Abnormal, multilayered eggs occasionally occur in extant amniotes and result from a variety of noxious stimuli. Microscopic examination of some fossil dinosaur eggshells (Fig. 1A) reveals similar structural abnormalities. The eggshell exhibits an unusually thick shell with two superimposed eggshell layers (Erben, 1970; Erben et al., 1979; Kerourio, 1981; Hirsch, 1994; Hirsch et al., 1989; Zelenitsky and Hills, 1997; Vianey-Liaud et al., 1994). The lower shell layer may be of normal thickness and structure, whereas the overlying layer may be structurally organized but thinner. Similar multi-layered eggshell occurs with relative frequency in hard-shelled eggs of extant turtles (Fig. 1B) and results from egg retention by the female, often a result of stress (Erben et al., 1979; Ewert et al., 1984).

Although very rare, multiple eggshell layers also occur in birds (Fig. 2). This eggshell condition represents one form of structural abnormality referred to as ovum in ovo (“egg within an egg”) in the older avian literature (Hargitt, 1897; Von Nathusius translated by Tyler, 1964; Curtis, 1916; Asmundson, 1931; Romanoff and Romanoff, 1949). In avian eggs, ovum in ovo includes a variety of conditions. These range from an egg separated from an additional eggshell layer by membrane (described here) to an egg separated from an additional eggshell layer by albumen and/or yolk. Although ovum in ovo is documented in the early avian literature, descriptions focused primarily on forms that include albumen and/or yolk rather than superimposed eggshell layers (Hargitt, 1897; Von Nathusius translated by Tyler, 1964; Curtis, 1916; Asmundson, 1931; Romanoff and Romanoff, 1949). Furthermore, these descriptions were written prior to the use of scanning electron microscopy for study of eggshell structure. Documentation includes only hand-drawn illustrations of microscopic thin sections of eggshell and schematic diagrams of abnormal eggs. The abnormal structure, therefore, is often ambiguous and difficult to interpret.

More recent studies of egg abnormalities (Grau and Kamei, 1949; Sykes, 1955; Tyler and Simkiss, 1959; Hughes et al., 1986; Solomon et al., 1987) document and categorize various deposits of calcitic material on the eggs of domestic fowl, but do not include a detailed description of the structural features of ovum in ovo. In an excellent review of eggs of domestic fowl, Solomon (1997) published a scanning electron micrograph of the superimposed shell layers of a double-shelled egg and a brief statement about abnormal egg formation. The oblique angle of the micrograph, however, revealed little detail of the eggshell structure.

The absence of documentation of superimposed, multilayered eggshell (lacking albumen and/or yolk) in extant birds resulted in a hypothesized “reptilian-like” reproductive system in some dinosaurs (Hirsch, 1989, 1994; Hirsch et al., 1989; Carpenter et al., 1994; Zelenitsky and Hills, 1997). This hypothesis was questioned based on evidence of multilayered eggshell in a domestic hen (Jackson et al., 1998). Recently, it was suggested that the second eggshell layer in this hen egg (Solomon, 1997:fig. 76; Jackson et al., 1998) might represent an amorphous deposit rather than the structured layer found in true multilayered dinosaur eggs and therefore an inappropriate comparison for fossil material (Hirsch, 2001). Furthermore, Hirsch (2001) indicated that the eggshell pathology resulted from artificial adrenaline injection and therefore multilayered eggshell had not been reported in birds as a result of naturally stimulated adrenaline due to stress. However, the author mistakenly attributed the double-shelled hen egg to an earlier study by Solomon et al. (1987), a study that contains no reference to double-shelled eggs resulting from the experimental adrenaline injection.

The apparent confusion over the occurrence and morphology of double-layered eggshell in extant birds suggests that careful documentation of structural characteristics of egg abnormalities is essential for inferences of soft tissue anatomy in dinosaurs based on modern analogs. We describe the eggshell structure of a multilayered egg from a Japanese quail (Coturnix japonica), and discuss phylogenetic bracketing for interpretation of reproductive anatomy of dinosaurs.

**METHODS AND MATERIALS**

Thirty unincubated Japanese quail eggs (Coturnix japonica) were obtained from a random-bred colony maintained by the Department of Animal Science, University of California, Davis. The birds receive water and commercial chicken breeder ration ad libitum. Quail hens lay eggs from about six weeks of age until approximately seven to nine months. The birds are then culled from the colony due to low fertility and poor shell quality. Specimens included 29 “normal” eggs with tan, black and brown pigmentation and one pale blue egg of similar size.

All eggs were produced and stored under similar conditions. The pale blue egg sustained damage during shipment, resulting in the fortuitous discovery of the double eggshell layers. The eggs were measured and fragments were examined from a normal egg and the abnormal, double-shelled egg.

Scanning electron microscopic examination using a J. R. Lee Instrument and Jeol 6100/Noran SEM included coated (10 nanometers of gold) and uncoated specimens. The abnormal specimen, MOR-ES-0015, and normal specimen, MOR-ES-0016, are curated at the Museum of the Rockies (MOR), Montana State University, Bozeman, Montana. Eggshell structural terminology used in this paper follows Nys et al. (1999).

**RESULTS**

The 29 normal Japanese quail eggs measure 27.6 mm × 23.3 mm to 32.0 mm × 24.0 mm. They exhibited brown, tan, and black pigmentation characteristic of this species. Examination of eggshell fragments from a normal egg (MOR-ES-0016) shows typical eggshell structure (Fig. 3A). The mineralized portion of the eggshell, excluding membrane and cuticle, is approximately 183 μm thick.

The anomalous pale blue egg (approximately 30 mm × 23 mm) is slightly crushed, revealing an egg with yolk, desiccated albumen, and normal pigmentation beneath the outer blue eggshell that covered the entire egg. The two distinct shell layers are 344 μm (Fig. 2A). Little or no membrane remains at the base of the mammillary cones of the inner eggshell and the mineralized portion of this shell is 185 μm thick. Manual separation of the two eggshell layers shows that the inner egg has pigmentation typical of a Japanese quail egg, and the “crazed” surface texture (Fig. 2D) characteristic of the cuticle. Although extremely thin in one fragment, the cuticle of the inner egg generally ranges from 15 μm to 21 μm in thickness.

Immediately above the cuticle, membrane fibers (Fig. 2C) attach to the mammillary cones (Fig. 2B) of the outer, blue eggshell. This additional shell layer consists of only mammillary and palisade layers, with no evidence of external or cuticle layers. The outer, mineralized portion (“true” shell) measures approximately 118 μm thick. Another shell...