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How Do Embryonic Turtles Process Yolk?

Evidence from the Snapping Turtle, *Chelydra serpentina* (Chelydridae)

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RUNNING HEAD: YOLK PROCESSING IN A TURTLE

19 Pages; 3 Figures; 0 Tables

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**Abstract:** Compared to amphibians, oviparous reptiles and birds lay large eggs that contain abundant yolk. Because the yolk is extracellular, it must be taken up by cells of the yolk sac and metabolized, so that products of yolk digestion can be transported to the embryo to fuel development. In birds, the yolk is processed by cells that line the inside of the yolk sac. A very different developmental pattern recently has been demonstrated in lizards and snakes, in which the yolk sac cavity is converted to a compact mass of blood vessels lined by endodermal cells. In this study, we used scanning electron microscopy to determine which of these developmental patterns (if either) occurs in a representative chelonian, the North American snapping turtle, *Chelydra serpentina* (Linnaeus 1758). Our observations reveal that yolk-filled endodermal cells progressively fill the yolk sac cavity. These cells become organized around anastomosing blood vessels, forming elongated strands that are morphologically well-suited for yolk digestion and vascular transport of nutrients. This developmental pattern shares features with that of squamates, but differs markedly from that of birds. These observations indicate that mechanisms of yolk processing in lizards and snakes have an evolutionary history that predates the squamate clade.

**Keywords:** Development, Embryology, Morphology, Oviparity, Turtles, Snapping Turtle, *Chelydra serpentina*
Introduction

Developmental and reproductive morphology of reptiles are subjects of active research by biologists seeking to answer fundamental questions about function and evolution. Most published work in this area has focused on squamates, as shown by recent compilations of major reviews on lizards (Rheubert et al. 2015) and snakes (Aldridge and Sever 2011). Such features have received much less attention in chelonians, despite reviews of turtle embryology (Ewert 1985; Miller 1985) and reproduction (Kuchling 1989; Miller and Dinkelacher 2007; also see Wynecken et al. 2008). Among many other aspects, the pattern by which embryos cellularize and process yolk is as yet unknown in turtles.

Two distinct patterns of yolk processing have been documented in Sauropsida. In birds, the liquid yolk is held in a sack-like structure (the yolk sac) that is lined internally by endodermal cells. These cells take up and digest yolk material, and release its digestive products into underlying blood vessels for delivery back to the embryo (Lambson 1970; Bauer et al. 2013). A very different pattern has recently been documented among squamates (Elinson and Stewart 2014; Elinson et al. 2014). In snakes (Powers and Blackburn 2017a, b) as well as lizards (Blackburn et al. 2018), endodermal cells invade the yolk sac cavity and phagocytose yolk. Subsequently, blood vessels invade and each becomes surrounded by a monolayer of yolk-filled endodermal cells. As a result, the yolk sac cavity becomes filled with a compact mass of “spaghetti-like” strands of blood vessels coated by yolk-filled endodermal cells. This developmental pattern allows extracellular yolk material to be cellularized, digested, and transported to the embryo for development and growth.
The present investigation draws on electron microscopy to analyze the mechanism of yolk processing in a representative chelonian. Our goal was to determine whether turtles process yolk as do lizards and snakes or by the very different means documented in birds. Our study species is the snapping turtle, *Chelydra serpentina* (Linnaeus 1758), an aquatic, omnivorous species that inhabits lakes, streams, bogs, and brackish tide pools (Ernst and Barbour 1989; Ernst and Lovich 2009). This species is widely distributed in eastern and central North America including southern Canada (Gibbons et al. 1988; Shaffer 2008; Ernst and Lovich 2009). Female *C. serpentina* reproduce seasonally, laying a single clutch of eggs per year (White and Murphy 1973; Congdon et al. 1987). Clutch sizes of 25-45 eggs are common (Ernst and Lovich 2009), although average clutch size varies geographically from 18 to 83 eggs (Iverson et al. 1997; Congdon et al. 2008). Eggs are spherical with flexible (leathery) shells and typically measure about 26-28 mm in diameter (Iverson et al. 1997; Congdon et al. 2008). Incubation in nature commonly takes 75-95 days (Ernst and Lovich 2009), depending on locale and temperature conditions.

Our study tests the hypothesis that the pattern by which lizards and snakes process yolk is also found among chelonians, thereby revealing whether or not this pattern is likely to predate the squamate clade, perhaps even being ancestral for Reptilia. Our findings therefore contribute to an evolutionary reconstruction of yolk-processing patterns in Reptilia (including birds), and potentially, in amniotes in general.
Materials and methods

*Chelydra serpentina* eggs were purchased from a dealer in Arkansas, USA and were sent by overnight mail to Trinity College in Connecticut. Eggs were buried in moist vermiculite held in plastic containers with lids, and incubated in a reptile colony at 26°C on a 14L/10D light cycle. Procedures for housing the eggs were in accord with the institutional guidelines of Trinity College. Eggs were dissected and examined via a Wild stereomicroscope equipped with a Micropublisher 5.0 digital camera (QImaging Corp., Surrey, British Columbia). Embryos were removed and placed in Ringer’s solution (Humason 1962), photographed, fixed in 10% formalin, and stored in 70% ethanol. Embryonic development was characterized according to Yntema’s (1968) classification system for *C. serpentina* (as reproduced by Porter 1972). Although many of the eggs died during transport, a total of 45 usable tissue samples were obtained from 11 eggs. These contained embryos of stages from throughout most of the developmental period: stages 17-18, 23, 24, and 25, the last of these being the stage just before hatching.

For electron microscopy (EM), pieces of yolk and other yolk sac contents were dissected free, rinsed in Ringer’s solution, fixed in Karnovsky’s solution (3% paraformaldehyde and 1.5% glutaraldehyde in 0.1 mol/l sodium cacodylate buffer), and refrigerated in buffer. Small pieces were post-fixed on ice in a 2% osmium tetroxide/ buffer solution. These pieces were dehydrated in an ethanol series and dried with liquid CO$_2$ in a critical point dryer (Leica EM CPD300). They were then mounted on aluminum stubs and coated with gold/palladium in a Denton DV-502A sputter coater. The tissue samples were examined and photographed using a Zeiss EVO LS15 scanning electron microscope (SEM). Photomicrographs were minimally
processed and labeled using Adobe Photoshop Elements 8.0 (Adobe Systems Incorporated, San Jose, CA, USA).

Results

General observations

Eggs and embryos in our study ranged from a limb-bud stage through the stage just before hatching. In the earliest stages observed (Yntema stage 17-18), five digits on the forelimb are well-demarcated, the lower jaw extends beyond the level of the lens, and marginal lamina of the carapace are evident. By stage 23, the body form is fully established, the claws are heavily pigmented, and the body and shell are pigmented and brown in color (Fig. 1b). By stage 25, the size and proportions of the body approach that of the hatchling; likewise, black pigmentation has is evident on the head and carapace (Fig. 1c).

Under dissection, the yolk and yolk sac contents appear in three different forms that represent a developmental sequence. In eggs with limb-bud embryos, most of the yolk material is liquid and oozes from incisions in the eggshell along with albumen. Liquid yolk is increasingly replaced during development by clumps of material of a more solid consistency. These clumps represent the cellularized yolk apparent via SEM. In eggs as late as stage 23, considerable liquid yolk is still present. Elongated strands of cells increasingly predominate in stages 23-25. However, even eggs of stage 25 contain cell clumps that have not undergone transformation into the elongated strands.
Liquid yolk

Liquefied yolk consists of spherical elements immersed in a matrix. As seen via SEM, its composition appears similar in eggs of stages 17 through 23, although liquid yolk diminishes in quantity during that developmental period. The “yolk spheres” (*sensu* Bellairs 1964) exhibit smooth, featureless surfaces (Fig. 2a). Most are spherical (as their name implies), although some appear distorted, presumably due to specimen preparation. They range widely from ~2 μm to over 100 μm in diameter, while many have diameters of 30 to 50 μm (Fig. 2b). In our SEM specimens, the matrix took the form of delicate sheets of fibers and elongated thread-like components that surrounded and sometimes coated the yolk spheres.

Cell clumps

By stage 23, the yolk sac cavity contains abundant clumps of endodermal cells intermixed with the liquid yolk. The clumps of cells form around the periphery of the yolk sac, with liquid yolk being concentrated towards the center. Upon examination with stereomicroscopy via transmitted light, the cells appear spherical, translucent, and filled with rounded inclusions.

SEM examination shows that the endodermal cells are rounded and ovoid bodies that form tightly packed groups (Fig. 2c). The cells in our samples commonly ranged from 75 to 120 μm in diameter, with some being as large as 150 μm. Their external surfaces bulge prominently with the intracellular yolk droplets that they contain (Fig. 2d-f). (As in other work, we use the term “yolk droplets” to distinguish these cellular inclusions from the extracellular “yolk spheres”: Powers and Blackburn 2017a). As viewed through the external surfaces of cells, visible yolk droplets commonly measured about 25-50 μm in diameter, but ranged up to 85 μm in size.
specimens that showed large extracellular yolk spheres scattered among the cell clumps, these spheres were similar in caliber to bulges formed by the intracellular yolk droplets (Fig. 2e, f).

**Elongated vascularized strands**

In late-stage eggs (stage 25), the yolk sac contents are mainly represented by elongated, yellowish “spaghetti-like” strands. As viewed by stereomicroscopy, the strands appear to be branched and interwoven (Fig. 3a). Other specimens consist of compact masses in which the strands must be teased apart to be distinguished. Examination at high magnification reveals that the strands contain spherical cells like those of the cellular clumps (Fig. 3b). Transillumination indicates that the cells are translucent and filled with numerous droplets. A blood vessel lies at the core of each strand (Fig. 3a, b).

SEM examination of late-stage specimens reveals that the elongated strands branch and interweave with one another (Fig. 3c-e). They range widely in diameter from 165 µm to at least 420 µm, with most being about 200-250 µm. The strands are lined with closely-packed endodermal cells whose surfaces bulge with intracellular yolk droplets. In stage 25 specimens, the cells average about 100 µm in diameter with a range of ~45 to 160 µm. The external bulges formed by the yolk droplets that they contain range in diameter from very small (~10 µm) to large (up to 65 µm). Many of the droplets appear large relative to the cells that contain them (Fig. 3d, e). In a few of our SEM images, cells evidently had been artifactualy shed during specimen preparation, exposing a thin blood vessel that lies at the core of the strand (Fig. 3f).
Discussion

This investigation was stimulated by the discovery that embryos of the corn snake, *Pantherophis guttatus*, process yolk very differently than do birds (Elinson and Stewart 2014; Elinson et al. 2014). Recent work has documented details of the corn snake pattern and extended it to other lampropeltine snakes and to the lizard *Sceloporus undulatus* (Powers and Blackburn 2017a, b; Blackburn et al. 2018). The present study shows that yolk processing in embryonic *Chelydra serpentina* is similar (but not necessarily identical) to the pattern found in those squamate reptiles that have been examined.

General observations

Eggs and embryos of *C. serpentina* in this investigation extend from the post-pharyngula limb-bud stage to the stage attained at hatching (Fig. 1). By reference to Yntema's (1968) study, one could infer that the earliest eggs were roughly 9-10 weeks post-oviposition (halfway through the developmental period). However, such an inference is tentative, given that rate of early development in snapping turtles is so temperature dependent (Yntema, 1978). In any case, this study is equivalent in developmental scope to the recent analyses of yolk processing in snakes and lizards (Powers and Blackburn 2017a, b; Blackburn et al. 2018). Yolk of the analyzed eggs encompass a full developmental sequence from liquid yolk through yolk cellularization to establishment of elongated strands of endodermal cells (Fig. 2a and 3a).

Although details of fetal membrane morphology are beyond the scope of this study, we noted that a vascularized chorioallantois spreads to line the eggshell in the embryonic (dorsal)
hemisphere. In this respect, *C. serpentina* follows the general reptilian/avian pattern (Blackburn 1993; Stewart 1997). Likewise, the vitellus (the yolk body) becomes surrounded by tissues of the yolk sac. Given composition of the reptilian yolk sac (Stewart 1993, 1997), its endodermal lining clearly is the source of cells that invade the yolk sac cavity in *C. serpentina*.

**Yolk and its cellularization**

As shown by SEM, the liquid yolk material consists of yolk spheres surrounded by a matrix (Fig. 2a, b). The fibrous character of the matrix as viewed under SEM probably results from its chemical fixation (Powers and Blackburn 2017a). The size of the yolk spheres in the snapping turtle (~4-60 μm in diameter) is equivalent to that of recently-studied squamates (Powers and Blackburn 2017a, b; Blackburn et al. 2018). Yolk in snapping turtles mainly consists of protein and lipid, in a dry mass ratio of 1.6 to 1 (Wilhoft 1986), figures that are well within the range of other reptiles, including turtles (Thompson and Speake 2003, 2004). Whether the various sizes of yolk spheres (Fig. 2a) vary in their composition awaits future investigation.

Yolk material that has a solid consistency during dissection corresponds to the clumps of endodermal cells that progressively fill the yolk sac cavity (Fig. 2c, d). What holds the clumps together was not evident in our samples. In squamates, such clumps result from the fact that the endodermal cells remain connected after mitosis by thin intercellular bridges (Powers and Blackburn 2017a, b; Blackburn 2018). Although we attempted gently to tease apart fixed samples of *C. serpentina* to visualize such structures (as we have done with squamate yolk samples), fragility of the tissue made that impossible without destruction. The endodermal cells bulge with prominent, intracellular yolk droplets (Fig. 2d-f). We infer that the intracellular
droplets resulted from phagocytosis of yolk spheres, although our samples yielded few potential images of that process (Fig. 2d, f).

**Elongated strands of cells**

In late-stage eggs, the yolk sac cavity is filled with “spaghetti-like” strands, each of which is formed by endodermal cells arranged around a blood vessel (Fig. 3a, b, f). These structures have the same appearance and composition as the strands described among snakes and lizards (Elinson and Stewart 2014; Powers and Blackburn 2017a, b; Blackburn et al. 2018). Our observations have not revealed how the strands develop, nor the source of the contributing blood vessels. By reference to squamates, the strands presumably form through a re-arrangement of the clumped endodermal cells around invading vitelline blood vessels. The cells that form these strands bulge prominently with large yolk droplets, even into late development (Fig. 3e). This observation may seem surprising, given that late embryonic development is when yolk digestion is maximized (Morris et al. 1983). However, in snapping turtles and other chelonians, residual (undigested) yolk can fuel metabolism and growth for weeks or months after hatching (Rhen and Lang 1999; Wilhoft 1986; Lance and Morafka 2001). This aspect may explain the retention of large yolk inclusions until the final stages of development.

**Functional issues**

Our observations indicate that embryonic yolk processing in *C. serpentina* is very similar to the pattern observed in lizards and snakes. In squamates, the process involves three steps (see Powers and Blackburn 2017a, b; Blackburn et al. 2018). First, proliferative endodermal cells
invade the yolk sac cavity and phagocytose yolk spheres, growing massively in size from taking up yolk. As the cells multiply, they remain connected by intercellular bridges, eventually forming large cellular clumps. Second, small blood vessels that arise from omphalopleuric circulation invade the cell clumps. Third, the yolk-filled endodermal cells become arranged into monolayers around these vessels, forming elongated strands. The overall process provides an effective means by which yolk material is taken up into cells and digested, with the products of digestion then being delivered into adjacent small blood vessels for transport back to the embryo (Blackburn et al. 2018).

Snapping turtle eggs exhibit aspects of each of these developmental steps, as illustrated by large amounts of liquid yolk in early development (Fig. 2a, b); the establishment of clumps of yolk-laden endodermal cells (Fig. 2c-f); and formation of elongated vascularized strands of cells (Fig. 3). While we were unable to verify the presence of intercellular bridges in C. serpentina, such structures would account for how the proliferating endodermal cells form cellular clumps. In addition, although phagocytosis was only occasionally observed in our samples, this process would account for how yolk material becomes intracellular. Finally, our tissue samples did not reveal blood vessels in the process of invading the yolk sac cavity. However, we infer that such invasion must have occurred, given the widespread distribution of vascularized strands of cells in late development (Fig. 3a,b, f).

One potential difference between squamates and the snapping turtle lies in the contribution of extracellular digestion to yolk processing. In lampropeltine snakes and the lizard Sceloporus undulatus, the intracellular yolk droplets found in proliferating cells and cell clumps are usually 2-10 µm in diameter, much smaller than many or most of the extracellular yolk spheres. These
observations offer indirect evidence that the yolk spheres undergo extracellular digestion before being phagocytosed (Powers and Blackburn 2017a; Blackburn et al. 2018). In contrast, in the snapping turtle the intracellular yolk droplets are relatively large even into stages 24 and 25, and similar in size to the extracellular yolk spheres (see Fig. 3c-e). Consequently, we postulate that extracellular digestion plays a less important role in yolk processing in the snapping turtle than in the squamates examined. In another potential difference, our observations on C. serpentina did not reveal whether small cells bud mitotically from larger ones and grow massively in size through yolk uptake, as occurs in squamates (Powers and Blackburn 2017a; Blackburn et al. 2018). The absence of a wide range of sizes range of endodermal cells such as seen in squamates may reflect the limited sample size in our study. Further examination could consider this issue, as well as whether these taxa differ in the pace and timing of yolk processing relative to embryo development.

The pattern that we have observed in squamates and the snapping turtle differs dramatically from that of birds. In the avian pattern, the yolk is not invaded by proliferating cells and no vascularized “spaghetti-like” strands form in the yolk sac cavity. Yolk processing begins with substantial extracellular digestion (Yoshizaki et al. 2004). Endodermal cells that line the yolk sac cavity then phagocytose yolk material, digest it intracellularly, and send digestive products into the blood vessels of the underlying omphalopleure for delivery to the embryo (Lambson 1970; Freeman and Vince 1974; Mobbs and McMillan 1979, 1981). Yolk phagocytosis in birds does not begin until after vascularization of the splanchnopleure, and cell receptors facilitate uptake of specific nutrients (Yoshizaki et al. 2004; Bauer et al. 2013). In contrast, in squamates and the snapping turtle, phagocytosis begins well before blood vessels invade the yolk sac cavity.
Evolutionary considerations

The mechanisms of yolk processing in birds and squamates represent two functional solutions to the problem of how to cellularize and efficiently process large amounts of yolk. The functional “problem” is a consequence of the evolution of the large-yolked amniotic egg (see Elinson and Beckham 2002; Elinson and Stewart 2014; Elinson et al. 2014). Our observations indicate that yolk processing in snapping turtles bears similarities to that of the squamate pattern, but is entirely different from that of birds. Accordingly, a form of the pattern found in lizards and snakes may be ancestral not only for squamates (Blackburn et al. 2018) but for Reptilia. This interpretation would hold regardless of whether chelonians are interpreted as a diapsid sister group to archosaurs (Zardoya and Meyer 1998; Iwabe et al. 2004; Crawford et al. 2012) or as a derivative of a basal reptilian stock (Laurin and Reisz 1995; Lee 1997).

In summary, similarities between squamates and the snapping turtle in the mechanisms of yolk processing have significant functional and evolutionary implications. Details of the chelonian pattern require confirmation through examination of other species. Ultimately, a full understanding of the evolutionary history of yolk processing patterns in Reptilia will also require equivalent studies on crocodylians in comparison to birds.
AUTHORS’ CONTRIBUTIONS

DB supervised the study and wrote the manuscript; LL assisted with manuscript revision; KP and DB harvested the yolk samples; and LL, MB, and KP processed the samples and examined them via SEM.

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References


Crawford, N.G., Faircloth, B.C., McCormack, J.E., Brumfield, R.T., Winker, K., and Glenn, T.C. 2012. More than 1000 ultraconserved elements provide evidence that turtles are the sister group of archosaurs. Biol. Lett. 8: 783-786.


FIGURE LEGENDS

Figure 1. *Chelydra serpentina* embryos.  A. Stage 18 (a limb bud stage).  B. Stage 23.  C. Late stage 25 (close to the time of hatching).  Scale bars:  A = 2 mm; B, C = 5 mm.

Figure 2. *Chelydra serpentina*, yolk and endodermal cells as viewed by scanning electron microscopy.  A, B. Extracellular yolk, showing yolk spheres in a precipitated matrix.  C. Clumps of endodermal cells.  D-F. Clumped endodermal cells, showing bulges formed by their intracellular yolk droplets.  Asterisks label yolk spheres that may be in the process of phagocytosis.  Embryo stages:  A, B, D = stage 18; C, E, F = stage 25.  Scale bars:  A, C = 100 μm; B = 50 μm; D = 75 μm; E = 30 μm; F = 20 μm.

Figure 3. *Chelydra serpentina*, elongated strands, as viewed by stereomicroscopy (A) and scanning electron microscopy (B-F).  A. “Spaghetti-like” strands.  The specimen was illuminated via transmitted light.  B. Composition of the strands, showing endodermal cells clustered around blood vessels (v).  C-E. Elongated strands, showing yolk-filled endodermal cells that form them.  F. A strand with cells removed, showing its central blood vessel (v).  Embryo stages:  A, C-E = stage 25; B, F = stage 24.  Scale bars:  A = ~0.5 mm; B = ~125 μm; C = 150 μm; D, E = 100 μm; F = 50 μm.
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Graphical Abstract. In embryonic snapping turtles, the yolk sac becomes filled with vascularized strands of endodermal cells that take up and digest the yolk. This mechanism of yolk processing also occurs in lizards and snakes, but is entirely different from the avian pattern. Our findings emphasize a dichotomy in how embryos of Reptilia process yolk for development.