is laid at one time (Aitken and Solomon, 1976).

Extant crocodilians show a divergence from other reptiles in reproductive anatomy. Eggshell membrane is produced in one region of the oviduct, while the calcite eggshell is produced in a more distal area. This separation of oviduct function in the formation of eggs is shared with birds and represents an “archosaurian” mode of reproduction (Palmer and Guillette, 1992). Crocodilians, however, retain the ancestral condition of simultaneous ovulation of eggs and mass egg laying, whereas birds exhibit sequential ovulation, with each egg shelled and laid individually, at a 24 hour or greater interval.

Taxonomic Assignment of Abnormal Fossil Eggs

Taxonomically referable fossil eggs provide the most reliable basis for inferences regarding the reproductive biology of extinct taxa, particularly when studied within the context of the Extant Phylogenetic Bracket (EPB) (Witmer, 1995). Definitive identification of any fossil egg, however, requires the presence of an *in ovo* embryo with diagnostic features (Chiappe et al., 1998). For this reason, taxonomic assignment of pathological eggshells represents a significant problem. Although Ewert et al., (1984) document a brief post-hatching survival of an embryo from a pathological turtle egg, multilayered eggshell typically results in embryonic death (Erben et al., 1979; Ewert et al., 1984; Hirsch, 2001; Jackson and Varricchio, 2003). The additional, abnormal shell layers restrict embryonic gas exchange with the atmosphere and the embryo suffocates. Therefore, multilayered eggs that appear hatched produce uncertainty about the presence of a pathological condition in fossil material. Hirsch (2001), for example, questioned the alleged pathological condition reported by Schleich and Kästle (1988) in hatched gecko eggs from the Oligocene of Germany.

Without embryonic remains, taxonomic referral of fossil abnormal eggs or eggshell fragments requires caution. Reliable assignment of an abnormal egg is possible when the specimen occurs in the same clutch with other eggs that contain identifiable embryonic bones, for example, the Cretaceous turtle egg described in this study. The titanosaur eggshell from the Auca Mahuevo site also allows reasonable taxonomic assignment because the inner eggshell microstructure is identical to eggs containing embryos from the same egg-bearing stratum (Chiappe et al., 1998; Chiappe et al., 2001; Salgado et al.,

2005). The inner eggshell layer of the abnormal hadrosaur specimen from the Willow Creek anticline also exhibits identical microstructure as hadrosaur eggs and eggshell associated with hatchlings from the same locality (Hirsch and Quinn, 1990). However, due to its presence in a different subfacies (Lorenz and Gavin, 1984), the specimen provides less confident identification.

Cladistic methodology may also prove useful in referring abnormal eggs at higher taxonomic levels. For example, I interpret ES-127 (Fig. 3.5) as an abnormal specimen of prismatic eggs recently reported from the same locality. The calcite shells of the new eggs consist of three structural layers: mammillary, prismatic and external (Figs. 3.5A, B). Although diagenetically altered, similar structural features are also present in the abnormal specimen. Normal eggs from this site often display detachment of the external layer, similar to the separation of the inner and outer laminae of the abnormal eggshell described here. Because these new prismatic eggs lack embryonic remains, their phylogenetic affinity was assessed by including the egg in a cladistic analysis of egg characters, allowing assignment to an avian or non-avian theropod (Varricchio and Jackson, 2004a). However, diagenetic alteration of important structural features in the abnormal specimen requires a more cautionary assignment of the eggshell to this theropod taxon.

Magnitude and Timing of Stress

Microstructural features of eggshell provide information regarding stress events and the stage of egg formation at which those events occurred (Hughes et al., 1986; Solomon et al., 1987; Solomon, 1997; Jackson and Varricchio, 2003; Jackson et al.,

2004). The normal microstructure in the hadrosaur and titanosaur inner eggshell layers suggests that the stress that resulted in egg retention most likely occurred immediately before the time of normal oviposition. With the exception of the titanosaur eggshell from Auca Mahuevo egg bed 3, the outer abnormal layers in all specimens display aberrant calcite or aragonite microstructure. These abnormalities include one or more of the following features: absence of nucleation sites, additional shell fragments intercalated with membrane, decreased eggshell thickness, and atypical ornamentation. This suggests that the stress which produced egg retention by the female effected shell deposition during formation of the outer, abnormal eggshell layer. As documented in modern birds, stress of sufficient magnitude may influence eggshell structure for a considerable length of time (Solomon, 1997; Jackson and Varricchio, 2003).

In contrast to the dinosaur eggshells, the inner eggshell layer of the Cretaceous turtle eggshell differs significantly in thickness, shell unit width, and ornamentation from normal eggs in the same clutch. The inner eggshell measures 290 μm in thickness, compared to the 676 μm thickness of normal eggs (Fig. 3.1A, B). This suggests that the physiological or environmental stress that produced the abnormality most likely occurred before or during an early phase of mineral deposition. The decreased shell thickness of the inner eggshell layer observed in the Cretaceous turtle egg is not typical of abnormal dinosaur eggs, nor has this morphology been documented in modern avian eggs. Modern turtles also display a greater range of abnormal egg morphology than reported in extant archosaurs (Erben et al., 1979; Ewert et al., 1984; Hirsch, 2001). The number of superimposed shell layers far exceeds that reported in fossil dinosaur eggs or living birds

(Solomon, 1997; Jackson and Varricchio, 2003; Hayes, 2005) and most likely reflect differences in reproductive anatomy (Ewert et al., 1984; Jackson and Varricchio 2003; Jackson et al., 2004).

Recognition of Egg Abnormalities in the Fossil Record

Study of abnormal fossil eggs from taxonomically referable specimens may elucidate questions regarding abnormal shell formation and the evolution of reproductive anatomy in modern amniotes. However, the usefulness of abnormal eggs for inferences of reproductive biology in extinct taxa requires differentiation of pathological conditions that result from biological processes (egg retention) from taphonomic processes which produce similar morphology. Here I identify six features of abnormal eggshells from this and the previous study (Chapter 2) that are useful for distinguishing these two processes in fossil material.

Morphological Features

Abnormal eggs may differ in microstructure, surface ornamentation, and shell thickness from other eggs in the same *in situ* clutch or eggs in the same stratum. For example, abnormal titanosaur eggshells from Auca Mahuevo typically exhibit robust surface ornamentation that consists of unusually wide (> 1 mm) and prominent tubercles on the eggshell surface (Fig. 3.4A). This unusual surface ornamentation provides contrast to normal eggs and facilitates identification in the field.

If eggs are recently exposed to weathering, the abnormal shell layer may still cover a substantial portion of the abnormal specimen. Multilayered fragments often occur

in the immediate vicinity of the clutch and are unusually thick compared to normal specimens. A 10-power hand lens typically allows identification of individual eggshell layers. If poorly cemented, however, the superimposed shells may separate. These shell layers are easily mistaken for normal but somewhat thinner eggshell. In rare cases such as PVPH 255 (Fig. 3.4A), the abnormal outer layer may exhibit identical thickness and structure to normal eggs, making their pathologic origin impossible to recognize if separation of the two layers occurs.

Without careful examination, pathological conditions may be overlooked in the field or laboratory. This is particularly true if the egg remains intact or if weathered, multilayered eggshells are no longer present at the site due to erosion and transport. For example, the closely superimposed eggshells of the abnormal Cretaceous turtle egg are not obvious in hand sample. Presumably this results from the thinness and irregular distribution of the pathological layer. The discovery of the abnormal condition resulted from routine microscopic examination of eggshells removed from randomly selected eggs. Further investigation revealed an apparent absence of embryonic remains in this egg, possibly due to suffocation of the embryo during early development. Careful inspection of all eggs in a clutch, therefore, may yield abnormal specimens that are undetected in the field.

Eggshell Orientation

Stacked eggshells that result from lithostatic compaction and pedogenic processes are easily misidentified as a pathological condition (Sabath, 1991; Carpenter, 1999; Hirsch, 2001). Egg bed 4 of the Auca Mahuevo locality often contains abundant, highly

compressed, and strongly cemented, crushed eggs. Some of these egg amalgamations are comprised of 24 or more eggshell layers, with alternating concave-up and concave-down shell fragments. These specimens most likely represent collapsed and compressed egg clutches. To rule out a taphonomic cause often requires examination, using scanning

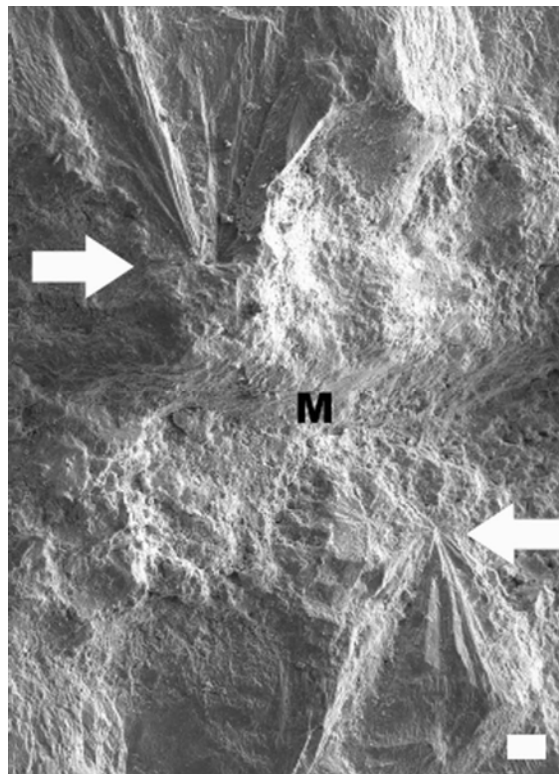


Figure 3.6. Titanosaur eggshells. SEM image of mammillary-to-mammillary contact; arrows show nuclei, separated by sediment matrix (M). Scale bar = 10 μ m.

electron microscopy. The unusually thick specimen displays mammillae to mammillae contact and a thin layer of sediment separates the shell fragments (Fig. 3.6), a feature less apparent under the lower magnification of light microscopy. If strongly cemented,

stacked layers of eggshell that result from hatching are often difficult to distinguish from a pathological condition, particularly if little or no sediment separates the eggshell layers (Carpenter, 1999; Grellet-Tinner et al., 2006). This phenomenon may have contributed to the perception of an increase in abnormal eggshells towards the Cretaceous-Tertiary boundary in southern France, as reported by Erben et al., (1979).

Eggshell Membrane

Although rare at most localities, many abnormal titanosaur eggs from Auca Mahuevo display permineralized protein membrane between multiple eggshell layers (Fig.3.4B). In the absence of *in situ* eggs, this membrane provides evidence of a biologic origin in isolated multilayered eggshell fragments. Comparison of membrane degradation in extant eggs to fossil specimens also provides clues to the diagenetic history and timing of permineralization (Bravo, 2003). For example, a modern avian egg from a recent study remained intact for over six months before damage revealed the two superimposed eggshell layers (Jackson and Varricchio, 2003). The albumin evaporated prior to damage and the membrane of the inner eggshell was absent or extremely desiccated and compressed. In contrast, the inner and outer eggshell layers protected and preserved the three dimensional morphology of the protein membrane fibers between the eggshell layers (Jackson and Varricchio, 2003, p. 701, Fig. 12B,C). Once broken, the multiple eggshell layers separated within days to weeks due to desiccation of the membrane.

Similarly, the abnormal fossil eggs often lack membrane at the base of the inner eggshell, while preserving permineralized membrane between the abnormal, multiple

eggshell layers (Fig. 3.1; 3.4). Preservation of the three dimensional, open mesh-like structure of the membrane protein (Fig. 3.4D) suggests that rapid permineralization (possibly of intact eggs) occurred prior to egg crushing, sediment dewatering, and compaction. Compaction would most likely result in collapse of the soft tissue, with accompanying reduction of the open spaces within the protein structure.

Membrane may also contribute to low preservation potential of the Type I morphology present in PVPH 255 (Fig. 3.4A,B). Compared to Type II and III morphology (Chapter 2:fig. 2.7), the increased thickness of the membrane separating the eggshell layers results in a greater surface area exposed to desiccation once the egg is broken. This may result in more rapid separation of the superimposed layers. Because the two eggshell layers display identical thickness and microstructure as normal eggs, no evidence of an abnormal condition exists once separation occurs. The unusual diagenetic history that resulted in rapid replacement of soft tissue (i.e., eggshell membrane and embryonic skin) at Auca Mahuevo (Chiappe et al., 1998; Grellet-Tinner et al., 2004; Schweitzer et al., 2005) may explain why Type I morphology occurs at this site but remains unreported from other localities.

Some eggshells with Type I morphology (Fig. 3.3A,B) show significant replacement of membrane by authigenic minerals, yet preserve evidence of their biological origin. Radiating calcite spherulites at the base of the abnormal outer eggshell layer are laterally adjacent to one another and extend equal distances above the surface of the underlying, unweathered eggshell layer (Fig. 3.3A, B). In thin section, these spherulites produce distinctive pseudouniaxial crosses under cross polars. Their equal

and consistent distance above the underlying shell surface suggests that the outer eggshell layer resulted from egg retention by the female dinosaur, rather than compaction and cementation of stacked eggshell fragments.

Topographic Conformation of Eggshell

The Cretaceous turtle, hadrosaur, and titanosaur specimens display an abnormal layer that is “seeded” directly to the retained egg (Figs. 3.1C; 3.2A-C; 3.4B). This additional eggshell layer lacks nucleation sites at the base of the shell and the inner surface of the outer abnormal layer closely follows the contour of the underlying egg ornamentation. In normal titanosaur eggshell, crystal expansion from adjacent nucleation sites truncates mineral growth, giving the shell units their distinctive shape and surface ornamentation. In the abnormal titanosaur specimen the absence of nucleation sites at the inner edge of the additional eggshell layer allows unimpeded lateral expansion of the calcite spherulites. This results in the broad (> 1 mm), rugose surface ornamentation that is easily recognized in the field (Fig. 3.3B).

Optical Continuity

The radiating calcite crystals in the shell units of the inner layer act as a template for continued radial calcite growth into the abnormal outer layer. Calcite crystals in the superimposed eggshells are often syntaxial (i.e. in crystalligraphic continuity) in some areas of the egg. The abnormal outer eggshell layer maintains the crystal orientation established in the underlying, lower eggshell layer. The extinction pattern extends across both eggshell layers simultaneously with rotation of the stage under crossed nicols,

showing the crystals to also be in optical continuity across the inner and outer eggshell layers.

To test whether similar optical effects can result from eggshell fragments superimposed during lithologic compaction, two fragments of the same eggshell were glued together, one upon the other, embedded in polyester resin, thin sectioned, and examined under a petrographic microscope. Extinction sweeps through calcite crystals of both layers, creating the illusion of optical continuity across multiple eggshell layers. Therefore, caution should accompany use of optical continuity since extinction pattern alone is not diagnostic of a pathological condition. Accurate interpretation requires additional supporting features.

Surface Morphology

Eggshells that exhibit prominent surface ornamentation (e.g., turtle, hadrosaur, and titanosaur) are unlikely to exhibit a smooth transition between the “stacked” shell layers that result from taphonomic processes. For example, normal prismatic theropod eggs from the Sevenmile Hill locality exhibit raised nodes on the egg surface (Varricchio and Jackson, 2004a). Therefore, the smooth contact between eggshell layers in ES-127 provides convincing evidence of their pathological nature.

In contrast, misidentification of superimposed eggshells as a pathological condition may occasionally occur when an egg lacks nodes or tubercles on the shell surface. For example, *Troodon* eggs from the Two Medicine Formation of western Montana display a smooth surface and therefore stacked eggshell fragments may

resemble a pathological condition (Fig. 3.7). However, the *Troodon* egg illustrated here occurred in a paleosol with numerous slickensides that resulted from pedogenic and

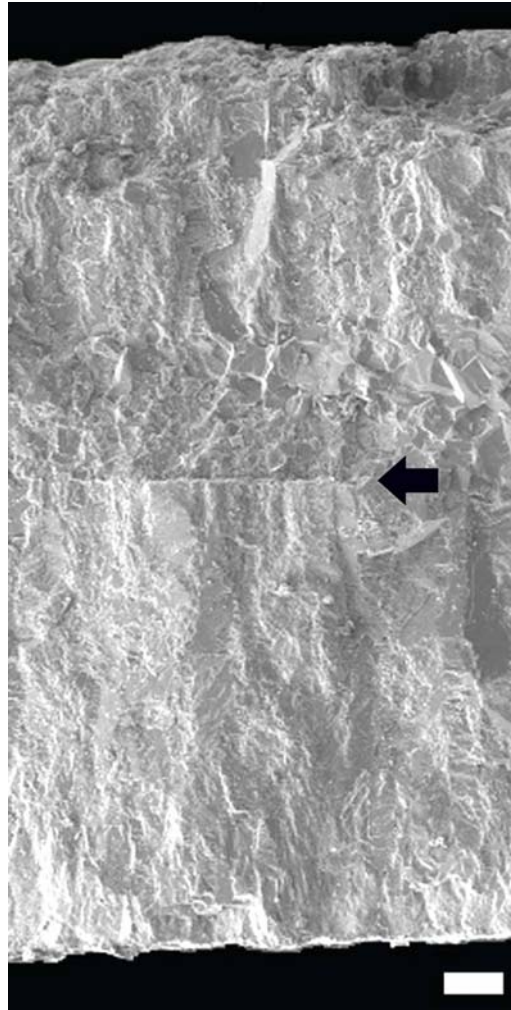


Figure 3.7. *Troodon* eggshells. SEM image shows two superimposed *Troodon* eggshells resulting from taphonomic juxtaposition. Black arrow indicates contact between superimposed fragments. Scale bar = 100 μm .

tectonic deformation. Soil movement or tectonic sheering most likely produced the superimposed eggshell fragments, rather than egg retention by the female theropod. Examination of additional fragments from the same egg reveals normal calcite

microstructure; the egg also contains well-ossified embryonic remains. Since an additional, abnormal eggshell typically occludes pores and limits embryonic gas exchange, the presence of ossified bone suggests embryonic death was unrelated to egg pathology. However, most pathological specimens reported in the literature represent isolated fragments rather than *in situ* eggs. Therefore, distinguishing taphonomic from biologic processes may prove more difficult in eggshells that lack surface ornamentation.

Other Structural Relationships

Eggshell microstructural characteristics often provide supporting evidence for a biological origin of multiple eggshell layers. For example, truncation of pores, intercalation of shell fragments with membrane (Fig. 3.4B), and absence of sediment between superimposed eggshell layers further support a biological interpretation of multilayered specimens.

Conclusions

The absence of embryonic remains in abnormal fossil eggs is problematic for taxonomic identification. However, reasonable taxonomic assignment can be made under the following conditions: 1) abnormal, multilayered specimens occur in clutches containing normal eggs containing identifiable embryonic remains; 2) abnormal eggshell exhibits identical microstructure to eggs containing embryos from the same stratum; and 3) cladistic analysis allows referral of a normal egg of the same oospecies to a particular clade.

Site taphonomy represents a critical component in taxonomic assignment and inferences of physiology, anatomy and paleoenvironment of extinct taxa. The following characteristics allow differentiation of biologic from taphonomic processes that result in similar multilayered eggshell morphology. The features are listed in the order of decreasing reliability; the more features present, the greater the confidence in an interpretation of a pathological condition in fossil eggs.

1). Multiple eggshell layers are separated by permineralized eggshell membrane, recognizable under SEM. Additional support for this interpretation is provided by radiating spherulites of calcite that emanate from nucleation sites contained within membrane that separates the eggshell layers. To the contrary, a pathological condition may be ruled out when sediment separates the eggshell layers or when mammillary-to-mammillary contact occurs between shell layers.

2. The base of the outer abnormal eggshell layer closely conforms to the unweathered surface ornamentation of the underlying egg when viewed in thin sections or SEM.

3. Structural relationships often provide supporting evidence for a biological condition in the following circumstances: pore truncation by the abnormal shell, intercalation of shell and membrane, smooth contact between superimposed shell layers when the egg normally exhibits ornamentation, and nucleation sites that occur at a consistent height above the upper surface of the inner eggshell.

4. Optical continuity exists between the calcite crystalline structure of the superimposed eggshell layers and the extinction pattern crosses both layers

simultaneously in some areas of the shell when the stage is rotated under crossed polars. However, evidence provided by this characteristic must be supported by additional structural features.

CHAPTER 4

TAPHONOMY OF THE PINYES FOSSIL EGG LOCALITY,
COLL DE NARGÓ, LLEIDA PROVINCE, SPAINIntroduction

The south-central Pyrenean mountain range of northern Spain contains excellent outcrop exposures of Upper Cretaceous terrestrial strata and a rich fossil record of dinosaur faunas (Fig. 4.1; Galbrun et al., 1993; Ardèvol et al., 2000; Lopez-Martinez et al., 1998, 2001; Lopez-Martinez, 1999; Vila et al., 2006). From the mid 1950s, discoveries in the Tremp basin in Catalonia, northern Spain included dinosaur bones, track horizons, and fossil eggs (Lapparent, 1958; Erben et al., 1979; Lopez-Martinez, 1999 and references therein). While paleontologists focused research primarily on dinosaur tracks and osteological remains, commercial collectors exploited the much more abundant fossil eggs.

Beginning in the late 1970s, the importance of southern Pyrenean basin for studies of the Cretaceous extinction event brought renewed interest in the fossil egg localities. For example, Erben et al. (1979) concluded that paleoenvironmental degradation produced abnormalities in sauropod eggs from the upper Tremp Formation, thereby contributing to gradual sauropod extinction prior to the Cretaceous-Tertiary boundary. In contrast, eggshells collected from the same formation near the village of Coll de Nargó allegedly provided evidence that sauropods survived into the Tertiary (Ashraf & Erben, 1986). Radiometric dates for the Tremp Formation, however, now

support a Late Cretaceous age (Martinez-Lopez, 1999 and references therein).

Numerous localities are present within the Tremp Formation and yield important information on egg microstructure, paleobiology, and biochronology (Kohring, 1989, Moratalla 1993; Sanz et al., 1995, Sander et al., 1998, Peitz, 1999; 2000; Lopez Martinez, 1999, 2003; Bravo et al., 2005). *In situ* eggs and clutches, for example, were mapped from at least five localities: Basturs and Coll de Nargó (Sanz and Moratalla, 1997; Peitz, 1999, 2000); Suterranya (Ardévol et al., 1999); Biscarri (Martinez-López et al., 2000); and Faidella (Bravo et al., 1999). In 2003, a new megaloolithid nesting locality was discovered in outcrops approximately 20 km west of the village of Coll de Nargó, in Lleida Province, Spain. The Pinyes locality, named for an abandoned farmstead near the site, occurs within a heavily forested tributary drainage that flows southeast from the mountains and joins the Riu Sallent. Excavation of this site began in 2005 with a collaboration among researchers from the Institut de Paleontologia of Sabadell, the University Sutonoma of Madrid, and myself. My responsibilities on this project included quarry mapping and taphonomic data collecting.

Geologic Setting

The Pyrenees are an Alpine fold-thrust belt extending east to west along northern Spain and southern France (Fig. 4.1A). The orogen formed as a result of Late Cretaceous to early Miocene crustal contraction, due to the collision of the European and Iberian plates (Ardévol et al., 2000; Vergés et al., 2002). The South Pyrenean Zone

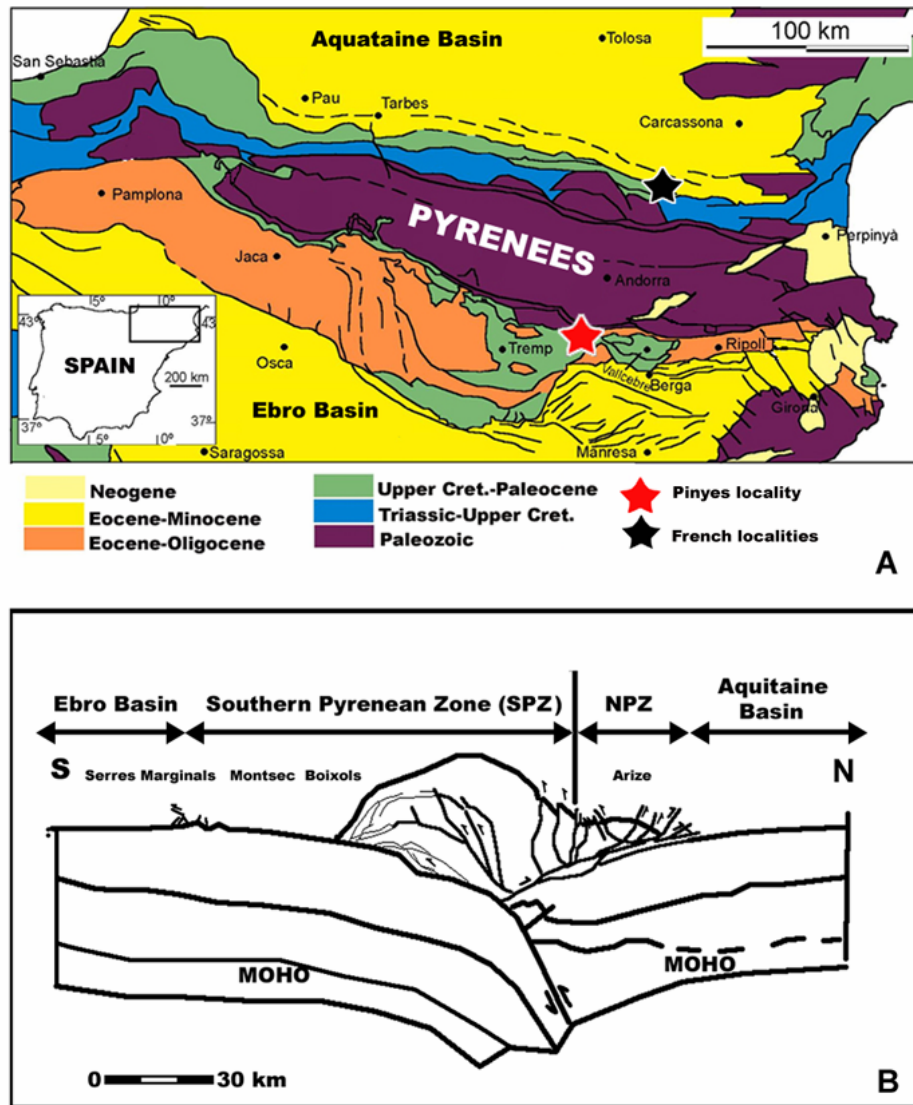


Figure 4.1. Location and geology of the Pinyes locality. (A) Geologic map of the Pyrenees; (B) schematic cross section. (A, courtesy of O. Oms; B, modified from Martin-Chivelet et al., 2002).

(SPZ) represents the former north Iberian continental margin, while the North Pyrenean Zone (NPZ) was once the European continental margin (Martin-Chivelet et al., 2002).

This convergent zone was superimposed on structures of the former divergent margin, related to the opening of the Bay of Biscay during Late Jurassic to Early Cretaceous time.

After the initial stages of collision, two major foreland basins (northern Aquitanian and southern Ebro basins) developed, one on either side of a central double-wedge of basement rocks (Capote et al., 2002). The southern thrust system produced various structural features including the Boixols, Abella, and Nargo Anticlines and the Tremp, Coll de Nargo, and Vallcebre synclines, among others (Nagtegaal et al., 1983; Díaz Molina 1987; López-Martínez et al., 2000). These structures now expose dinosaur bones and extensive strata containing fossil eggs.

Sediments that accumulated within the southern, east-west trending basin record the maximum extension of the Cretaceous sea in the Pyrenean domain (Martin-Chivelet et al., 2002). The foredeep filled with 3400 m of basinal turbidites and pro-delta shales, followed by deltaic deposits (López-Martínez et al., 2001). The Arén Sandstone, interpreted as the deposits of a barrier island-lagoon system (Nagtegaal et al., 1983; Díaz Molina 1987), is transitionally overlain by the fluvial deposits of the Tremp Formation (Soler-Gijón and López-Martínez, 1998; López-Martínez et al., 2001 and references therein). Both formations record regression from marine to lagoonal setting, and finally to continental environments.

The Tremp Formation

Continental Upper Cretaceous-lower Paleocene rocks comprise the Tremp Formation in the southern Pyrenees (Mey et al., 1968). Historically referred to as the

“Garumnian” facies, the Tremp Formation consists of two major units (Rosell et al., 2001). The lower unit varies from grey to red, attains a thickness of 500 m, and consists of thin intercalated coals, calcareous mudstones, and limestone beds. These deposits are interpreted as sediment that accumulated within lagoon and marsh environments (Díaz Molina, 1987; Soler-Gijón and López-Martínez, 1998; López-Martínez et al., 2001; Sander per. comm.). Towards the upper portion of this unit, the color of these facies changes from gray to red up-section. The mudstone intercalates with small to medium-size sandstone bodies (< 4 m thick); both facies exhibit caliche nodules, mottling, and evidence of extensive bioturbation. These rocks are interpreted as sediment deposited within lagoon, marsh, or fluvial environments (Díaz Molina 1987; Plaziat, 1981). The upper Tremp Formation includes the lacustrine Vallcebre limestone, in addition to mudstone, sandstone, and conglomerate deposits representing a variety of continental depositional environments.

Dates purposed for the lower Tremp Formation typically vary from late Campanian to entirely Maastrichtian, while the upper portion of the formation is thought to be early Paleocene in age (Fig. 4.2) (Plaziat 1970, Liebau 1971; Erben et al., 1979). However, sequence stratigraphy correlation (Ardèvol et al., 2000) and magnetostratigraphy (Galbrun et al., 1993; Oms and Canudo, 2004; Oms et al., in press) suggest diachronous relations within the Tremp Formation deposits. The Pinyes locality falls within the middle portion of the lower Tremp Formation, approximately at the transition between the gray and red mudstone facies.

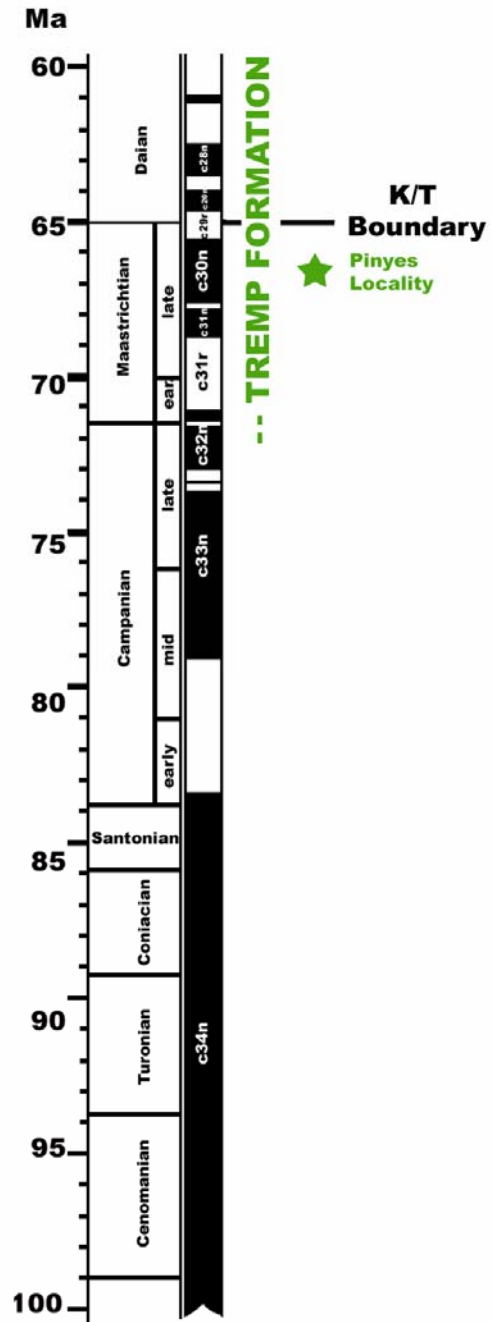


Figure 4.2. Time scale showing approximate location of Pinyes Locality. (Modified from Lopez Martinez, 2003).

Site Selection and Data Collection

A study area was selected after field reconnaissance, based on the presence of well-defined *in situ* eggs (Fig. 4.3). Chisels and hand tools were used to expose the eggs and pneumatic jackhammers facilitated removal of egg clutches. Ten sites containing



Figure 4.3. The Pinyes study area. Numbers identify sites containing one or more egg clusters. Note that the beds dip 30°; north is to the lower left corner of photograph.

one or more clutches were mapped using a metric grid and graph paper. Taphonomic data recorded for each site included the state of egg preservation, number and size of eggs, and the eggshell orientation within eggs exposed in cross-section. Collection of

multiple data points around each egg circumference by Trimble Total Station allowed high-resolution mapping of each specimen. *AutoCAD*® or *Carrara Pro*® software provided a three-dimensional model of five sites, based on over 800 x, y, z coordinates. However, due to complications in the field, reconstructions are not yet available for all sites. Excavated egg clusters were covered with aluminum foil and surrounded by cardboard panels prior to the application of a 2-part polyurethane that provided protection during transport (see <http://www.sprayfoam.com>).

A stratigraphic section was drawn for the 18 m-thick interval that contains four egg-bearing horizons, and sedimentological features documented using an Olympus C-750 digital camera. Strike and dip direction were noted on the map. Four rock samples were collected from the freshly exposed surface of a 0.5 m-thick interval immediately underlying an egg cluster at site 17E05 and labeled with arrows indicating original orientation. The samples were analyzed using Scintag X1 x-ray diffractometer (XRD). Eggshells were removed from an egg at site 18E02 and prepared as described in Methods and Materials in Chapter 1.

Results

Sedimentology and Facies Analysis

The 18 m-thick measured stratigraphic section consists of three lithofacies: (A) laterally extensive, massive calcareous silty mudstones, (B) massive, very fine- to fine-grained sandstone bodies, and (C) a parallel stratified, medium to coarse-grained sandstone (Fig. 4.4). Repeated intervals of facies A and B occur within the lower 10.5 m

of the section, with the thickness of the sandstone units generally decreasing in an upward direction. An erosional contact separates the uppermost 6.5 m-thick mudstone from the overlying medium to coarse-grained sandstone (facies C). This uppermost unit preserves primary sedimentary structures.

Facies A. This facies consists of reddish brownish to chocolate-brown, silty mudstone; the weathered exterior appears red in color. Calcareous cementation varies within facies A, with some intervals exhibiting more pronounced induration. While comparable in textural characteristics to the less indurated portions of the mudstone facies, these strata are resistant, recognizable in outcrop, and laterally continuous throughout the study area. Ubiquitous bioturbation characterizes facies A, and the massive mudstone units retain no evidence of sedimentary structures. Extensive mottling varies in color from yellow-brown to blue-gray or gray (Fig. 4.5A,B). Small (~ 1.0 cm), dispersed calcareous nodules occur within most mudstone facies. Approximately 8.5 to 10.3 m above the base of the section, these nodules increase to ~1.5 cm in diameter. In contrast, the mudstone unit from 10.6 to 14.8 m shows a marked absence of calcareous nodules (Fig 4.4). Increasingly abundant and slightly larger caliche nodules (2.0 – 2.5 mm) occur from 14.8 m to the erosional contact with the overlying sandstone, 17 m above the base of the section.

Tremp Basin Sequence

Pinyes Egg Locality
Coll de Nargo, Spain

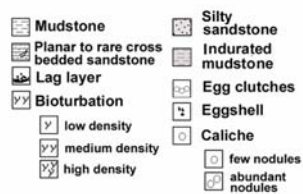
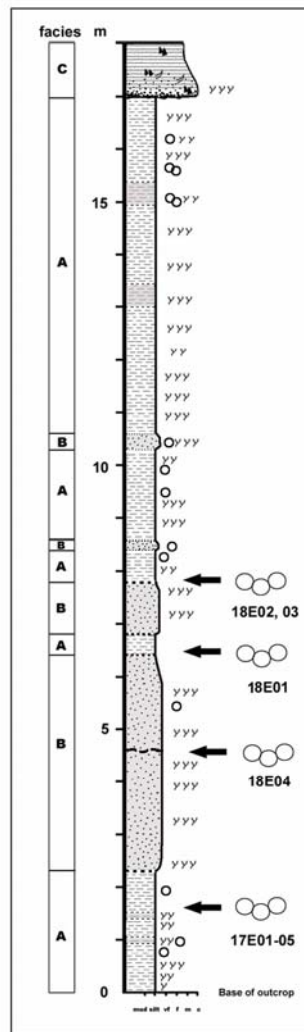


Figure 4.4. Stratigraphic section showing 4 egg- bearing horizons and facies decription

Facies B. The thickness and continuity of these massive, highly bioturbated, very fine to fine-grained sandstones vary laterally, with units generally thinner in an upward direction within the section. Contacts between facies A and B are gradational, and the two facies display similar color, texture, and extensive bioturbation. The sandstone units often include small (~1 cm) calcium carbonate nodules, similar to those present in most mudstone facies.

Facies C. Approximately 17 m above the base of the section, an erosional contact separates the final mudstone in the sequence from a well cemented, fining-upward, medium to coarse-grained, poorly-sorted, dark brown sandstone. Exposure of Facies C is limited within the study area. Small carbonate nodules, mud rip-up clasts, and abraded eggshell fragments occur at the base of the unit. Minor bioturbation occasionally disrupts the sedimentary structures, which include parallel stratification, rare cross stratification, and faint ripple cross-laminations. Moderately abundant fossil eggshell; macerated plant remains; and vertical to subvertical, bifurcating, three-dimensional pedotubules occur throughout the sandstone.

Pedogenic Features

Facies A and B exhibit extensive bioturbation, macroscopic fine root traces, sparse organic material, and abundant overlapping, branched and unbranched pedotubules (Fig. 4.5). Sparse, 20 cm-long corkscrew-shaped rhizoliths taper in a downward direction and exhibit thin branches that extend laterally from the main body of the structure (Fig. 4.6). Larger pedotubules are typically simple, unlined features with



Figure 4.5. Bifurcating tubules with gley mottling in red mudstone. Note egg in the lower right corner of the photograph.

red or blue-grey colored sediment fill that resembles the host rock in texture. In facies A and B, these structures often show complex cross-cutting relationships (Fig. 4.7). For example, grey reduced zones contain red pedotubules, that are in turn intersected by grey or yellow-orange tubular structures of relatively low morphological diversity. The vertical to subvertical features display sharp or diffuse boundaries that occasionally display more intense red color along the edge. In cross-section, they appear circular to



Figure 4.6. Corkscrew-shaped rhizolith

elliptical in shape. Some cylindrical structures exhibit moderately arcuate, poorly to reasonably well-defined meniscate backfilling (Fig. 4.8A,B). These meniscate structures measure 0.4 - 2.2 cm in diameter and 5 cm or more in length; some exhibit a pelleted

appearance in longitudinal cross-section (Fig. 4.8A). These features occur both within the sediment-filled fossil eggs and the surrounding mudstone. Occasionally, a tubule

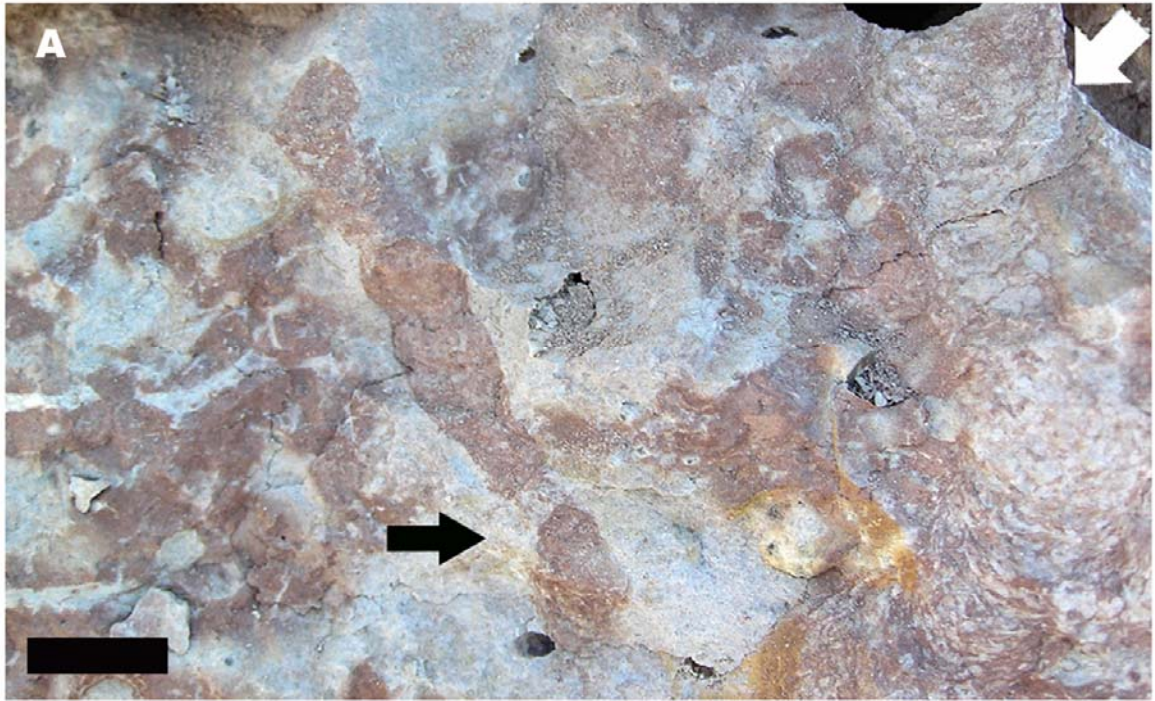


Figure 4.7. Complex intersecting pedotubules. Black arrow indicates cross-cutting relationship of structures, and white arrow shows meniscate burrow. Scale bar equals 1 cm.

changes direction at the base of an egg and follows the contour of the fossil specimen (Fig. 4.8C). An egg inadvertently removed from an egg cluster displays a sediment-filled pedotube that extends from the mudstone below the specimen into the egg interior (Fig. 4.9). When intersecting the eggs, the structures often produce minor disruption and displacement of the eggshell.

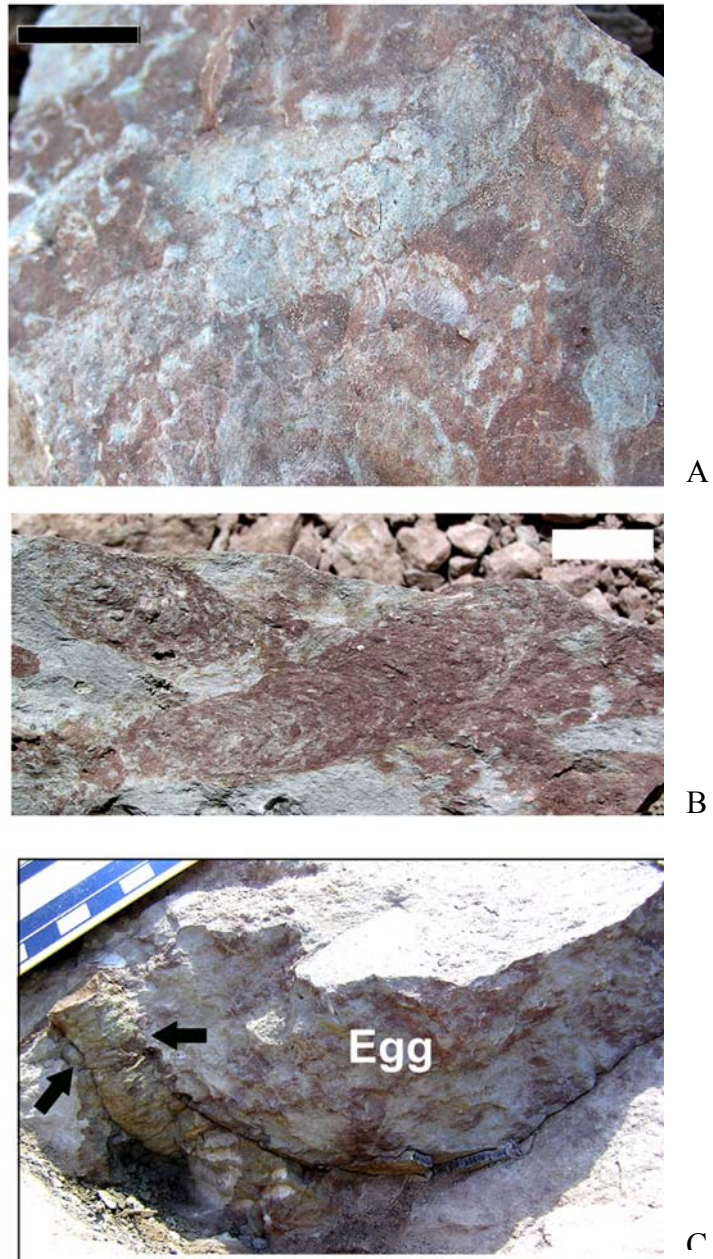


Figure 4.8. Meniscate burrow and egg. (A) Mensicate burrow exhibiting a pelleted appearance; (B) cross cutting burrow with arcuate structure; (C) Black arrows indicate burrow that follows egg contour. Note similar mottling inside egg.



Figure 4.9. Egg inadvertently removed from a clutch during excavation. Note lower surface, relative to bedding plane, exhibits tubular structure.

Site Taphonomy

The degree to which specimens were exposed in the field varies; therefore, egg dimensions represent estimates that may change with specimen preparation. Although the following description pertains primarily to site 17E05, most egg clusters exhibit similar taphonomic characteristics, unless otherwise noted.

Egg Horizons. Strata within the study area dip steeply to the north at 30°, contributing to rapid erosion of the tilted, fine-grained rocks (Figs. 4.3). The egg-bearing layers are laterally traceable for approximately 1 km or more, until covered by vegetation. At least four egg horizons occur within the measured section, at approximately 1.5, 5.0, 6.2 and 7.5 m above the base of the outcrop (Fig. 4.4). Egg clusters examined for the study include five sites from facies A (17E01-17E05) in the

lower three meters of the section, and one (18E04) from the middle of the overlying unit, facies B. The remaining three sites (18E01-18E03) occur at the upper contacts between facies A and B units, at 6.5 and 7.75 m above the base of the section. Although 18E01—03 occur at the contact between facies A and B, the gradational character and extensive bioturbation of both units prohibits definitive recognition of nest structure such as a bowl-shaped depression containing the eggs.

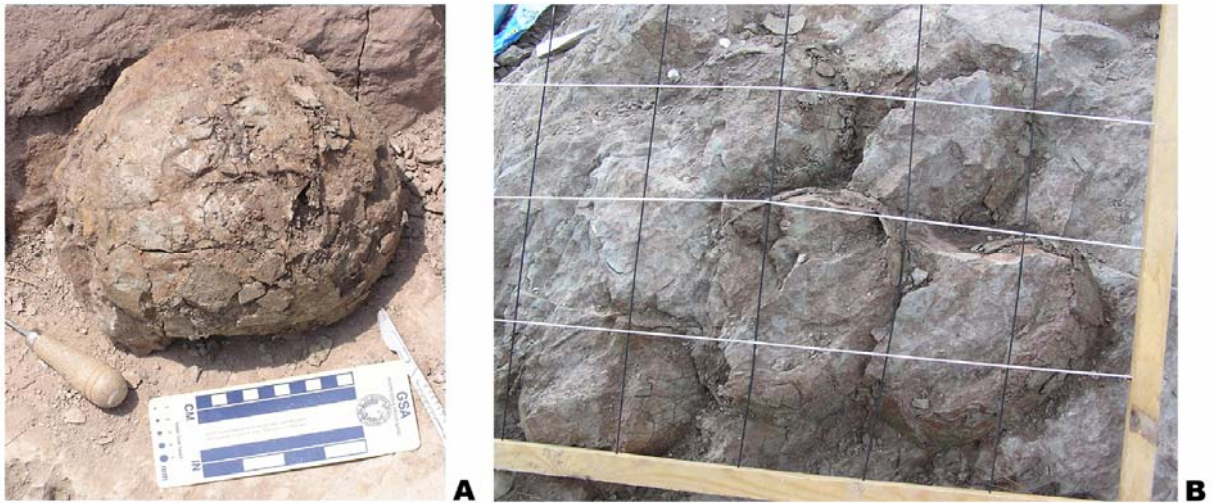


Figure 4.10. Pinyes eggs. (A) Round egg from site 17E05; (B) elliptical eggs from same site.

Eggs and Egg Clusters. Most eggs are incompletely preserved (Fig. 4.10), round to subround, and vary between 16 to 24 cm in diameter. Eggs within the clusters occur in a random arrangement in two, possibly three superimposed layers, relative to the bedding plane. Some specimens exhibit an ellipsoidal shape (Fig. 4.10B), and the mean long axis orientation of the eggs ($n=41$) is 42° and therefore consistent with tectonic stress in the region (Figs. 4.11; Vila per. comm.). Eggs typically occur in close contact with one another and at least four sites (18E01, 18E02, 18E04, 17E04, 17E06) reveal superimposed

specimens. Five sites (18E01, 18E02, and 18E04; 17E04 and 17E 05) are known to contain egg clusters that include one to possibly four whole eggs.

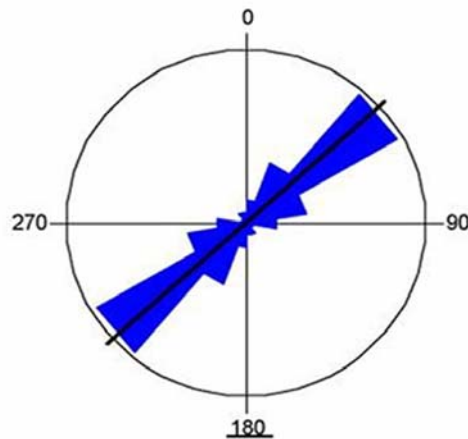


Figure 4.11. Rose diagram showing long axis egg orientation.

Moderate to abundant eggshell debris typically characterizes the immediate area surrounding the clutch, and large eggshell fragments (>6 cm) occasionally occur within the adjacent mudstone. These large pieces may represent the upper portion of a hatched egg. Some eggs exposed in cross-section reveal numerous fragments within the mudstone matrix that fills the egg interior, typically concentrated in the lower 1/3 of the specimen. The majority of these eggs ($n = 59$) contain one or two layers of broken eggshell fragments: 15% of these eggshell fragments occur concave downward, 53% concave upward, and 32% show mixed orientations. However, in contrast to most specimens, a cluster at site 16E02 includes one or more crushed eggs that consist of 9 to 10 superimposed eggshell layers (concave upward and downward). The layers comprising these egg portions are separated by sediment and thus differ from the other 10 eggs within the same cluster.

Other Fauna. Aside from the fossil eggs, no additional vertebrate remains (i.e., embryonic, juvenile, or adult bone) of any taxon were documented within the study area. The invertebrate fauna at the site includes a small (< 1 cm) unidentified, poorly preserved, high-spiral gastropod and a 3.5 x 2.5 cm internal mold of a bivalve. These specimens occurred within the mudstone facies, approximately 10 cm below specimens at site 17E05.

Petrographic and Other Analyses

Analysis of the well indurated mudstone and three additional samples removed from a 0.5 m interval below 17E05 reveals kaolinite clay, quartz, calcite, hematite, magnetite, and a notable absence of feldspars in all samples. Petrographic thin sections of the indurated layer also contain abundant clay, very fine quartz grains, minor amounts of opaque mineral with calcite overgrowths, sparse organic matter, and tapering pedotubules that contain sparry calcite. The quartz grains are angular to subrounded and often exhibit a cloudy appearance. The gradational contact, distinguishable in thin section, occurs between the indurated layer and the underlying mudstone. The very fine to fine-grained quartz grains decrease to silt-grade quartz in a downward direction. This subtle change in lithology and apparent increase in calcium carbonate cement represents the primary differences between the indurated layer and less indurated mudstone.

Eggshell Microstructure. Eggshells vary in thickness from 2.4 to 2.9 mm (Fig. 4.12). Thin section and SEM images of shell fragments reveal a single structural layer of

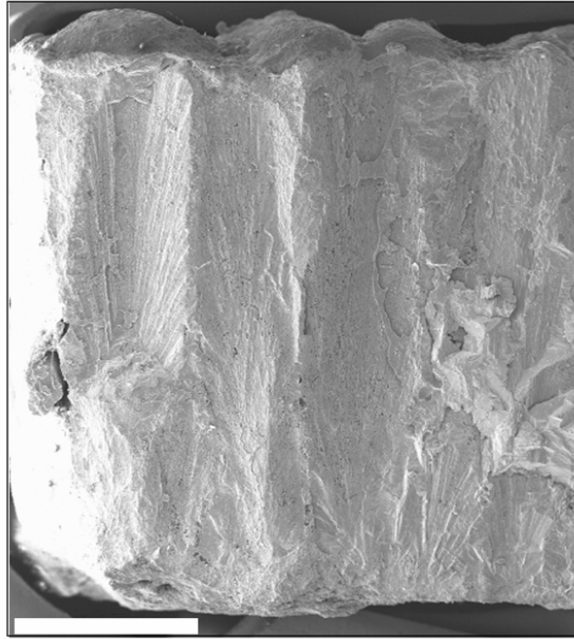


Figure 4.12. SEM of Pinyes eggshell, *Megaloolithus siruguei*. Outer shell surface is toward the upper portion of the image.

calcite. Radiating spherulites extend from nucleation sites at the inner shell surface until truncated by crystal growth from adjacent nuclei, forming the slightly flared, narrow shell units. The distance between adjacent nuclei is approximately 0.59 mm and shell unit diameter at mid-point of the shell thickness measures 0.59 mm to 0.62 mm. Shell units terminate in tuberculate surface ornamentation. Radial thin sections reveal arched accretion lines within the shell unit and relatively straight pores. Branching pore canals are also present in some specimens, and tangential thin sections show abundant, evenly distributed, sparry calcite-filled pore structures.

Discussion

All eggs at the Pinyes locality are referable to oospecies *Megaloolithus siruguei*, based on size, tuberculate surface ornamentation, shell thickness, and eggshell microstructure. Eggs and eggshells of this oospecies are well documented from various sites in northern Spain and southern France (Vianey-Liaud & Lopez-Martinez 1997, Bravo et al., 1999, Lopez-Martinez 1999, Lopez-Martinez et al., 1999, Panadés I Blas 2002, 2005; Sander per. comm.). Egg horizons within the Tremp Formation were once continuous within the southern foreland basin that developed as a result of the collision of the European and Iberian plates. The uplift of the Pyrenees from Late Cretaceous to Miocene time produced structural deformation of the egg-bearing strata. The deformation that characterizes the Pyrenees today impacts interpretations of dinosaur reproductive biology. In the following section, I discuss the geologic and taphonomic attributes of the nesting locality and their influence on interpretations regarding the reproductive biology of the egg-laying taxon.

Depositional Setting of the Nesting Sites

The three facies present within the study area (Fig. 4.4) comprise a typical overbank sequence in a fluvial system (Walker and James, 1992). The sequence consists of highly bioturbated, calcareous mudstone (facies A), incised by laterally discontinuous, bioturbated, small to medium-sized (< 5 m thick) channel fill or crevasse splay deposits (facies B). The sequence is capped by a fining upward, medium to coarse-grained, primarily parallel-stratified sandstone (facies C). Coarse material reworked from the

flood plain (e.g., eggshell, caliche nodules, mud chips) floors this channel deposit. The poorly preserved ripples are most likely superimposed on the surface of migrating dunes by way of accretion, producing the fining upward sequence in facies C (Walker and James, 1992). In modern fluvial systems, fine-grained sediments such as mud, silt and very fine sand are carried as suspension load by rivers and indicate deposition in distal floodplain areas (Miall, 1996). These depositional environments often include abandoned areas of normally active channels.

Bioturbation within the Pinyes sequence varies in intensity. According to the bioturbation index developed by Tucker (2003), facies C preserves distinct bedding and represents grade 2 sediments, exhibiting ~5-20% bioturbation. In contrast, facies A and B correspond to grade 6, characterized by the absence of primary sedimentary structures, thus representing 95-100 % bioturbated sediment. These extensively bioturbated, fine-grain mudstones contain abundant *Spirographites ellipticus* traces (Fig. 4.7; 4.8A-C). Mayoral and Calzada (1998) interpret these meniscate back-filled structures from the Tremp Formation as sediment reworking by arthropods such as crustaceans, spiders, beetles, or other insects. This extensive sediment reworking typifies many Late Cretaceous terrestrial localities. The evolution of angiosperms and resulting explosion of insects in the Cretaceous correspond to an increase in terrestrial, non-marine crustaceans and their traces in this time period, compared to pre-Cretaceous deposits (Walker and James 1992). The thick, red, clayey Pinyes profile also preserves bifurcating rootlets, 20 cm-long corkscrew-shaped rhizoliths (Fig. 4.5, 4.6), and drab-colored, tapering tubular structures that occasionally contain plant remains. The blue-gray color of these and other

traces results from chemical reduction of elements such as iron and manganese during anaerobic decomposition of organic matter remaining after burial of the soil profile (Retallack 2003).

The uniformity and extensive bioturbation of these fine-grained sediments and the absence of well-defined soil horizons or color banding at the study site (and in the middle Tremp Formation in general) suggest slow, incremental rates of deposition, where bioturbation and pedogenic processes kept pace with sedimentation (Kraus and Bown 1993; Tucker 2003). In modern environments, most bioturbation takes place shortly after deposition, at or just below the depositional interface; typically, this activity occurs within depths of a few tens of centimeters. While most non-marine biogenic structures are surficial and therefore ephemeral (Walker and James, 1992), the extensive and overlapping trace fossils and pedogenic features at the Pinyes locality indicate a stable land surface existed for an extended time period. This stability facilitated use of these well-developed soils for dinosaur nesting sites. Floodwaters, however, periodically inundated the floodplain, and suspension settling of fine-grain sediments filled the remaining portion of the hatched eggs, thereby resulting in their preservation in the rock record. Exhumation and exposure of the eggs may have resulted in subsequent erosion. The multiple egg-bearing horizons in the study area record the periodicity of these events.

The decreasing number and thickness of incised channels and crevasse sand lobes in the upper half of the stratigraphic section correspond to an absence of nesting horizons (Fig. 4.4). This may reflect less optimum conditions for nesting, possibly due to climatic change, a shift in depositional environments due to increased distance from the sediment

source, or changes in the water table. Alternatively, the absence of nesting horizons within this interval may reflect a preservational bias, lack of available resources for the adult, increased predation, or other unknown factors. Deposits of the uppermost three meters of the section accompanied increasingly arid conditions that produced more abundant and larger caliche nodules within the soil profile. Unlike the mudstone facies, soil development on the abandoned channel deposit was inadequate to obliterate most primary sedimentary structures.

Paleoclimate

In modern soils, the types of clays formed represent the product of weatherable minerals and are attributable to the amount of rainfall available to the soil (Retallack 2003). Soils of wet regions of low relief typically show enrichment in clay and fine-grained, weather-resistant minerals such as quartz, magnetite, and hematite. Organic acids produced by plants, microbes, and other soil-forming processes produce relatively acidic soils in these warm, wet environments (Retallack 1990). High leaching rates result in low ratios of less stable soil components such as feldspar and mafic minerals. Mudrocks of the Pinyes locality exhibit kaolinite, hematite, quartz, and magnetite, characteristic of soil development in warm, wet regions. This interpretation also corresponds well to paleoclimatic reconstructions by Scotese (2000) that suggest southern Europe experienced a warm temperate to warm tropical climatic regime in Late Cretaceous time (Fig. 4.13). These paleoclimatic reconstructions are based on soil types, palynomorphs, coal deposits, and other factors compiled from the published literature.

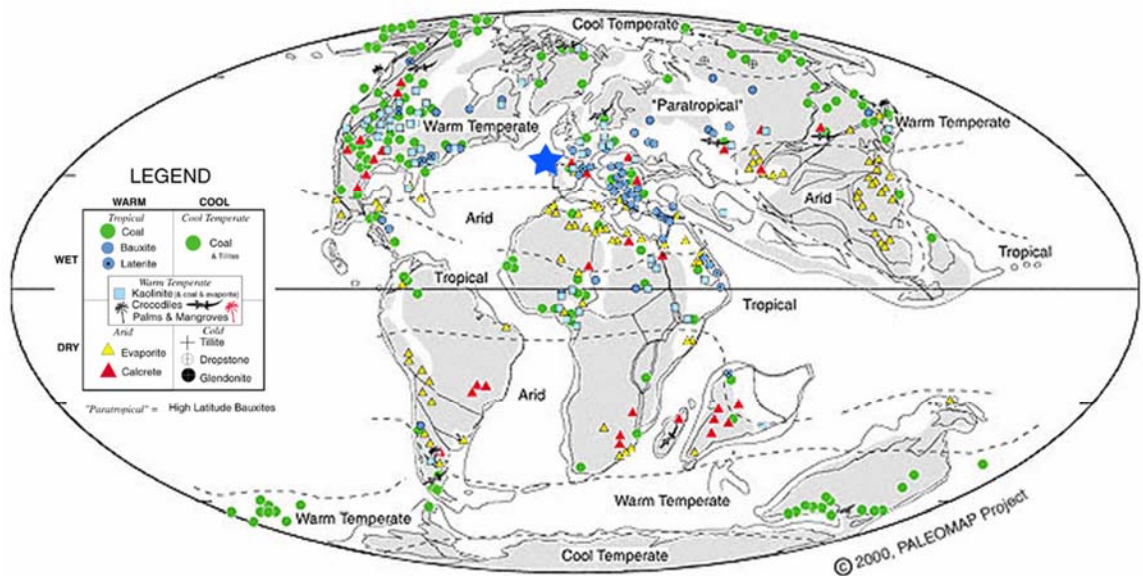


Figure 4.13. Late Cretaceous paleoclimate reconstruction. Blue star indicates approximate location of Spain. Modified from Paleomap project (Scotise, 2000)

This paleoclimate interpretation, however, requires distinguishing primary kaolinite from secondary clay formed through diagenetic processes. If conditions under which a soil originally formed change over time, the clay mineralogy may change as well. For example, through erosional lowering of a ground surface, montmorillonite at depth may eventually become part of the profile that is most conducive to kaolinite formation and alter to this mineral (Birkeland 1999). Illite and smectite are also known to undergo similar alteration. While the yellow brown hue of goethite characterizes humid regions, the red colors present throughout the study area, and the middle Tresp Formation in general, suggest a well-drained soil of a drier climate and therefore may be inconsistent with a wet regime. Furthermore, acidic soils are generally noncalcareous and show little evidence of plant decay, burrowing activity, or sediment reworking

characteristic of the Pinyes site (Retallack 1990). The presence of weathering-resistant minerals and the absence of feldspars may reflect more an indication of time and greater soil age than paleoclimate (Birkeland 1999). The Pinyes locality, therefore, requires further study for accurate paleoclimate reconstruction.

Eggs and Clutches

Eggs at the Pinyes locality vary from relatively intact specimens to fragmented, partial eggs. The eggs are round to subround and exhibit tuberculate surface ornamentation (Figs. 4.10A). Although megaloolithid eggs are sometimes described as ellipsoidal in shape, the mean long axis orientation measured in 41 eggs at the Pinyes site is 42° (Fig. 4.10B, 4.11), and therefore consistent with the tectonic stresses in the region (Vila, pers. comm.). Large-scale geologic processes, therefore, adversely impact measurements of egg size and volume (compare Fig. 4A and B) that are important to some calculations (e.g., determining water vapor conductance rate, morphometric analyses). Small-scale taphonomic processes also influence egg preservation. These include pedogenesis, sediment reworking and burrowing activity by Mesozoic arthropods and other organisms that fragment and displace eggshell, resulting in more rapid erosion.

At least four egg-bearing horizons occur within the lower 8 m of the study area (Fig. 4.4), and sites contain clusters of 4 to 27 eggs. Some clusters are clearly separate and distinct from others. For example, site 18E04 preserves substantial portions

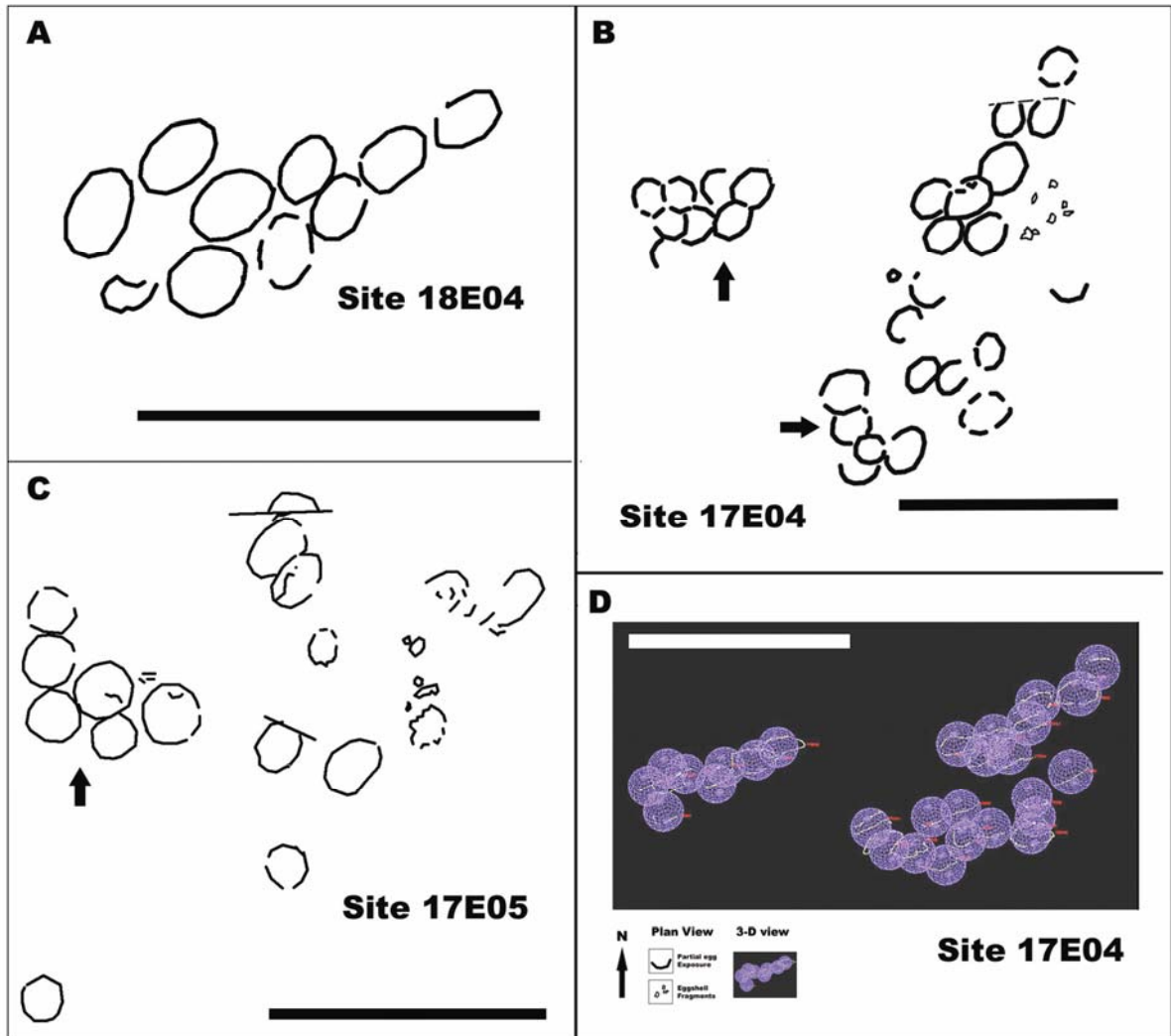


Figure 4.14. Pinyes clutches. (A-C) eggs in plan view and (D) three-dimensional representation of 17E04. Figure D shows lateral view (looking north) of same clutches in B (plan view), reconstructed from Total Station data and corrected for dip. Two egg clusters on right may represent different nesting horizons. Scale bars equal 1 meter.

of nine eggs (Fig. 4.14A), and the cluster in the northeast portion of site 17E04 includes eight eggs (Figs. 4.14B). All eggs in these clusters occur in close association or touching one another, and excavation around the specimens revealed no additional specimens. I interpret these groups as *in situ* egg clutches, representing oviposition by a

single individual. In contrast, the remaining eggs at site 17E05 and those of 17E04 (Fig. 4.14B-D) include larger groups of at least 19 eggs that include possible subgroups of 8 to 12 specimens (see arrows Fig. 4.14B,C). These subgroups resemble the geometry and close egg contact of the interpreted clutches. Data points recorded with the Trimble Total Station around each exposed egg at site 17E04 allow correction for dip and the identification of possible multiple, superimposed egg horizons (Fig. 4.14D). These superimposed egg levels are far less apparent in traditional, horizontal-oriented, two-dimensional maps, which intersect the bedding plane and thereby obscure the dip angle and egg geometry (compare Fig. 4.14B,D).

The close egg contact and superimposed egg levels in some clutches at the Pinyes site also occur at modern nesting localities. Similar close egg packing in buried turtle clutches facilitates adequate oxygen exchange by reducing the amount of sediment surrounding the eggs (Ackerman 1980). Limited availability of suitable substrates also leads to closely spaced, superimposed egg clutches; the nesting activity of the females at modern sites occasionally disrupts previously buried clutches. In addition, some modern turtle species show a strong tendency to lay eggs near their previous nest. Similar to fossil nesting horizons, different generations of nests are often difficult to identify (Erben et al., 1979; Sahni and Tripathi, 1990; Weishampel et al., 2003).

Egg Distribution

Determining horizontal egg and clutch distribution at the Pinyes locality also proves problematic. Although egg horizons are laterally continuous within the study area, eroded gullies truncate these strata, thereby providing little opportunity to measure

the distance between clutches at a scale that allows analysis of clutch distribution (Fig. 4.3). In addition, the homogeneous nature of the enclosing sediment makes precise stratigraphic position of the clutches difficult to determine. For example, three egg clutches are likely present at site 17E04 (Fig. 4.14D); however, it remains unclear if they occur on the same or slightly different paleosurfaces within the egg-bearing horizon.

Without clear, unambiguous identification of a single nesting horizon, two-dimensional mapping (Fig. 4.14A-C) provides little information about reproductive behaviors such as colonial nesting or method of egg-laying. For example, a previous study from the French Pyrenees analyzes egg and clutch distribution in detail, applying methods used in archeology, including vertical excavation in 10 cm depth increments (Cousin et al., 1989; 1994, 1999). The two-dimensional field maps are then used for interpretations of sauropod reproductive behavior. Adult female sauropods are hypothesized to have rotated their body during oviposition, thereby producing eggs arranged in an arc pattern that allegedly correlates with a radii proportional to female body size (Cousin et al., 1989; 1994). However, if the field maps are not corrected for the dip of the beds, the resulting egg distribution most likely has no biological significance (discussed below).

Many descriptions of dinosaur nesting localities in the Pyrenees of France and Spain provide little information on tectonic deformation in the study area (Cousin et al., 1989, 1994; Sanz et al., 1995; Bravo et al., 1999; Lopez-Martinez 1999; Peitz 1999; Martinez et al., 2000; Vianey-Liaud and Garcia 1999). Alternatively, the authors may report the bedding attitude, but behavioral interpretations are made without further

reference to corrections that compensate for structural deformation (Sanz et al., 1993; Garcia et al., 2003). The steeply dipping strata in mountainous regions, however, can contribute to misinterpretation of reproductive behavior. For example, clutches at sites 17E04 and 17E05 occur within the same stratigraphic interval (Fig. 4.15A,B), yet due to tectonic uplift, 17E04 appears topographically higher in outcrop (Figs. 4.3,4.4, 4.15C). Disregarding the 30° dip of the strata could result in misinterpretation of a single stratum containing eggs as vertically repeated egg-bearing horizons, typically interpreted as “site fidelity” in the literature.

Site Fidelity. Although at least four egg-bearing layers occur within the Pinyes stratigraphic section (Fig. 4.4), these repeated egg horizons simply indicate that dinosaurs

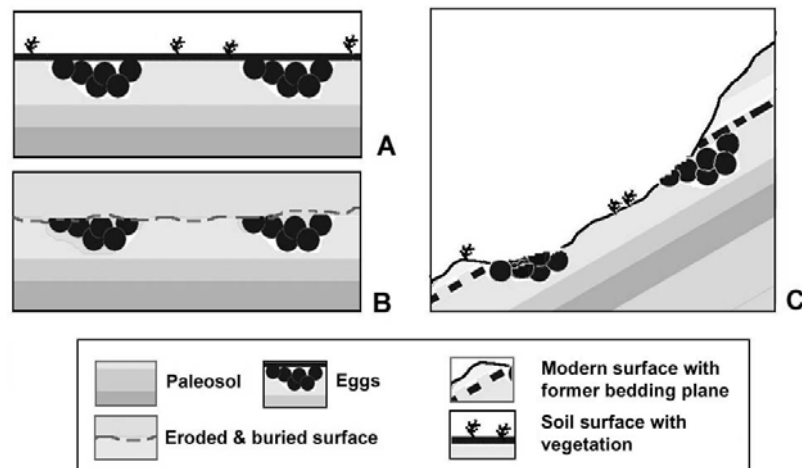


Figure 4.15. Pinyes Site 17E04 and 17E05. (A) Two buried clutches within the same stratum. (B) Original surface eroded and overlain by similar fine-grained sediment, with subsequent soil development that produces homogeneous profile. (C) Tectonic uplift and modern erosion. Original bedding (shown for illustration purposes) is no longer discernable due to extensive bioturbation. 17E04 (upper right) appears topographically higher than 17E05 (lower left).

nested in the general area over a lengthy time interval. However, this does not correspond to site fidelity, as the term is currently used in the ecological literature. Centimeters to meters of sediment separate the multiple nesting horizons at the Pinyes site. This time interval between nesting events most likely represents decades to perhaps hundreds of thousands of years, as evidenced by the well-developed soil horizons that characterize the site. In modern ecology, site fidelity typically refers to an individual animal returning to a previous nesting site where reproductive success occurred in the past (Nordmoe et al., 2004; Kruckenberg and Borbach-Jaene 2004), usually because of certain attributes (soil condition, low predation, resource availability) that characterize the locality. When applied to a fossil egg locality, those features that provided optimum nesting are unlikely to persist for such extended periods of time due to the dynamic nature of fluvial environments. Within a vertical section, multiple dinosaur nesting horizons may share some general physical and environmental characteristics. However, dinosaurs of the same species were not returning to the same nest, locality, or necessarily to the same habitat. Inferences of reproductive behavior such as site fidelity far exceed the resolution of the rock record. Therefore, “repeated use” represents a more accurate term for multiple nesting horizons within a stratigraphic section.

Egg Incubation Mode

Trace fossil nests are extremely rare in the fossil record (Varricchio et al., 1999; Chiappe et al., 2004). Interpretations regarding nest construction and incubation strategy employed by extinct taxa typically rely on two lines of evidence: 1) superimposed eggs within a clutch, and 2) water vapor conductance rates calculated from fossil eggs (see

Chapter 5). With a few exceptions (Dughi and Siruge 1958; Cousin et al., 1994), nearly all previous studies infer substrate burial of megaloolithid eggs (Seymour, 1979; K  rourio, 1981; Williams et al., 1984; Sabath 1991; Girgorescu 1994; Sahni et al., 1994; Peitz, 2000; Deeming 2002; 2006; Sander per. comm.). Based on sedimentology and egg geometry, inferences regarding egg incubation at the Pinyes locality remain inconclusive for the following reason.

Like most *Megaloolithus* nesting localities, the Pinyes clutches are preserved in uniform, fine-grained bioturbated mudstone, with no evidence of sedimentary structures. This presents a possible alternative hypothesis to that of underground egg incubation: oviposition occurred in an open nest, excavated in fine-grained sediment. The depressions later filled with sediments identical to the host strata and subsequent bioturbation removed crucial lithologic evidence necessary to distinguish an open nest from eggs that were buried in the substrate. In addition, preservation of vegetation that would support a high humidity/low oxygen incubation environment (e.g., vegetation mound) rarely occurs in well-drained, oxidized sediments (Retallack 1990). Substrate burial of eggs at the Pinyes site, therefore, cannot be substantiated by sedimentological evidence. However, the high water vapor conductance rate calculated for the Pinyes egg provides support for previous inferences of underground egg burial (see Chapter 5).

Hatching Method

Most eggs from the Pinyes site exhibit an upper surface with sizeable areas that lack intact eggshell (Fig. 4.10B), and the mudstone immediately surrounding the eggs typically contains abundant eggshell fragments. Unusually large fragments are

sometimes associated with the clutches. For example, a 6 x 8 cm fragment occurred concave downward in the sediment, 2.4 cm from an egg in 17E05. Displacement of the upper portion of the egg during hatching may account for the sizable openings in some specimens and the large shell fragments in the adjacent sediment. These eggshell fragments were most likely incorporated into the disrupted soil surrounding the eggs as the hatchlings exited the nest.

Eggshells at the Pinyes locality also occur within the eggs. Examination of 59 specimens exposed in cross section reveals that 53% contain eggshell with concave-upward orientation within the egg interior, 15% contain eggshell resting concave-downward, and 32% exhibit mixed orientations. Nearly all specimens examined display one or two eggshell layers within the sediments that filled the egg interior. However, 16E03 includes an unusual specimen. Initially, the specimen was thought to represent a single egg, but further examination revealed 9 to 10 eggshell layers within the egg, with varying concave upward/downward orientations. The individual fragments are closely superimposed and separated by sediment and, therefore, do not represent a pathological egg condition (Chapters 2,3). The specimen most likely represents multiple crushed eggs. The unique condition of this specimen, compared to the other nine eggs in the clutch, suggests a different (and uninterpretable) taphonomic history. The similar preservation of the other nine eggs suggests successful hatching. Taphonomic data regarding hatching method remains equivocal in this and most eggs in the study area (discussed below). Nevertheless, as suggested by Hayward et al., (2000), comparison of

data obtained from fossil and modern egg localities may eventually prove useful for differentiating taphonomic from biological processes and patterns in the fossil record.

Previous Hypotheses About Hatching Mode

Several hatching behaviors are hypothesized for sauropod dinosaurs, based on eggshell fragments associated with fossil eggs. Cousin et al., (1994) interpreted the presence of large pieces of concave-up eggshell at the bottom of megaloolithid eggs from Rennes-le-Chateau, France as a “hatching window”. Presumably, as the juvenile escaped from the egg, large eggshell fragments slid into the egg interior and rested concave side up. Mueller-Töwe et al., (2002) concur with their conclusions, based on computed axial tomography (CAT scan) study of fossil eggs. They suggest that the interpreted hatching window was inconsistent with shell entering the eggs through lithostatic compaction or a “gradual escape” during the hatchling; the latter term, however, remained undefined by the authors. Furthermore, they hypothesized the presence of an “egg tooth” in titanosaurs, similar to that found in many modern egg-laying amniotes. Presumably, the embryo used this structure to perforate the shell surface, producing a large, regularly shaped opening in the upper egg surface. Unfortunately, percentages for the orientation of concave up and concave down eggshell fragments that would allow comparison to the present study are not reported. Recording such information in the future may be beneficial to interpretations.

Mueller-Töwe et al., (2002) also suggest that shell fragments that primarily occur inside the egg (rather than in the surrounding sediments) provide strong evidence for underground egg incubation and hatching. They suggest that shell surrounding a clutch

incubated above ground would have been broken, fed upon, washed away, and trampled by the hatchlings or other animals, thus leaving few large fragments in the sediment. However, abundant eggshells occur within the matrix surrounding the Pinyes clutches, yet water vapor conductance rates suggest these eggs were buried (Chapter 6). In addition, Deeming and Unwin (2004) argue that eggshell fragments in the adjoining sediments are characteristic of modern, successful reptile nests. Therefore, differences between the sites described by Mueller-Töwe et al. (2002) and the Pinyes locality most likely reflect variation in taphonomic history. Finally, actualistic experiments by Bravo et al. (2003) reveal that a large opening in the upper egg surface may also result from gas collection and expansion, due to the decay of organic matter in buried eggs. Therefore, interpretations of hatching strategies from the rock record require caution.

Conclusions

The Pinyes locality occurs within the middle portion of the lower Tremp Formation, at approximately the transition from the gray to red mudstone facies. The Upper Cretaceous continental deposits of the study area dip steeply to the north at approximately 30° and contain four egg bearing horizons. The local section represents a typical overbank sequence of a fluvial system and consists of three lithofacies. In ascending order, facies A-C include (1) a highly bioturbated, calcareous mudstone, (2) small to medium-sized (< 5 m thick) channel fill or crevasse splay deposits, and (3) a fining upward, medium to coarse-grained, primarily parallel-stratified sandstone. Facies A and B exhibit extensively bioturbated sediments containing bifurcating tubules,

rhizoliths, blue-grey mottling, small calcareous nodules, and meniscate back-filled structures (*Spirographites ellipticus*), interpreted as sediment reworking by arthropods such as crustaceans, spiders, beetles, or other insects.

The extensive and overlapping trace fossils and pedogenic features at the Pinyes locality indicate a stable land surface some distance from an active stream channel. This surface existed for an extended time period, and the stability facilitated use of these well developed soil profiles for dinosaur nesting sites. Floodwaters, however, periodically inundated the floodplain, and suspension settling of fine-grain sediments covered the nests or filled the remaining portion of the hatched eggs, thereby preserving the specimens in the rock record. Exhumation and exposure of the eggs may have resulted in subsequent erosion. The weathering-resistant minerals suggest a wet/humid climate, an interpretation consistent with paleoclimatic reconstructions of the Late Cretaceous of the Iberian Peninsula. However, the red oxidized sediments indicate a well-drained soil, thus contributing to the difficulty of interpretation; the mineralogy may reflect greater soil age, rather than representing a paleoclimate indicator.

The round to subrounded Pinyes eggs are attributable to the oospecies *Megaloolithus siruguei*. Ten clutches were mapped in plan view, with high resolution point data collected for each egg with a Trimble Total Station. A three-dimensional model provides more accurate representation of clutch geometry. Clutches are comprised of multiple egg levels and contain 4 to 12 randomly distributed eggs that occur in close contact with one another. Larger clusters of 19 or more eggs are interpreted as superimposed nests of the same or multiple nesting seasons. Most eggs are eroded or

hatched, but four clutches are known to contain from one to four whole eggs. The abundant eggshell debris that surrounds the clutches suggest some, if not most eggs are hatched; the eggs, however, lack definitive evidence for hatching mode. The uniform, homogeneous mudstone prohibits identification of nests on the basis of sedimentological criteria, but the bowl shape of the clutch suggests the eggs were deposited in a shallow excavated depression. Whether the clutches were buried underground, or if the excavated depression were open and later filled with similar fine-grained sediment remains equivocal due to extensive bioturbation and uniformity of the enclosing mudstone.

The homogeneous sediments and steep dip angle contribute significantly to the difficulty of interpreting the relationship among clutches, both at the outcrop scale and individual sites. Two-dimensional mapping and taphonomic data recorded for each egg, as well as the portion of each egg preserved, supplement the total station data points when reconstructing egg location. Removal of the dip angle with the computer software program provides a more accurate representation of each egg position, allowing more accurate identification of multiple, superimposed egg horizons. Without these techniques, excavations that intersect the bedding plane result in “time-averaging” of eggs from different clutches, horizons, and nesting seasons; the superimposed egg levels are indistinguishable in traditional plan view maps. In steeply dipping strata, therefore, traditional mapping techniques provide little information about reproductive behaviors of the egg-laying taxon, including egg and clutch distribution, gregarious nesting, or site fidelity.

CHAPTER 5

GAS CONDUCTANCE OF *MEGALOOOLITHUS PATAGONICUS*, A TITANOSAUR
EGG FROM AUCA MAHUEVO, ARGENTINA, WITH COMPARISON TO
MEGALOOOLITHUS SIRUGUEI EGG FROM SPAINIntroduction

In the last 20 years, a growing interest in understanding extinct animals as living organisms has produced considerable discourse in the scientific literature regarding dinosaur reproductive behaviors. Inferences of egg brooding (Norell *et al.*, 1995; Dong & Currie, 1996; Varricchio *et al.*, 1997), life history strategy (Weishampel and Horner 1994), parental care, and neonate development mode (Horner & Makela, 1979; Horner 1984) continue to generate controversy (Geist and Jones 1996; Carpenter 1999; Ruben *et al.*, 1996; Jackson and Varricchio 2003; Jackson *et al.*, 2004; Deeming 2002; 2006; Grellet-Tinner *et al.*, 2006). Although fossil eggs and nesting horizons provide potentially valuable evidence about the egg-laying taxon, reproductive behaviors are often difficult to interpret from the fossil record (Hirsch 2001; Jackson and Varricchio 2003; Jackson *et al.*, 2004; Grellet-Tinner 2006; Chapters 2, 4,6).

In his seminal paper on gas conductance of dinosaur eggs, Seymour (1979) argued that, in searching for evidence of dinosaur reproductive behavior and physiology, it is desirable to form conclusions that depend directly on measurements available from the fossil material. In modern taxa, the shell conductance value of an egg closely corresponds to the type of incubation environment, thereby providing important insights into the nesting strategy (Seymour, 1979; Deeming 2006). The amount of theoretical and

empirical information available on shell conductance and water loss in bird eggs provides a model for comparing fossil specimens (Seymour, 1979). For example, most avian eggs are exposed to the atmosphere during incubation and exhibit low water vapor conductance rates (\dot{G}_{H_2O}) in order to conserve water that would potentially be lost to evaporation. In contrast, reptiles typically incubate their eggs in high humidity/low oxygen conditions such as underground burial or in vegetation mounds, and the eggs generally exhibit higher gas conductance values than avian eggs. These differences between avian and reptilian egg \dot{G}_{H_2O} are often used to interpret the egg incubation environment of extinct taxa (Seymour, 1979; Williams et al., 1984; Sabath 1991; Girgorescu 1994; Deeming 2002; 2006; Grellet-Tinner and Chiappe, 2004; Grellet-Tinner et al., 2006).

Fossil eggs of the oogenus *Megaloolithus* are often assigned to sauropod dinosaurs. This oogenus, however, exhibits substantial diversity of eggshell structures and currently includes over 25 oospecies (Carpenter 1999). All analyses of *Megaloolithus* eggs to date reveal a significantly higher water vapor conductance rate than modern avian eggs of comparable size. Previous studies, therefore, conclude that sauropods buried their eggs underground or in incubation mounds (Seymour, 1979; Williams et al., 1984; Sabath 1991; Girgorescu 1994; Deeming 2002; 2006; Sander per. comm.). Furthermore, two studies (Seymour, 1979; Seymour and Ackerman 1980) suggest that sauropods may have been forced to limit their clutch size to 13 eggs. Theoretically, this would have prevented unacceptably high levels of carbon dioxide and depletion of oxygen in the nest during the latter part of the incubation period when

embryonic metabolic activity reached a maximum. However, eggs identifiable as sauropod on the basis of embryonic material are not included in any of the previous analyses (Seymour, 1979; Seymour and Ackerman 1980; Williams et al., 1984; Sabath 1991; Sahni et al., 1994; Deeming 2002, 2006) and, therefore, taxonomic identification of the eggs remains uncertain.

The 1997 discovery of the Auca Mahuevo locality in Argentina allowed the first definitive correlation of the oospecies *Megaloolithus patagonicus* (Calvo et al., 1997) to a sauropod dinosaur, based on osteological remains within an egg (Chiappe et al., 1998). Detailed taphonomic studies conducted at this site show that the number of eggs in these South American titanosaur clutches far exceeds the proposed limit on sauropod clutch size. While European and Indian clutches typically contain fewer than 15 eggs (Kerourio 1981; Cousin et al., 1994; 1999; Mohabey 1996, 1999, 2001; Sahni and Khosla 1994; Sander et al., 1998; Peitz 1999, 2000; Lopez Martinez et al., 2000; Garcia et al., 2003), the Argentine titanosaur clutches commonly contain 20 to 40 eggs (Chiappe et al., 1999; 2005; Jackson et al., 2004). Furthermore, six trace fossil nests documented at the Auca Mahuevo locality indicate that the nests were open and the eggs were not buried underground (Chiappe et al., 2004). Two papers (Grellet-Tinner and Chiappe, 2004; Grellet-Tinner et al., 2006), however, now suggest that Auca Mahuevo titanosaur eggs were incubated in high humidity conditions consistent with vegetation in the nest, a conclusion that appears contradictory to the open nest hypothesis.

To examine these contrasting interpretations of incubation environment, I calculate the gas conductance rate for an Auca Mahuevo titanosaur egg, *Megaloolithus*

patagonius. To address potential problems of extrapolation from modern avian egg data to masses equivalent to dinosaur eggs (see Carpenter 1999), I added *Aepyornis* (elephant bird) eggshell to the extensive data set compiled by Ar and Rahn (1985). *Aepyornis* eggs are extremely large compared to most avian eggs and, therefore, valuable for extending the range of data and improving the regression analysis, to allow interpolation, rather than extrapolation from the data.

The unique combination of definitive egg identification, trace fossil nests, and calculated water vapor conductance rates allows a more rigorous evaluation of previous hypotheses regarding egg incubation and possible limits on sauropod clutch size. If Auca Mahuevo eggs were incubated in open nests (Chiappe et al., 2004), the eggs should show significantly lower conductance values than eggs incubated in high humidity environments that characterize substrate burial or a vegetation mound. Comparison of the Auca Mahuevo eggs to previous European and Indian studies of *Megaloolithus*, however, is difficult due to different field and laboratory methods employed by various workers. Therefore, the techniques used at Auca Mahuevo are also applied to a new *Megaloolithus siruguei* locality in Spain. Use of similar procedures at both sites allows the first direct comparison of South American and European eggs and clutches, in order to assess potential differences in reproductive biology.

Nesting Localities

Auca Mahuevo. Most eggs at the Auca Mahuevo locality occur in uniform, fine-grained overbank mudstone. Deposition of these sediments on the floodplain occurred at

some distance from an active stream channel, and the muddy deposits exhibit no evidence of sedimentary structures (Chiappe et al., 1999). In contrast, six titanosaur clutches at the Argentine locality are preserved in sandstone: five occur in the upper surface of a channel deposit, and a sixth clutch is preserved in a crevasse splay sand lobe at another site within the study area (Chiappe et al., 2004). The depressions containing the six egg clutches truncate primary sedimentary structures of the host strata, and a massive

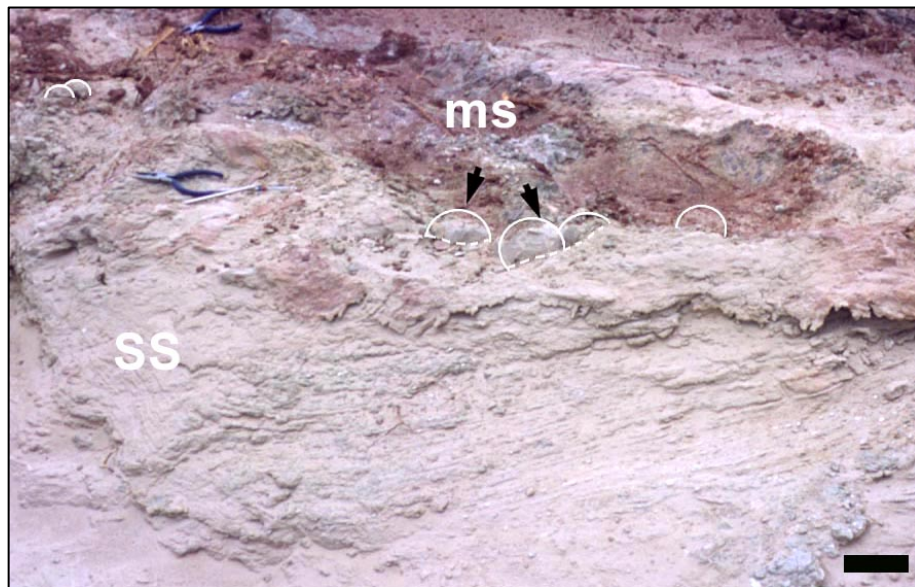


Figure 5.1. Auca Mahuevo trace fossil nest (NE05). White circles and two back arrows indicate eggs preserved within a depression that truncates cross-stratified sandstone (ss). Portions of massive sandstone rim occur below eggs on the right. Darker mudstone (ms) surrounds the eggs. Scale bar is 10 cm

sandstone rim encircles portions of each nest (Fig. 5.1). The eggs within all six depressions are surrounded by mudstone, deposited from suspension settling of fine-grain sediment during one or more flood events. The lithological difference between the truncated sandstone and the mudstone surrounding the eggs indicates that the nests were

open, and that Auca Mahuevo eggs were not incubated underground (Chiappe et al., 2004).

Pinyes Site. Egg clutches at the Pinyes sites also occur within uniform, fine-grained overbank sediments, deposited distal to an active stream channel (Chapter 4). The massive mudstone displays evidence of extensive pedogenesis and bioturbation, including sediment reworking by arthropods such as crustaceans, spiders, beetles, or other insects (Mayoral and Calzada, 1998). These processes resulted in an absence of primary sedimentary structures associated with the egg clutches. Unlike Auca Mahuevo, trace fossil nests have not been documented at the Pinyes locality.

Specimens

Titanosaur Egg. MCF-MVPH-775 comes from the egg bed 3 quarry at the Auca Mahuevo locality; this stratum yields abundant eggs containing embryonic remains (Chiappe et al., 1998; Chiappe et al., 1999; 2001; Grellet-Tinner et al., 2004). The *M. patagonicus* egg selected for this study, however, does not contain visible embryonic bones or skin. The egg exhibits an intact lower surface, relative to the bedding plane (Fig. 5.2A), while the upper surface is compressed downward. Due to the variation that occurs among Auca Mahuevo specimens, average values for some attributes are used in this study. For example, Auca Mahuevo clutches range from 15 to over 40 eggs (Fig. 5.2B), therefore, 25 eggs are considered an average clutch size for calculating total clutch \dot{G}_{H_2O} (Chiappe et al., 1999; Jackson et al., 2004; Grellet-Tinner et al., 2004).

Similarly, the egg dimensions (15 x 14 cm) are estimated from measurements of MCF-

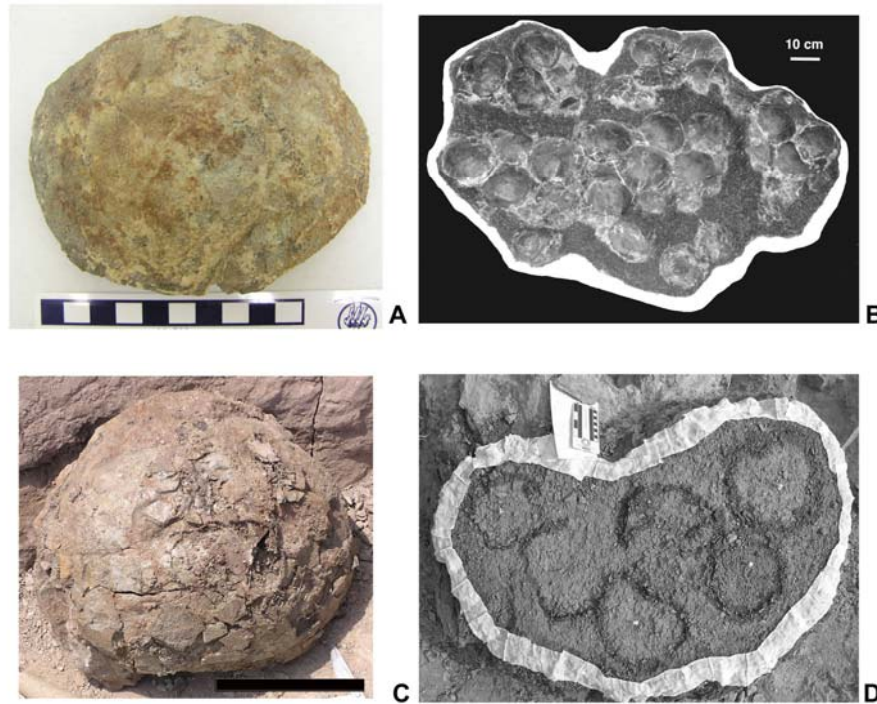


Figure 5.2. Two *Megaloolithus* eggs and clutches. (A) Auca Mahuevo titanosaur egg (MCF-PVPH-775) showing the intact, lower egg hemisphere. (B) Auca Mahuevo titanosaur clutch (MCF-PVPH-258) containing approximately 40 eggs, many with embryonic remains. (C) Pinyes *Megaloolithus siruguei* egg (18E03-E). (D) Field jacket with *M. siruguei* clutch (17E05) containing 6 eggs. Scale bars are 10 cm.

MVPH-775 and other eggs from the same locality (Chiappe et al., 1999, 2003, 2005; Jackson et al., 2004; Grellet-Tinner et al., 2004).

Megaloolithus siruguei. For comparison, an egg was removed from clutch 18E02, in egg layer 2 at the Pinyes locality in northern Spain (Fig. 5.2C,D; see also Chapter 4). This oospecies is commonly assigned to sauropod dinosaurs, based on sauropod bones in the same horizon or formation. However, eggs from the Pinyes site (and all *M. siruguei* localities) lack embryonic remains and, therefore, taxonomic identification of the egg remains uncertain. In addition, it should be noted that the 20 x

22 cm size of the analyzed egg compares favorably with other *M. siruguei* specimens (Vianey-Liaud et al., 2003), but falls at the upper size limit for well-preserved eggs excavated at the Pinyes locality.

Aepyornis. A complete egg of the recently-extinct "elephant bird" was unavailable for the study. Therefore, radial and tangential thin sections of eggshell were examined for diagenesis, and calculations performed on a tangential specimen (Fig. 5.3). Additional egg attributes (e.g., size, volume) were obtained from the literature (Long et al., 1998). These data were added to the extensive tables of Ar and Rahn (1978, 1985) and regression analyses performed.

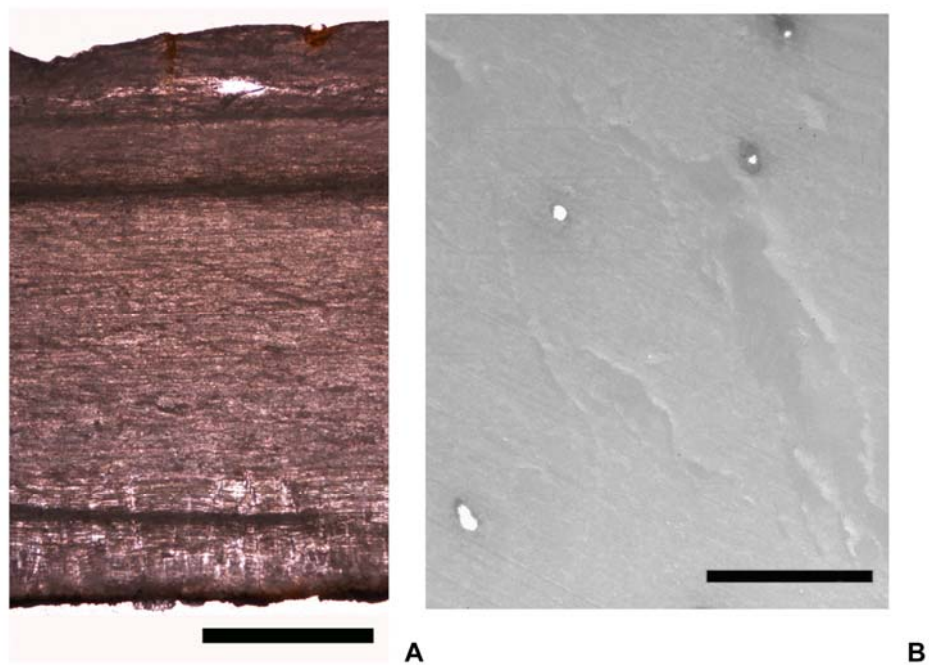


Figure 5.3. *Aepyornis* eggshell. (A) Radial thin section of eggshell. (B) Tangential thin section showing four pores.

Eggshell Preparation

Megaloolithus patagonicus eggshells from the Auca Mahuevo locality often exhibit diagenetic dissolution between adjacent nucleation sites at the inner shell surface;

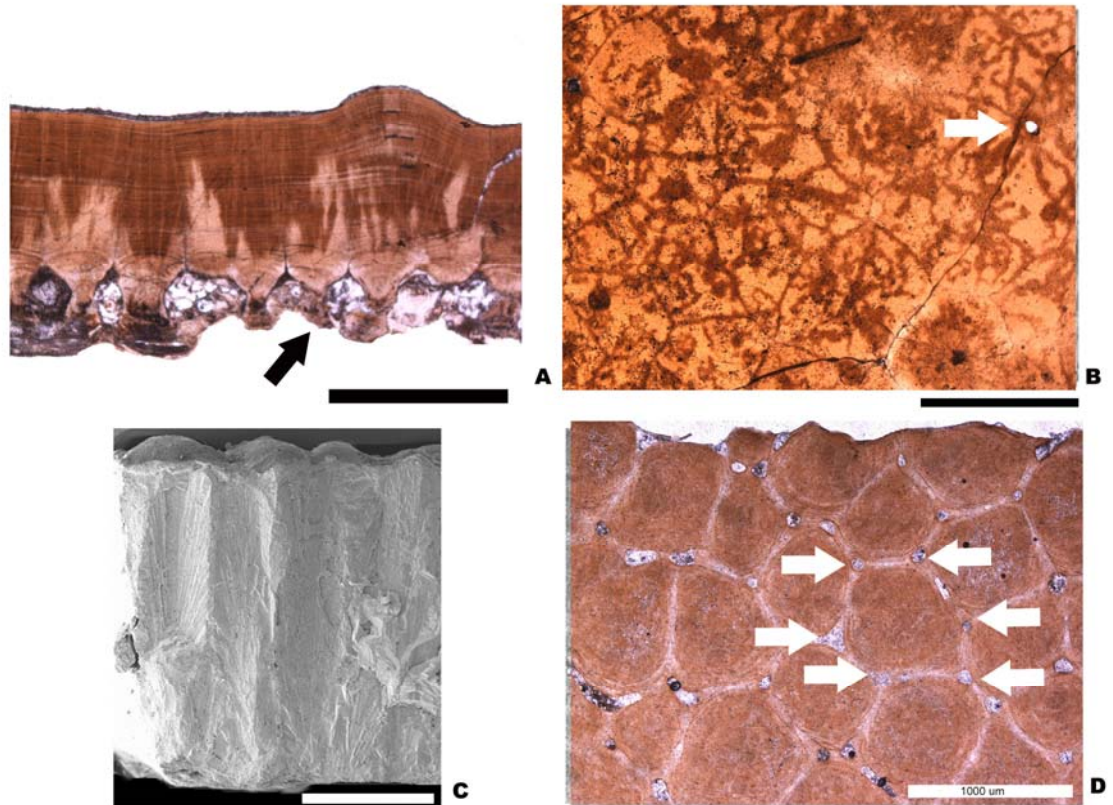


Figure 5.4. *Megaloolithus* eggshells from the Auca Mahuevo and Pinyes localities. (A) Radial thin section of a Argentine titanosaur egg (MCF-PVPH-112). Outer surface of the egg is at the top of the image. Note the calcite dissolution that occurs between nucleation sites at the inner shell edge and remnants of preserved membrane. (B) Tangential thin section of Auca Mahuevo eggshell (MCF-MVPH-775); single pore filled with diagenetic sparry calcite at upper right. (C) SEM in radial view of a Pinyes eggshell (ES 131). (D) Tangential thin section of Pinyes eggshell showing abundant and even distribution of calcite-filled pores (18E02). Five to six pores surround each shell unit. Scale bars are 1 mm.

this dissolution often affects one fourth to one third of the shell thickness (Fig. 5.4A).

Eggshell samples, therefore, were examined under a dissecting microscope, and ten

specimens displaying minimal dissolution were then embedded in polyester resin. Tangential sections were cut from each specimen, mid-way between the inner and outer eggshell surfaces (Fig. 5.4B). Five eggshells removed from the Spanish *M. siruguei* egg are thicker and do not exhibit extensive dissolution at the interior surface of the shell (Fig. 5.4C). Therefore, tangential thin sections were obtained from a commercial laboratory (Fig. 5.3D). All thin sections measure 30 μm thick. Additional eggshells removed from the two eggs were coated with gold (10 nm), mounted on aluminum stubs, and imaged as radial sections under a J.R. Lee Instrument Personal SEM and/or JEOL 6100 SEM with Backscattered Electron Imaging (BEI) capabilities, coupled to a Noran Voyager Energy Dispersive X-ray (EDX) system.

Determining Pore Characteristics

The tangential thin sections were viewed under a Nikon Eclipse E600 petrographic microscope equipped with a digital camera. Approximately 10 non-overlapping images were photographed during systematic transects of each slide, in order to obtain maximum sample coverage. The images ($n = 247$) were imported into Adobe Photoshop. After examining several thin sections from each specimen, criteria were determined for including or excluding pores, determining original pore size, and distinguishing pores from diagenetic features. For example, some pores are filled with opaque fine-grained sediments; enhancement of brightness and contrast in Photoshop often revealed the true diameter. Other pores are enlarged by diagenetic dissolution. Rather than estimating the diameter based on “normal” pores, the area was calculated for the enlarged opening. This potentially results in a slightly higher estimated eggshell

porosity. In some cases, however, the shell appears extremely thin as a result of the egg curvature and thin-sectioning process, rendering that portion of the shell area unusable. This unusable region was therefore excluded from the calculations. Subjectivity and human error in accurately determining exact pore circumference using established criteria will likely be consistent throughout the study and therefore compensatory.

The image was then imported into the Scion image analysis program, available through the National Institute of Health (NIH) web site (<http://rsb.info.nih.gov/nih-image/default.html>). Each pore was numbered on the image, the area calculated, and total area computed. Shell thickness was measured from additional specimens with a scanning electron microscope (SEM) or from digital images with the image analysis software. Shell thickness, pore area, pore number, pore percentage, and pore radius values were determined for all specimens.

Variables and Equations

Calculation of water vapor conductance rates in this study is similar to methods used in previous studies (e.g., Ar et al., 1974; Seymour, 1979; Williams et al., 1984; Deeming 2006), with minor variations noted below. Table 5.1 lists the variables, with units, and constants used in calculations in this study, and the middle two columns of Table 5.2 provide the formulas, sources, and reference of data. Assuming Fick's 2nd law of diffusion, water vapor conductance rates were determined for the three specimens using calculated data and direct measurements of egg attributes as discussed below. The calculated rates for water vapor conductance represent theoretical maximum values for a maximal pressure gradient. For determining the effective pore radius and subsequent

water vapor conductance rates, I assume that the pore canals are circular in cross section and of uniform radius throughout their lengths. This assumption is common to all previous studies. Branched pores, however, could potentially alter effective radius and gas conductance rates. While some branching occurs in both *M. patagonicus*

Table 5.1. Variables and Constants Applicable to the Study.

Variable	Definition	Units
L	Maximum egg length	mm
W	Maximum egg width	mm
M	Egg mass	g
L_s	Shell thickness	mm
A_s	Surface area	mm^2
N	Total number of pores	per egg
D	Pore density	$\#/\text{mm}^2$
r	Pore radius	μm
A_p	Total pore area	mm^2
A	Mean individual pore area	μm^2
$\dot{G}_{\text{H}_2\text{O}}$	Water vapor conductance	$\text{mg}_{\text{H}_2\text{O}}/(\text{dayTorr})$
$_{\text{sp}}\dot{G}_{\text{H}_2\text{O}}$	Mass-specific water vapor conductance	$\text{mg}_{\text{H}_2\text{O}}/(\text{dayTorr g})$
$_{\text{p}}\dot{G}_{\text{H}_2\text{O}}$	Water vapor conductance per pore	$\mu\text{g}_{\text{H}_2\text{O}}/(\text{dayTorr pore})$
E	Mean eggs per clutch	
$_{\text{c}}\dot{G}_{\text{H}_2\text{O}}$	Total gas conductance per clutch	$\text{mg}_{\text{H}_2\text{O}}/(\text{dayTorr clutch})$
Constants		
c	constant from empirical avian data	$c = 1.56 \times 10^9 \text{ sec mg day}^{-1} \text{ mol}^{-1}$
R	Universal gas constant	$6.24 \times 10^4 \text{ cm}^3 \text{ Torr mol}^{-1} \text{ }^\circ\text{K}^{-1}$
T	Temperature	$T = 303 \text{ }^\circ\text{K}$ (= assumed 30°C)
$d_{\text{H}_2\text{O}}$	Diffusion coefficient for water vapor	$0.292 \text{ cm}^2 \text{ sec}^{-1}$

Table 5.2. Results of measurements and calculations on the Auca Mahuevo titanosaur, unidentified Pinyes, and extinct *Aepyornis* (elephant bird) eggs.

	Description	Units	Formula/source	<i>Aepyornis</i>	Auca Mahuevo (Argentina)	Pinyes (Spain)
L	Egg length	cm	Data	31.7	15	22
B	Egg breadth	cm	Data	25.8	14	20
V	Egg volume	cm ³	$V = 0.51(L \times B^2)$	10,760	1500	4488
M	Egg mass	grams	$M = \rho \times V$	11,620	1620	4850
ρ	Egg density	g/cm ³	Assumed from avian egg data	1.08	1.08	1.08
L_s	Shell thickness.	mm	Data	3.25	1.48	2.4
A_s	Surface area	cm ²	$A_s = 4.928 V^{0.668}$	2432	652	1356
D	Pore density	#/mm ²	Data	0.831	0.195	4.41
N	# pores/egg	no.	$N = D \times A_s$	202,000	12,714	598,000
r	Pore radius	μm	Data	41.1	61.62	49.25
% pore area	%	--	Data	0.441	0.370	3.36
A_p	Total pore area	cm ² /egg	$A_p = A_s \times \% \text{ pore area}$	10.72	2.41	45.56
A	Mean indiv. pore area	μm^2	$A = \pi r^2$	5,307	11,929	7,620
\dot{G}_{H_2O}	Water vapor conductance	mg _{H₂O} / (dayTorr)	$\dot{G}_{H_2O} = \frac{c \cdot d_{H_2O}}{RT} \frac{A_p}{L_s}$	691.4	341.3	3978.9
Predicted \dot{G}_{H_2O}	For bird egg of equiv. size	mg _{H₂O} / (dayTorr)	Regression formula $\dot{G}_{H_2O} = 0.3786 M^{0.818}$	800.8	159.8	391.8
Calcul. \dot{G}_{H_2O} to predicted				0.9:1	2.1:1	10.2:1
$p\dot{G}_{H_2O}$	\dot{G}_{H_2O} per pore	$\mu\text{g}/(\text{dayTorr pore})$	$p\dot{G}_{H_2O} = \dot{G}_{H_2O} / N$	3.42	26.84	6.65
E	Number of eggs/clutch		Data	Unknown	~25	~9
M_c	Clutch weight	grams/clutch	$M_c = M \cdot E$	Unknown	40,500	43,650
$c\dot{G}_{H_2O}$	per clutch	mg/(dayTorr pore)	$c\dot{G}_{H_2O} = \dot{G}_{H_2O} \cdot E$	Unknown	8,532	35,810
$spc\dot{G}_{H_2O}$	Gas conductance per gram clutch wt.	mg/(dayTorr gram)	$= c\dot{G}_{H_2O} / (M \cdot E)$	Unknown	0.211	0.820

(Grellet-Tinner et al. 2006) and *M. siruguei*, this pore morphology appears rare in *M. patagonicus*. Finally, I consider the “lateral pore system” reported in Auca Mahuevo eggs (Grellet-Tinner et al., 2004, 2006) the result of diagenetic dissolution, a common feature at the base of *M. patagonicus* eggshell (Jackson et al., 2004).

Results

Megaloolithus Eggs

Table 5.2 provides the results summarized here. *Megaloolithus patagonicus* clutches from Auca Mahuevo typically contain 20 to 40 eggs, while *M. siruguei* clutches from the Pinyes locality average 9 eggs (Chiappe et al., 1999; Chapter 5). However, the smaller, 15 cm Argentine egg has an estimated volume of 1500 cm³, compared to 4488 cm³ of the Pinyes egg. It should also be noted that the volume of the Auca Mahuevo egg significantly exceeds the incorrect volume (800 cm³) reported by Chiappe et al., (1998) and recently used to calculate titanosaur egg mass (Varricchio and Jackson, 2003). As typical for smaller egg size (Ar and Rahn, 1985), the Auca Mahuevo eggshell is thinner than that of the larger Pinyes egg (Table 5.2). The Auca Mahuevo egg has 47 times fewer pores, but the individual pores are each 1.57 times larger than those of the Pinyes egg. The Argentine egg also exhibits a more irregular pore distribution. For example, pores are very abundant in all tangential thin sections from the Pinyes egg, while 20% of the analyzed images from all quadrants of the Auca Mahuevo egg are completely devoid of pores. The \dot{G}_{H_2O} of the *M. patagonicus* and *M. siruguei* eggs are 341 and 3979 mg H₂O/(dayTorr), respectively.

Aepyornis Egg

The 11.6 kg *Aepyornis* egg exceeds both *Megaloolithus* specimens in diameter, mass, volume, and eggshell thickness (Table 5.2; Long et al., 1998). In addition, the egg is far larger than the largest known extant avian egg, the 1.4 kg ostrich egg (Vleck and Hoyt 1990). If representative of the entire egg, the number of pores/mm² calculated for the *Aepyornis* eggshell yields approximately 202,000 pores per egg, with a water vapor conductance rate of 691.4 mg_{H₂O}/ (Torr day). These data were added to the avian data set of Ar and Rahn (1985) in order to extend the regression analysis range and produced only minor changes in the regression formula (Tables 5.3, 5.4). This suggests the sample was representative of the egg and adequate for the analysis. Furthermore, the values calculated for the *Aepyornis* egg fall very close to the avian values predicted by previous regression formulas (Ar and Rahn 1985), thereby validating the original methodology and the expansion of the data set.

Table 5.3. Avian Data Set Regression formulas with inclusion of *Aepyornis*.

Egg Attributes	With <i>Aepyornis</i>	Correlation coefficient
L_s	$L_s = 0.057839 M^{0.4248}$	$r^2 = 0.92$
N	$N = 373.54 M^{0.7457}$	$r^2 = 0.86$
D	---	
A_p	$A_p = 0.0106 M^{1.2337}$	$r^2 = 0.95$
\dot{G}_{H_2O}	$\dot{G}_{H_2O} = 0.3786 M^{0.818}$	$r^2 = 0.92$

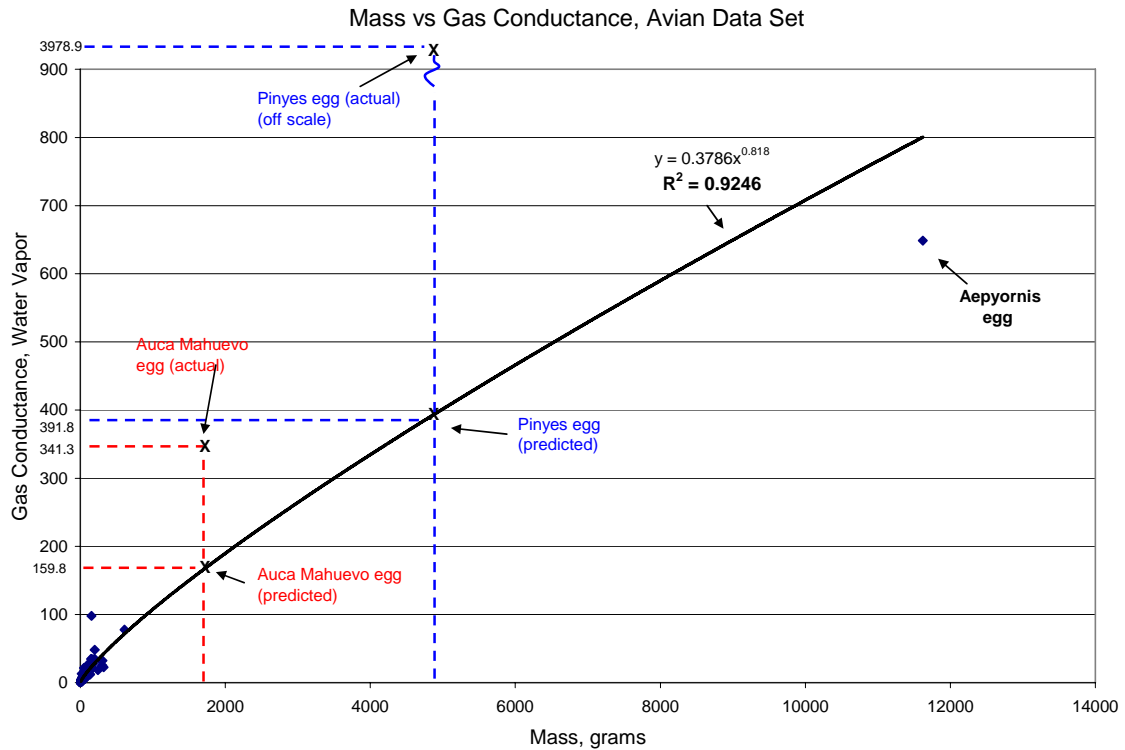


Figure 5.5. Regression analysis of eggshell water vapor conductance data sets (Ar et al., 1974, Ar and Rahn 1985) with addition of *Aepyornis*. Includes actual and predicted conductance rates for *Megaloolithus patagonicus* and *M. siruguei* eggs compared to avian eggs of comparable size. Note that *M. siruguei* value of 3878.9 extends far beyond the graph.

Discussion

Megaloolithus exhibits a wide range of egg morphology, and temporal and paleogeographic distribution (Carpenter 1999). In the current study, significant variation in reproductive attributes (e.g., egg and clutch size, shell thickness, microstructure) distinguishes the South American titanosaurs from the European egg-laying taxon (Table 5.2). In addition, the Aucá Mahuevo titanosaur egg displays substantially lower \dot{G}_{H_2O} than the Pinyes egg (Table 5.2). The water vapor conductance rates of *M. patagonicus*

and *M. siruguei* are 2.1 and 10.2 times greater, respectively, than avian eggs of comparable size.

Water vapor conductance rates calculated for modern avian eggs are often used to hypothesize the incubation environment for extinct taxa (Deeming 2006); however, such comparisons are often complicated. For example, the \dot{G}_{H_2O} of the Auca Mahuevo egg is similar to that of the extant megapode (*Alectura lathamii*) that incubates eggs in vegetation mounds (Table 5.4). In most avian species the diffusive conductance of the eggshell remains relatively constant throughout incubation (Ar and Rahn 1985). In megapode eggs, however, dissolution of the inner surface occurs late in embryonic development. Significant eggshell thinning (12-21%) greatly increases \dot{G}_{H_2O} in response to decreasing oxygen availability in the vegetation mound (Booth and Thompson 1991). Furthermore, the male bird continuously turns over mound material, thus mitigating the severity of the hypoxic gaseous environment (Seymour and Ackerman 1980). This behavior and the complex branching pore system of megapode eggs contribute to the difficulty in comparing these eggs to the titanosaur specimen.

The \dot{G}_{H_2O} of the Auca Mahuevo egg, however, is lower than that of the pied-billed grebe (*Podilymbus podiceps*) and the common loon (*Gavia immer*) that exhibit \dot{G}_{H_2O} values that are 3 and 4 times higher than predicted for bird eggs of similar size (Table 5.4). These birds incubate their eggs in humid or even wet conditions (Ar and Rahn 1985), thereby accounting for the higher conductance values necessary for adequate oxygenation of the embryo.

Table 5.4. Calculated and predicted \dot{G}_{H_2O} in rigid-shelled reptile eggs, compared to avian eggs of comparable size. Four additional avian eggs are also included in the table for comparison.

Taxon	Species	Ref.	Nest Mode	\dot{G}_{H_2O} mg _{H₂O} / (dayTorr)	Predicted \dot{G}_{H_2O}	Difference
Gecko	<i>Sphaerodactylus</i>	3	O	1.1	5.5	0.2 x
	<i>Lepidodactylus lugruberis</i>	5	O	1.7	3.4	0.5 x
	<i>Hemidactylus garnoti</i>	5	O	2.6	4.4	0.6 x
Crocodilian	<i>C. porosus</i> (infertile)	2	M	622	810	0.77 x
	<i>C. porosus</i> (fertile)	2	M	2812	810	3.4 x
	<i>C. acutus</i>	4	B	1185	825	1.4 x
	<i>Alligator mississippiensis</i>	1	M	2902	685	4.2 x
Turtle	<i>Emydura macquarii</i>	6, 7	B	1268	150	8.3 x
	<i>Trionyx spiniferus</i>	1	B	810	150	5.46 x
Bird	<i>Gallus gallus</i>	8	O	14	9.19	1.57 x
	<i>Rhea Americana</i>	8	O	78	58.04	1.34 x
	<i>Gavia immer</i> (loon)	8	O/WH	98	23.3	4.2 x
	<i>Podilymbus podiceps</i> (grebe)	8	O/WH	13.02	4.51	2.9 x
	<i>Alectura lathamii</i> (megapode)	9	M	48.15	28.75	1.7 x

1) Packard et al., 1979; (2) Grigg and Beard, 1985; (3) Dunson and Bramham 1981; (4) Lutz et al., 1980; (5) Dunson 1982; (6) Thompson 1985; (7) Harrison et al., 1978; (8) Ar et al., 1978; (9) Ar and Rahn 1985. Nest mode: O=open; M=mound, B=buried, O/WH=open/wet or humid

While water vapor conductance rates are available for over 161 avian species (Ar et al., 1974; Ar and Rahn, 1985), only a few studies report a calculated \dot{G}_{H_2O} for rigid-shelled reptile eggs (Table 5.4). The paucity of studies and the broad range of values reported for reptiles, along with the questionable use of constants from the avian data for calculations, contribute to the difficulty of comparing the eggs with the titanosaur

specimen. For example, *Crocodylus acutus* exhibits a \dot{G}_{H_2O} only 1.4 times greater than an avian egg of comparable mass (Lutz et al., 1980), while *Alligator mississippiensis* measures 4.2 times greater (Packard et al., 1979; Deeming and Thompson, 1991). In addition, variation in \dot{G}_{H_2O} among modern reptiles results from several factors, including the stage of embryonic development (Kern and Ferguson, 1997), intrinsic and extrinsic degradation of the eggshell that increases porosity during incubation (Ferguson 1982; Wink et al., 1990), and whether the egg is fertile or infertile (Grigg and Beard, 1985). For example, the \dot{G}_{H_2O} of an infertile *C. porosus* egg is less than that of a bird egg of comparable size, while fertile eggs are 3.4 times higher (Table 5.4). Similarly, some geckos incubate rigid-shelled eggs in atmospheric conditions like birds, yet their eggs exhibit lower conductance rates than equivalent-sized avian eggs (Dunson and Bramham 1981; Dunson 1982); the lower \dot{G}_{H_2O} most likely results from their lower metabolic rate compared to birds (Richlefs and Stark, 1998). Nevertheless, comparison of the Auca Mahuevo and Pinyes specimens provides important information on egg incubation when considered within the sedimentological and taphonomic context of the two localities. Factors that might influence variation in water vapor conductance rates between eggs from the two localities include (1) incubation strategy, (2) total clutch metabolism and embryonic growth rates (3) climate regime, and (4) evolutionarily disparate taxa.

Incubation Strategy

Auca Mahuevo Locality. Presumably the Argentine titanosaurs employed the same strategy when constructing their nests in sandy or fine-grained areas of the

floodplain. The specimen in this study comes from the mudstone facies of egg bed 3, and the low \dot{G}_{H_2O} independently supports the previous interpretation (based on nesting traces from the sandstone facies) that the eggs were not buried in the substrate (Chiappe et al., 2004). The limited soil oxygen diffusion capacities of the clay-rich vertisols (Loope et al., 2000) would amplify an already limited oxygen uptake potential that results from the egg morphology and pore structure. While substrate egg burial can be ruled out on the basis of sedimentological evidence, the nest environment remains uncertain.

Nevertheless, suggestions that the Auca Mahuevo titanosaurs incubated eggs in high humidity conditions, consistent with vegetation in the nest (Grellet-Tinner and Chiappe 2004; Grellet-Tinner et al., 2006), are misleading and warrant further discussion.

The hypothesized incubation environment (Grellet-Tinner and Chiappe 2004; Grellet-Tinner et al., 2006) was based on comparison of the Auca Mahuevo titanosaur eggs to megaloolithid eggs studied by Williams et al. (1984) from southern France. These French eggs (identified as Type 3.1 Sample B) exhibit a water vapor conductance rate calculated as 24 times greater than modern avian eggs of equivalent size. The Auca Mahuevo eggs were thought to exhibit pore size, shape, and geometry similar to the French eggs; therefore, the authors assumed that the Argentine eggs must have a similar gas conductance rate (Grellet-Tinner and Chiappe, 2004; Grellet-Tinner et al., 2006).

The 21 cm x 21 cm diameters of the French eggs, however, are considerably larger than the 12 cm x 14 cm Auca Mahuevo specimens, and exhibit a greater volume and lower surface area-to-volume ratio. The French eggs also have a thicker shell, which reduces \dot{G}_{H_2O} ; however, the much denser pore distribution, compared to the Argentine

titanosaur egg (compare Fig. 6.4B to Williams et al., 1984: figure 2B), more than compensates for the increased shell thickness, resulting in a much higher \dot{G}_{H_2O} .

Although Deeming (2006) corrects errors in the original calculations and reports \dot{G}_{H_2O} for the French egg as 2322 mg_{H₂O}/(Torr day), this value remains seven times higher than the Auca Mahuevo egg. The French eggs, therefore, are not an appropriate comparison for inferences about incubation environment at the Auca Mahuevo locality.

In addition, Grellet-Tinner and Chiappe (2004) and Grellet-Tinner et al., (2006) equate surface ornamentation with the presence of vegetation in a trace fossil nest described by Chiappe et al. (2004). They suggest, “the grain size of the siliciclastic (*sic*) sediment at the Auca Mahuevo site is overall smaller than the minimum internodal distance necessary for the pore to be functional”; vegetation, therefore, was considered necessary to prevent occlusion of the pores (Grellet-Tinner et al, 2006: p. 299).

However, several modern turtles species (including living tortoises nesting at the Auca Mahuevo locality) bury their eggs in fine-grained sediments with high clay content (Burbidge and Kuchling 1994; Horne et al., 2003; Epperson and Heis 2003). These eggs do not exhibit surface ornamentation, nor are they incubated in vegetation. Given the lack of modern analogs for megaloolithid ornamentation, its possible influence on gas conductance represents an untested hypothesis. Finally, organic material reported by Chiappe et al. (2004) from a trace fossil nest allegedly supported the presence of vegetation in the nest (Grellet-Tinner and Chiappe, 2004; Grellet-Tinner et al., 2006). However, these minute and macerated particles are identical to organic matter present on the bedding plane of the strata that underlies this clutch (unpubl. field notes). The most

parsimonious interpretation is that these organic materials, in both the nest and the underlying stratum, represent transported debris, rather than *in situ* nesting material.

Although Grellet-Tinner and Chiappe (2004) and Grellet-Tinner et al., (2006) provide inadequate evidence to support high humidity conditions or vegetation in the Auca Mahuevo titanosaur nests, data from the current study also results in uncertainty about the microclimate of the nest. The sedimentological evidence indicates only that the eggs were not buried underground, and the water vapor conductance rate further supports this interpretation. The \dot{G}_{H_2O} , however, is somewhat higher than that of most modern bird eggs and may reflect diagenetic alteration of the eggshell or differences in physiology, climate, or other factors not interpretable from the fossil record.

Pinyes Locality. In contrast to the Auca Mahuevo titanosaur clutches that contain small eggs that are unburied during incubation, *M. siruguei* eggs are larger, but the clutches contain substantially fewer eggs (Table 2). The \dot{G}_{H_2O} calculated for the Pinyes egg differs by more than an order of magnitude from that of the Auca Mahuevo egg. If incubated above ground, the abundance of pores would have resulted in substantial water loss from evaporation. In a buried clutch, however, the high \dot{G}_{H_2O} of *M. siruguei* compensated for elevated carbon dioxide and lower oxygen levels present in soils surrounding the clutch (discussed below). Although sedimentological evidence of a trace fossil nest is lacking at the Pinyes locality (Chapter 4), the calculated \dot{G}_{H_2O} supports previous interpretations of underground burial for this megaloolithid egg type (Deeming 2006).

Total Clutch Metabolism

The oxygen consumption rate of the reptilian embryo increases throughout incubation, with the highest consumption rate coinciding with hatching (Ackerman 1980; Lutz et al., 1990). The high eggshell porosity in a buried clutch facilitates the transport of oxygen and carbon dioxide through the shell. This is of critical importance when air trapped inside the nest chamber becomes hypoxic and hypercapneic in the latter stages of incubation (Seymour 1979; Ackerman 1980, Deeming 2006). In addition, the physical characteristics of the substrate (e.g., soil type, permeability, water content, aerobic microbial content) also place an upper constraint on the metabolic activity of the buried egg clutch (Ackerman 1980). In modern taxa that bury their eggs, and presumably extinct amniotes as well, this constraint is expressed as limitations on the clutch size and on incubation time (Ackerman 1980).

Based on the allometric relationship between egg weight and maximum egg metabolism in reptiles, Seymour (1979) and Seymour and Ackerman (1980) proposed that sauropods might have been forced to limited their clutch size to 13 eggs. The Pinyes clutches and those reported from Europe and India generally support this hypothesis: the eggs exhibit high water vapor and respiratory gas conductance rates, and clutches generally contain fewer than the predicted number of eggs per clutch (Chapter 4; Kerourio 1981; Cousin et al., 1994; 1999; Mohabey 1996, 1999, 2001; Sahni and Khosla 1994; Sander et al., 1998; Peitz 1999, 2000; Lopez Martinez et al., 2000; Garcia et al., 2003).

In contrast, Auca Mahuevo titanosaur clutches typically contain 20 to 40 eggs, but the eggs were not buried underground. Adequate O₂ and CO₂ exchange is much less problematic with greater exposure to the atmosphere, compared to a buried clutch. Specifically, CO₂ in soil air is often several hundred times more concentrated than in the atmosphere; oxygen concentration decreases accordingly and in some cases may be 5-10%, compared to 20% for atmospheric air (Brady and Weil 2002). Greater exposure to atmospheric gases compensated for the larger clutch size, but with a greater risk of excessive water loss due to evaporation.

Previous studies of water vapor conductance in dinosaur eggs calculate \dot{G}_{H_2O} for a single egg (Seymour, 1979; Williams et al., 1984; Sabath 1991; Girgorescu 1994; Deeming 2006). However, total clutch conductance represents a more important factor, since embryos mature as part of the total clutch environment. For example, the \dot{G}_{H_2O} of the Auca Mahuevo egg is 12 times lower than that of the Pinyes egg, but the total \dot{G}_{H_2O} per gram of clutch weight is only about 4 times lower. The titanosaur and *M. siruguei* clutches exhibit approximately equivalent clutch mass (Table 5.2). This suggests that water conservation was more important at Auca Mahuevo than the Pinyes locality and that respiratory gas exchange was a less significant factor.

In contrast to atmospheric incubation conditions, the hypercapneic and hypoxic environment of a buried clutch represents the selective pressure for increased shell conductance so that tissue gas concentrations remain tolerable during incubation (Seymour and Ackerman 1980). At the Pinyes locality, this was accomplished with a greatly enhanced total pore area.

Embryonic Growth Rates. Seymour (1979) and Seymour and Ackerman (1980) also assumed that sauropod metabolic rates were comparable to modern reptiles when calculating clutch limit. However, osteological characteristics indicate that growth rates in some sauropods were closer to those of modern birds than to reptiles, particularly in younger individuals (Ricqlés 1983, Ricqlés et al., 1991; Sander 2000; Castanet, et al., 1996, 2000, Horner et al. 2000; Curry-Rogers 2005). Because large animals grow at faster rates than small animals at all ontogenetic stages (Case, 1978; Padian et al. 2004), a metabolic rate closer to that of a modern bird would most likely produce even more rapid growth in a sauropod embryo, thus requiring a higher rate of oxygen consumption. The increased oxygen demands of a faster growth rate would represent a more significant problem in the buried Pinyes clutches than the unburied eggs of the Auca Mahuevo locality. Since other physiological factors such as tolerance to hypoxia, incubation length, presence of unpreserved soft tissues such as pore plugs, and influence of ornamentation on \dot{G}_{H_2O} are unknown, further assessment remains difficult. Nevertheless, the functional attributes that characterize both egg types represent a successful balance between water conservation and adequate oxygen and CO₂ exchange.

Climate Regimes

Variation in climatic regimes between South America and Europe in the Late Cretaceous may also have contributed to differences in egg attributes and reproductive biology at the two localities. For example, paleovertisols at the Auca Mahuevo locality in Argentina indicate a semi-arid to arid climate regime (Chiappe et al., 1999; Loope et al., 2000). These ancient soils differed markedly from those present at the Spanish

nesting site. The highly bioturbated Pinyes soil profile contains primarily kaolinite and other weathering-resistant minerals such as quartz, hematite, and magnetite (Chapter 5). This mineralogy is typical of wet/warm regions with extensive leaching (Retallack, 1990; Birkeland, 1999). However, the red oxidized color of the mudrock results from hematite at the Pinyes site and may indicate well-drained soils of a drier region, thereby contributing to the difficulty of accurate interpretation. Paleoclimate reconstructions by Scotese (2002) suggest a semi-arid to arid climate in northern Patagonia during the Late Cretaceous period and a warm/wet regime for Spain during this same time interval (Chapter 4). If correct, differences in climatic regime may have resulted in adaptations for a more arid environment at Auca Mahuevo. The low porosity of the titanosaur eggshell limits water loss to evaporation in large clutches when the eggs were unburied by sediment. In contrast, incubation underground in a warm and wet climate most likely necessitated higher porosity for respiratory gas exchange and substantially higher water vapor conductance rates, features that characterize the Pinyes specimen.

Evolutionarily Disparate Taxa

Because eggs containing sauropod osteological remains occur only at the Auca Mahuevo locality, definitive taxonomic assignment of *M. siruguei* remains questionable. Although Mikhailov (1997) argues that the Megaloolithidae represents a single dinosaur clade, the Sauropoda, this assumption remains far from conclusive. Kohring (1989) identifies *Megaloolithus* eggs from Spain as those of hadrosaurs, and Grigorescu et al. (1994) assign similar eggs from Romania to *Telmatosaurus*, based on a hadrosaur embryo that occurred 0.5 m from an egg. These taxonomic assignments, however, are

controversial. Nevertheless, hadrosaur and titanosaur ranges overlapped in the Late Cretaceous (Barrett and Upchurch 2005). Recent cladistic analyses of egg characters also indicates considerable homoplasy in the evolution of hadrosaur and titanosaur eggshell microstructures (Varricchio and Jackson 2004a; Grellet-Tinner et al., 2006; Garcia et al., 2006), and Garcia et al., (2006) suggest this homoplasy most likely depended on reproductive physiology, anatomy, and incubation environment.

Egg Incubation in Other Saurischian Dinosaurs

Deeming (2006) reports the water vapor conductance of a variety of fossil eggs, including eggs of two additional Late Cretaceous saurischian dinosaurs known from embryonic remains: oviraptorids and *Troodon*. The \dot{G}_{H_2O} calculated for both the oviraptorid egg and the Auca Mahuevo titanosaur specimen reported here is 2.1 times greater than that of an equivalent-sized bird egg (Table 5.2). Furthermore, Deeming (2006) reports the \dot{G}_{H_2O} of a *Troodon* egg as equal to that of an avian egg of comparable size. Interestingly, all three Late Cretaceous saurischian dinosaurs are known from semi-arid or arid paleoenvironments. Despite significantly lower \dot{G}_{H_2O} values compared to other fossil eggs, Deeming (2006) concludes that all dinosaurs buried their eggs in the substrate. If *Troodon* eggs exhibit a \dot{G}_{H_2O} equal to an avian egg and the oviraptorid and titanosaur eggs are both 2.1 times greater, the question arises as to what water vapor conductance rate in dinosaur eggs would Deeming consider evidence for interpretation of partial egg burial or incubation in open nests. Stated another way, when interpreting

\dot{G}_{H_2O} for fossil material, what is the margin of error for eggs that are millions of years old?

While previous studies do not address this theoretical threshold value, I suggest that water vapor conductance rates of modern amniotic eggs provide a “first estimate”, rather than proof for the incubation environment of extinct taxa. Many factors contribute to the difficulty of comparing modern and fossil specimens, such as diagenetic alteration, different analytical methodology (dyes vs. thin section), and taphonomic processes that affect egg size, shape, and determination of original pore size. Therefore, calculated rates in fossil eggs that differ by at least an order of magnitude from an avian egg of equivalent size (e.g., *M. siruguei*) allow a reasonable interpretation of substrate egg burial. Conversely, when conductance rates of a dinosaur egg are equal to an avian egg (e.g., *Troodon*), the incubation environment most likely differed from modern reptiles that bury their eggs underground (contrary to Deeming 2006). However, when rates fall between these extremes of the fossil spectrum (e.g., oviraptorid and titanosaur eggs), interpretations based on \dot{G}_{H_2O} require supporting evidence. For example, an adult oviraptorid, preserved in a brooding position, occurs on top of a clutch of unhatched eggs (Clark et al., 1999: p. 16). Detailed taphonomic study of the site shows that the adult sternal elements, ribs and gastralia rest directly on eggs, thereby precluding complete substrate egg burial as proposed by Deeming (2002, 2006). Similarly, *Troodon* and Auca Mahuevo titanosaur nesting traces also provide sedimentological evidence that the eggs were not buried underground (Varricchio et al, 1997, 1999; Chiappe et al., 2004). Independent analysis of the sedimentologic and taphonomic evidence, therefore, may

substantially strengthen or refute inferences that are based solely on calculated water vapor conductance rates.

Conclusions

This study represents the first assessment of the water vapor conductance rate of a *Megaloolithus* egg definitively identified as that of a titanosaur sauropod dinosaur. In addition, this study allows the first direct comparison between a *Megaloolithus* specimen from Europe and South America. *Megaloolithus patagonicus* clutches from Argentina typically contain 20 to 40 eggs, while an average of 9 eggs comprise the *M. siruguei* clutches from the Pinyes locality in Spain. The 15 cm titanosaur eggs are significantly smaller than the 22 cm *M. siruguei*, and the latter exhibits three times greater volume. The *M. patagonicus* egg has a thinner eggshell, with 47 times fewer pores than *M. siruguei*; however, individual pores are 1.57 times larger, with a more irregular distribution. The water vapor conductance rates of the *M. patagonicus* and *M. siruguei* eggs are 371 and 3979 mg H₂O/(dayTorr), respectively. The \dot{G}_{H_2O} of the Auca Mahuevo and Pinyes eggs are 2.1 and 10.2 times greater than avian eggs of comparable size. The total \dot{G}_{H_2O} of a 25-egg Auca Mahuevo clutch is four times lower than the 9-egg Pinyes clutch. Data from the Auca Mahuevo site, in conjunction with the six trace fossil nests, indicate that eggs were not buried in sediment. However, the precise incubation environment remains unclear, and the presence of vegetation cannot be substantiated at this time by the physical evidence of organic remains in any Auca Mahuevo nest.

In contrast, the \dot{G}_{H_2O} of *M. siruguei* differs by more than an order of magnitude from the Auca Mahuevo egg, thereby allowing reasonable inference of substrate burial in the absence of sedimentological evidence of a nesting trace. Differences in reproductive biology that characterize the two sites may result from several factors: variation in nesting strategy, total clutch metabolism, climatic regime, or life history strategies. Large clutch size and potentially higher metabolic and growth rates may have prohibited underground incubation in some Late Cretaceous saurischian dinosaurs, including the Auca Mahuevo titanosaurs and some theropod dinosaurs. Water vapor conductance rates provide a first approximation of nesting environment in extinct taxa. However, detailed sedimentologic and taphonomic data provide essential information that may support or refute hypotheses based on water vapor conductance rates in fossil eggs.

CHAPTER 6

COMPARISON OF THE PINYES AND AUCA MAHUEVO LOCALITIES:
IMPLICATIONS FOR STUDIES OF DINOSAUR REPRODUCTIVE BIOLOGYIntroduction

This study represents the first direct comparison of eggs containing definitively identified titanosaur embryos from South American to purported sauropod eggs from Europe. In this chapter, I compare and contrast the geologic, taphonomic, and biologic attributes of the Auca Mahuevo and Pinyes *Megaloolithus* nesting sties and discuss the broader implications of this research for studies of other nesting localities and dinosaur species. The chapter closes with conclusions about the dissertation results and possible direction for future research.

Depositional Environment

The Auca Mahuevo and Pinyes localities are broadly similar in some respects, but differ in others. Deposition of both the Anacleto and Tremp Formations occurred within Late Cretaceous (Campanian or Maastrichtian) foreland basins of South America and Spain, respectively (Dingus 2000; Garrido 2000; Ardévol et al., 2000; Verges et al., 2002). The Auca Mahuevo study site consists of extensive outcrops of well-exposed, relatively flat-lying strata with a 2° dip (Dingus et al., 2000). In contrast, the Pinyes exposures are more limited due to vegetation cover, and the beds dip steeply to the north at 30° (Chapter 4). The sedimentary sequences of both localities represent fluvial deposition in a floodplain environment. Thick, massive, calcareous red mudstones

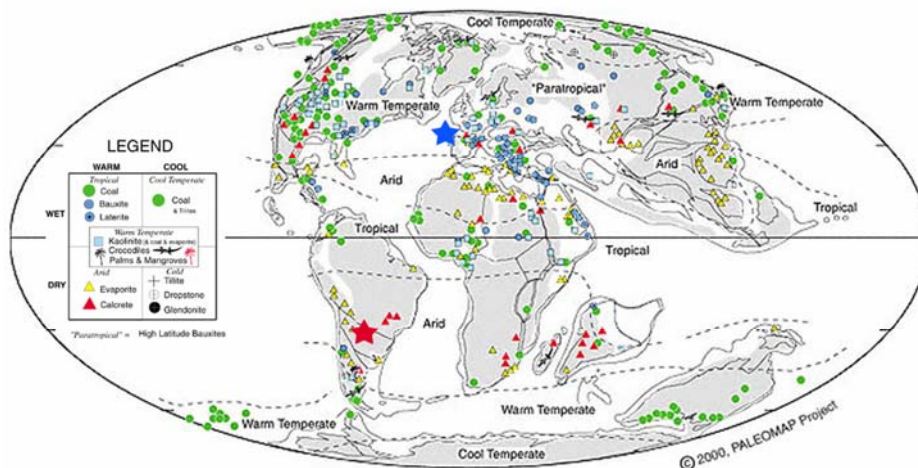


Figure 6.1. Auca Mahuevo and Pinyes localities in the Late Cretaceous Period.

are the dominant lithology at both sites, and these fine-grained mudrocks intercalate with thin, lenticular sandstones, interpreted as channel sand and crevasse splay sand bodies (Dingus et al., 2000; Chiappe et al., 2004; Chapter 4). The Pinyes and Auca Mahuevo nesting localities both occurred some distance from active stream channels, in areas characterized by infrequent flooding and well-developed paleosols (Dingus 2000; Chapters 1,2, 4).

Paleosols

The majority of clutches at both the Auca Mahuevo and Pinyes localities occur within the mudstone facies (Chiappe et al., 1999; 2004; Chapter 4). These fine-grained overbank deposits exhibit evidence of significant pedogenesis. However, the physical attributes of the paleosols differ markedly between the two sites. The egg-bearing horizons at Auca Mahuevo occur in paleovertisols, characterized by gilgai microrelief and abundant slickensides with highly variable orientations (Fig. 6.1; Chiappe et al.,

1999; Loope et al., 2000). Paleovertisol represents one of the few modern soil types easily identified in the rock record and indicate a subhumid to semi arid environment (Retallack 1997). Other pedogenic features at Auca Mahuevo include irregular blue-gray mottling and pedotubules (Retallack, 1997, 2003; Birkeland, 1999). Some tubular structures branch and taper in a downward direction, suggesting possible root traces, but preserve no evidence of *in situ* organic matter or vegetation other than transported material (Chapter 5).

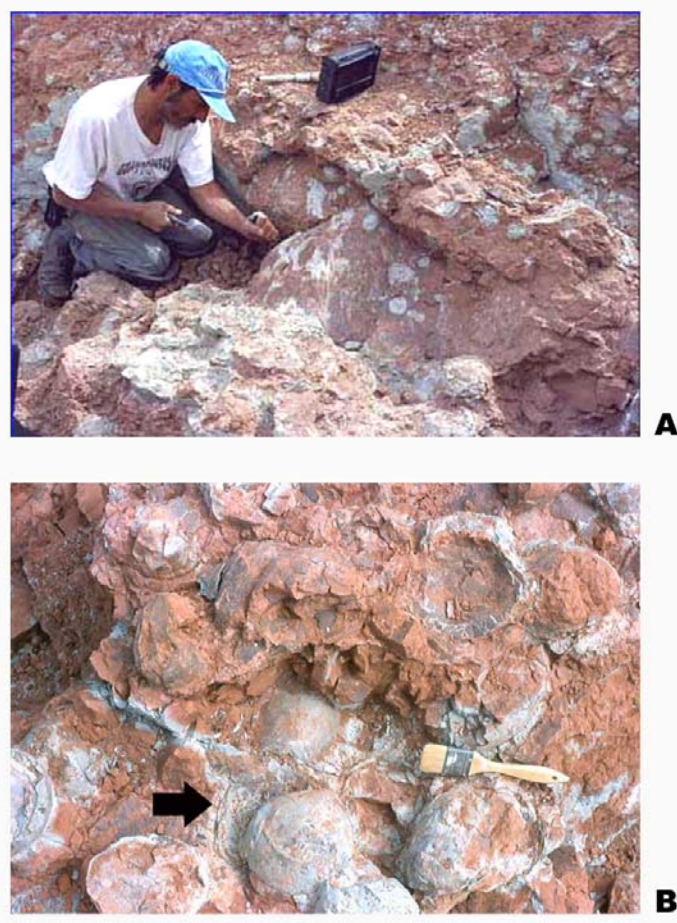


Figure 6.2. Auca Mahuevo paleovertisols. (A) Gilgai microrelief and crushed eggs in foreground (B) Eggs sheared along slickensides in the same quarry. Arrow shows offset egg hemispheres.

In contrast to the paleovertisol at the Auca Mahuevo locality, the Pinyes paleosols are more difficult to interpret (Chapter 4). The profile displays extensive bioturbation traces, corkscrew rhizoliths, and evidence of sediment reworking and burrowing activity by invertebrate organisms (Chapter 4: Figs. 4.5—4.8). Some pedotubules preserve organic matter, and the kaolinite clays contain weathering-resistant quartz, hematite, and magnetite that typify warm, wet climatic regions. However, the red oxidized colors and well-drained soils at the Pinyes locality may reflect a greater time interval, rather than a warm wet climate regime, thus contributing to the difficulty of assigning these paleosols to a modern soil type.

Eggs and Nesting Horizons

Both sites include laterally extensive egg-bearing strata, with four or more egg horizons within the stratigraphic sequence (Chiappe et al., 1999; Chapter 2,4). These repeated egg-bearing intervals vary from closely superimposed eggs to horizons separated by several meters of mudstone. The eggs from the Auca Mahuevo and Pinyes localities are referable to *M. patagonicus* and *M. siruguei*, respectively, and represent the only egg type documented at either site. Although several *Megaloolithus* oospecies share similar characteristics (Carpenter 1999), *M. siruguei* and *M. patagonicus* clearly differ in physical attributes such as egg size and volume, shell thickness, eggshell microstructure, pore density, and water vapor conductance rates (Chapters 4, 5). In addition, Pinyes clutches contain an average of 9 eggs, while Auca Mahuevo clutches typically contain 20 to 40 eggs. At both sites, the eggs occur in one or more layers within the clutch and display no discernible pattern of egg distribution (Chiappe et al., 1999, 2005; Jackson et

al., 2004; Chapter 4). More importantly, many Auca Mahuevo eggs contain embryonic remains that allow definitive taxonomic assignment of *M. patagonicus* to titanosaur sauropod dinosaurs (Chiappe et al., 1998; Salgado et al., 2005). In contrast, taxonomic assignment of the Pinyes eggs remains uncertain (Chapter 4).

Most egg clutches at both sites occur in uniform and homogeneous mudstone facies that provide no sedimentological evidence of nest structure (Chiappe et al., 1999; Chapter 4). However, six clutches at Auca Mahuevo occur in sandstone facies, representing the only trace fossil nests documented for megaloolithid eggs, based on lithological criteria. These six specimens are interpreted as open nests, and the eggs were not buried in the substrate (Chiappe et al., 2004). The low water vapor conductance rate calculated from an Auca Mahuevo egg also supports this interpretation. In contrast, the substantially higher water vapor conductance rate of the Pinyes egg suggests underground egg burial (Chapter 5), although this interpretation of egg incubation at the Pinyes site cannot be substantiated by sedimentological evidence (Chapter 4).

Eggs from the Pinyes locality often show elongation due to tectonic stresses in the region (Chapter 4), while Auca Mahuevo specimens display considerably distortion of egg size and shape. This deformation results from lithological compaction and soil movement associated with shrinking and swelling of the high clay content paleovertisols. For example, Auca Mahuevo eggs typically measure 12-15 cm in diameter; however, those associated with gilgai microrelief may be compressed to a thickness of a few centimeters and sheered in half along slickensides (Fig. 6.1). The vast majority of Auca Mahuevo eggs are unhatched (Chiappe et al., 1998, 1999) and little or no fragmented

eggshell occurs within or between clutches. For this reason, no assessment can be made of the presence or absence of a “hatching window” in the upper egg surface. In contrast, eroded or hatched eggs predominate at the Pinyes site. Abundant eggshell debris and the combination of intact and partial eggs in the same clutch suggest successful hatching occurred in at least some clutches. Although pathological *M. siruguei* eggshell fragments are occasionally reported from Spain and France (Erben et al., 1979; Vianey-Liaud et al., 1994), this abnormal condition remains undocumented at the Pinyes locality. In contrast, six clutches at Auca Mahuevo contain pathological eggs and represent the only *in situ* abnormal eggs known for any dinosaur species (Chapter 2).

Recent erosion, enhanced by the steeply dipping strata, limits lateral continuity of the egg-bearing horizons at the Pinyes locality; therefore, comparison of clutch distribution between the Auca Mahuevo and Pinyes localities is difficult despite three-dimension mapping efforts. Three to six meters often occur between Auca Mahuevo clutches (Chiappe et al., 2005), while some Pinyes clutches appear more closely spaced (Fig. 4.14). Precise time resolution within the stratigraphic sequence at both localities remains problematic. However, the vast number of egg clutches and the lateral continuity of these egg-bearing strata suggest that the areas were most likely used concurrently by multiple individuals over an extended but unknown time interval.

Faunal Composition

The faunal composition documented at the Pinyes and Auca Mahuevo localities also differs. In addition to fossil eggs containing embryonic remains in Argentina, rare adult titanosaur bones are occasionally present within the egg-bearing layers. In addition,

a horizon containing thousands of sauropod footprints occurs 2.5 m above egg bed 4 (Loope et al., 2000; Chiappe et al., 2004), and egg bed 3 preserves at least one sauropod footprint adjacent to an egg clutch (Schmitt, per. com.). Tracks, however, have not been documented at the Pinyes locality.

Invertebrate fossils reported at the Pinyes site include bivalve and gastropod casts, and meniscate burrows interpreted as evidence of arthropod behavior; these



Figure 6.3. Bivalve cast.

fossil are not present within the Auca Mahuevo section. Both sites, however, preserve evidence of insect boring associated with the fossil eggs (unpublished data, Chapter 4). The Argentine specimens also contain features interpreted as insect frons (FJ unpub. data) and arthropod body parts (Chiappe, pers. com.). Differences in the faunal components of the two study sites may reflect sampling bias from more fieldwork and larger crews at Auca Mahuevo over the past five years. Future research at the Spanish locality may significantly alter some attributes listed in Table 6.1 below.

Table 6.1. Comparison of the Auca Mahuevo and Pinyes localities

	Auca Mahuevo, Argentina	Pinyes Locality, Spain
General Information		
Tectonic Setting	Foreland basin	Foreland basin
Formation	Anacleto	Tremp
Climatic Regime	Semi-arid to arid	Indeterminate
Late Cretaceous Age	Campanian	Maastrichtian/?Campanian
Outcrop Exposures	Extensive, laterally continuous	Extensive but with vegetation cover
Bed Dip	< 2°	30° N
Depositional Setting	Distal alluvial plain	Distal alluvial plain
Lithological Features		
Dominant Facies	Overbank mudstone	Overbank mudstone
Color	Red	Red
Cement	Calcareous	Calcareous
Pedogenic Development	Extensive	Extensive
Pedogenic Features	Gleyed mottles, gilgai microrelief, rootlets, sparse pedotubules, abundant slickensides	<i>Spirographites ellipticus</i> , abundant mottles pedotubules, organic material in tubules, gleyed
Paleosol Type	Paleovertisol	Indeterminate
Biological Features		
Number of Horizons	> 4	> 4
Egg Type	<i>M. patagonicus</i>	<i>M. siruguei</i>
Embryonic Remains	Titanosaur sauropod bone, skin	None
Egg Horizons	Laterally continuous, exposed	Laterally continuous but limited by vegetation cover
Egg Size	12-15 cm	16-22 cm
Egg Shape	Round to subrounded	Round to subrounded
Shell Thickness	1.4 mm	2.4 mm
Typical Clutch Size	20-40 eggs	6-10 eggs
Egg \dot{G}_{H_2O}	341.3 mg _{H₂O} /(dayTorr)	3978.9 mg _{H₂O} /(dayTorr)
Clutch \dot{G}_{H_2O}	8,532 mg _{H₂O} /(dayTorr)	35,810 mg _{H₂O} /(dayTorr)
Nest Structure	Open nest, possibly some vegetation	Likely buried, based on eggshell conductance rate
Inter-Clutch Distance	3-6 m	3.5 m, limited observations
Egg Abnormalities	5 partial clutches & 1 complete clutch that contain abnormal eggs; other multi-layered eggshell fragments present	None
Taphonomic Features		
Eggshell Around Clutch	Little to none	Abundant
Egg Condition	Mostly unhatched, crushed	Mostly hatched and/or eroded
Features of Egg In-fill	Mudstone, slickensides, mottles	Mudstone, mottles, burrows
Other Faunal Remains in Nesting Horizon	Subadult titanosaur bones, foot print beside nest, insect fras and body parts, egg borings	Rare bivalve, gastropod, arthropod egg borings

Discussion

The association of megaloolithid eggs with titanosaur sauropods is deeply rooted in the paleontological history of the Pyrenees (Buffetaut and Le Loeuff 1994; Carpenter 1999). However, this study represents the first comparison of eggs containing titanosaur embryonic remains from South American to purported sauropod eggs (*Megaloolithus siruguei*) from Europe. The differences in nesting biology documented at the Auca Mahuevo and Pinyes localities produce further doubt about the hypothesized monophyly of the Megaloolithidae (Mikhailov 1997; Vianey-Liaud et al., 2003). Nevertheless, the context and spatial association documented from fossil egg horizons provide the essential framework for comparing dinosaur reproductive biology from different paleogeographic regions and time periods. Careful analysis of such taphonomic data can shed light on a wide array of palaeobiological questions (Rogers 1994).

Wood and Johnson (1978) affirm the importance of these analyses in the following statement: “If we fail to record the context, or if we misread or misinterpret that context, accurate interpretations are impossible.” Research at the Pinyes and Auca Mahuevo localities emphasizes several important aspects essential for accurate interpretation of dinosaur reproductive biology: use of appropriate modern analogs, collection of detailed taphonomic and sedimentologic data, assessment of tectonic deformation, and the importance of three-dimensional mapping. Two additional issues with broad implications for studies of fossil egg horizons warrant further discussion: (1) taxonomic egg identification and (2) pedogenic influence on inferences of dinosaur behavior.

Taxonomic Egg Identification

In the study of fossil eggs, some investigations do not require definitive egg identification—for example, determining \dot{G}_{H_2O} for comparison of egg incubation environments, or the use of eggshell horizons as stratigraphic markers. However, taxonomic egg identification is essential when comparing egg size to adult body size, for assessment of k- and r-strategies (Paul 1994), or determining the turning radius of an adult female dinosaur for inferences of nesting behavior (Cousin et al., 1994). Two additional types of investigations that require positive egg identification include assessment of dinosaur distribution and phylogenetic analyses that are based on reproductive characters.

Dinosaur Distribution. Fossil eggs are sometimes used for assessment of temporal and geographic distribution of dinosaur populations in the fossil record (Vianey-Liaud et al., 1994; Vianey-Liaud and López-Martínez, 1997; López-Martínez 2003; Vianey-Liaud et al., 2003; Vianey-Liaud and Garcia 2003). Furthermore, the eggs are often assigned to a specific clade, based on stratigraphic and geographical data. Megaloolithid eggs from Upper Cretaceous rocks of Europe, Asia, and South America are nearly always assigned to titanosaur sauropods, presumably because all other sauropods were considered extinct by Late Cretaceous time. For example, based on the absence of osteological remains, Buffetaut et al. (1997) suggest that titanosaurids disappeared in Europe some time prior to the late Maastrichtian, while López-Martínez et al. (2001) used *Megaloolithus* eggshells within a stratigraphic sequence to support a continuous titanosaur record from late Campanian to latest Maastrichtian in Europe.

Carrano and Wilson (2001) referred to this method of taxonomic assignment as “coincidence correlation” (in the case of dinosaur tracks). The authors argue that the limitation of this method is that it can only substantiate currently established taxon abundance and distributions, decreasing the likelihood that foot prints (or fossil eggs) will further promote examination or change in accepted distributional hypotheses that are based on osteological remains.

Over-generalization to a broader taxonomic group also represents a problem with indirect methods of egg and taxon correlation. For example, the discovery of embryos in Auca Mahuevo eggs provides evidence that at least one *Megaloolithus* egg type belonged to titanosaur sauropods. However, the tendency now is to extend this taxonomic identification to other egg types within the Megaloolithidae (López-Martínez et al., 2001; Garcia et al., 2003; 2006). As illustrated by the current study, however, some megaloolithid oospecies differ significantly from Auca Mahuevo specimens in egg and clutch size, shell unit shape, shell thickness, surface ornamentation, pore structure, and water vapor conductance rate (Chapters 4- 6; Carpenter 1999). Therefore, it appears questionable whether all megaloolithid eggs are those of titanosaurs, and the potential consequences of misidentification are significant.

Taxonomic Misidentification. If fossil eggs are incorrectly identified as titanosaur, this may misrepresent the spatiotemporal distribution of titanosaurs *and* the taxon actually responsible for producing the eggs. Furthermore, if this alleged sauropod taxon (based on misidentified specimens) implies an unusually early first occurrence in a geographical area, then the temporal and geographic distributions of all titanosaurs may

be falsely extended beyond the evidence provided by osteological remains (Carrano and Wilson 2002). The following example illustrates this potential problem. Titanosaurs are represented in the Maastrichtian in North America by *Alamosaurus*. In the United States, these titanosaur osteological remains are reported only from Utah, Texas, and New Mexico (Wilson 2005; Lehman et al. 2006). *Megaloolithus* eggshell, however, was tentatively identified from the Late Cretaceous Two Medicine Formation of Montana recently and resembles the Auca Mahuevo titanosaur specimens. Taxonomic assignment of the Two Medicine eggshell to a titanosaur (based on superficial similarity) would dramatically alter the known temporal and geographic distribution of titanosaur sauropods in the Late Cretaceous of North America. Adequate specimens, intact eggs, and additional reproductive characters (e.g., clutch size, egg arrangement) would allow the inclusion of the specimen into a cladistic analysis of egg and reproductive features. This would provide greater confidence in the assignment of the specimen to a titanosaur. However, taxonomic rather than parataxonomic assignments based on eggshell fragments should be viewed with caution because of the broader implications for paleobiogeography.

Characters Used in Cladistic Analyses. Hypotheses about evolutionary relationships also require definitive taxonomic identification of eggs representing outgroups used in phylogenetic analyses (Varricchio and Jackson 2004). Furthermore, a thorough taphonomic assessment is necessary to establish the validity of reproductive characters used in the study (e.g., egg and clutch size, eggshell microstructure, nest architecture, method of egg incubation). Taphonomic and sedimentologic context,

therefore, are essential for ensuring that observed features result from dinosaur reproductive biology or behavior, rather than geologic or pedogenic processes. The latter obviously provides no information pertinent to reproductive biology, paleoecology, or phylogenetic relationships. Many factors contribute to the difficulty of accurate interpretation of reproductive attributes (Chapters 2-6), but pedogenesis may represent the most significant barrier to studying dinosaur nesting horizons and accurately interpreting the reproductive behavior of extinct animals.

Pedogenesis: Inferences of Dinosaur Behavior

Most dinosaur nesting localities occur in pedogenically-modified mudstone (Lorenz and Gavin 1984; Cousin et al., 1994; Sanz et al., 1995; Varricchio et al., 1999; Chiappe et al., 1999; Mueller-Towe et al., 2002 Cojan et al., 2002, Lopez-Martinez 1999; Paik et al., 2004). The degree of soil development within these sedimentary sequences relates to the residence time of the sediment within the active zone of pedogenesis (Retallack 1990). The estimated time required for soil formation ranges from 10^3 - 10^5 years, depending on climate, soil characteristics, and other factors (Brady and Weil, 2002). This interval provides sufficient time for extensive modification or obliteration of the original soil fabric. As noted by Martin (1999), pedogenesis represents a major impediment to bridging the gap between ecological (short term) and geologic (long term) processes.

Recognition of a single nesting event, for example, is critical for accurate interpretation of paleoecology and reproductive behaviors such as site fidelity, colonial

nesting, and gregarious interactions of an extinct animal (Chapter 4). While nesting horizons are often considered mass mortality events (Rogers, 1993), biogenic and physical mixing of eggs within a stratigraphic interval can produce a time-averaged deposit (Chapter 4). Walker and Bamback (1971) define a time-averaged fossil assemblage as those fossils accumulated from a local living community during the time required to deposit the containing sediments. An inherent bias exists toward time-averaging in the rock record because biological generation times (and nesting events) are typically much shorter, relative to net rates of sediment accumulation (Martin 1999). For example, when sedimentation rates are low (e.g., Pinyes locality), intensive nesting activity over decades, hundreds, or even thousands of years may result in closely spaced, superimposed egg clutches, that are easily mistaken for a single nesting horizon.

Time-averaging also results from soil movement. For example, repeated shrinking and swelling of the high clay-content vertisols at Auca Mahuevo (Loope et al., 2000) resulted in the development of gilgai microrelief (Brady and Weil, 2002), thereby producing substantial compaction, shearing, and egg displacement (Fig. 6.1A,B). Egg clusters sometimes contain more than 50 eggs. This time-averaging of eggs and/or nesting horizons obscures the biological pattern and prohibits accurate assessment of sauropod reproductive behavior. Furthermore, the subsurface expression of this process produces intersecting arcs that form bowl-shaped depressions (Retallack 1997; Brady and Weil 2002). Misinterpretation of a nest may easily result when these gilgai micro-lows contain fossil eggs. Pedogenic processes, therefore, profoundly impact temporal

resolution over short time scales (i.e. years to centuries) (Martin 1999), contributing to the difficulty interpreting fossil egg assemblages.

Timing of pedogenesis relative to a nesting event also represents a significant problem for interpretations of reproductive behavior. Lopez-Martinez et al. (2000) consider reduced color of the mudrocks surrounding eggs at the Bicarri site in Spain as evidence that the eggs were not buried in the substrate; presumably, the water-logged sediments would inhibit adequate oxygen exchange necessary for embryonic development (Chapter 4). The authors argue that pedogenic features such as rhizoliths, burrows, and other soil features developed *after* the clutch was laid. However, lowering of sea level within a basin may initiate soil-forming processes, and the former mudflats may have occupied a significantly higher position, relative to sea level, when dinosaurs nested at this locality. Without preservation of primary sedimentary structures, interpretations of nest architecture and nesting behaviors are questionable (Chiappe et al., 2004).

Conclusions

Megaloolithid eggs from Europe and Asia have long been associated with titanosaur sauropod dinosaurs. This dissertation research provides compares *Megaloolithus patagonicus* eggs and nesting sites, definitively identified as those of titanosaur sauropods, to a *Megaloolithus siruguei* locality in Spain. This study combines taphonomy and eggshell microstructural studies to test hypotheses regarding titanosaur reproductive anatomy, physiology, and nesting behaviors. The results of these studies

indicates that biologically significant differences existed in the reproductive biology of the Auca Mahuevo titanosaurs of Argentina, compared to the taxon producing *Megaloolithus siruguei* eggs at the Pinyes locality in Spain. These differences include egg and clutch size, shell structure, eggshell porosity, water vapor conductance rates, and incubation mode. Numerous factors may have influenced this divergent pattern of reproductive characteristics: the eggs may reflect differences in embryonic physiology, climatic regime, and life history strategies. Careful assessment of reproductive attributes is essential in order to distinguish features that result from biological processes from those resulting from geologic, taphonomic, or pedogenic processes. Taxonomic identification, rigorous taphonomic procedures (three-dimensional mapping, facies analyses), use of appropriate modern analogs within the EPB of dinosaurs, and a conservative approach to interpretations will provide more reliable data for understanding dinosaur reproduction, distribution, and paleoecology.

Future Directions

Detailed comparative studies of dinosaur nesting horizons will hopefully lead to taphonomic models for the formation of fossil egg assemblages. These types of models are of heuristic value if they can demonstrate that at least some characteristics of fossil egg assemblages are predictable, despite the complexity of their formation (Martin 1999). Data generated from such models may eventually allow recognition of recurring patterns of preservation in a particular sedimentary context (Behrensmeyer, 1988). These patterns, and presumably the processes that produced them, may potentially reflect environmental phenomena such as population dynamics, continental fragmentation, speciation and

extinction, and reproductive behavior of extinct animals. Recognition of valid and biologically significant reproductive attributes, therefore, may provide important clues to the evolution of reproductive characteristics and dinosaur success throughout the Mesozoic.

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