Amer. Zool., 31:616–627 (1991)

The Development and Evolution of the Turtle Body Plan: Inferring Intrinsic Aspects of the Evolutionary Process from Experimental Embryology[1]

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Synopsis. The body plan of turtles is unique among tetrapods in the presence of the shell. The structure of the carapace involves a unique relationship between the axial and the appendicular skeletons. A common developmental mechanism, an epithelial–mesenchymal interaction, has been identified in the early stages of carapace development by means of basic histological and immunofluorescence techniques. By analogy to other structures initiated by epithelial–mesenchymal interactions, it is hypothesized that carapace development is dependent on this interaction in the body wall. Surgical perturbations were designed to test the causal connection between the epithelial–mesenchymal interaction in the body wall and the unusual placement of the ribs in turtles. By comparison to data available on body wall formation in avian embryos, these experiments also shed light on the segregation of somitic and lateral plate cell populations and the embryonic origin of the scapula in turtles.

This study specifically addresses the ontogeny of a unique tetrapod body plan. The ontogenetic information can be used to make inferences about the phylogeny of this body plan and how it could have evolved from the more typical primitive tetrapod. On a more general level this study explores the potential role of common developmental mechanisms in the generation of evolutionary novelties, and the developmental incongruities between homologous skeletal elements in different groups of tetrapods.

Introduction

The turtle body plan represents a classic problem of both ontogeny and phylogeny. The anatomy of the shell, composed of a dorsal carapace and ventral plastron, is unique among tetrapods. It is the basal synapomorphy for the order Chelonia and the monophyly of this group has never been questioned.

The nature of the carapace and plastron has intrigued morphologists for hundreds of years. The plastron, or lower shell, is composed entirely of dermal bones, and will not be discussed here. The carapace is a composite of the endochondral axial skeleton (the thoracic vertebrae and ribs) and a specialized dermis that overlaps and surrounds them. In many forms, this dermis ossifies to a greater or lesser extent in a characteristic bony mosaic (Owen, 1849; Zangerl, 1969).

An association of dermal bones and endoskeletal elements is typical in the cranial skeleton of vertebrates, but very rare postcranially. Even so, this association would not be so remarkable if it did not also alter the position of the ribs relative to the limbs and girdles. The dermal carapace overlaps the pectoral and pelvic girdles anteriorly, posteriorly and laterally. The ribs, because of their close association with this dermis, maintain a dorsal position and the girdle elements are situated ventrally, deep to the axial elements. This is in strong contrast to other tetrapods (Fig. 1).

How novel morphologies are produced is essentially an evolutionary question. However, ontogeny generates the variation we see in phylogeny and is thus an intrinsic aspect of the evolutionary process. Since every change in phenotype is the result of some change in developmental pattern, the study of ontogeny can potentially tell us more about the evolution of a particular morphology than the adult morphology itself. A broad comparative approach is crucial for such evolutionary studies. To date, much of the developmental data in the literature has been gathered from a limited number of organisms. Reptiles are under-

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[1] From the Symposium on *Experimental Approaches to the Analysis of Form and Function* presented at the Centennial Meeting of the American Society of Zoologists, 27–30 December 1989, at Boston, Massachusetts.

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represented for numerous reasons, not least of which are the practical difficulties involved in working on animals with seasonal reproduction and relatively small numbers of eggs. Many of these difficulties can be overcome, however, and the data generated have added greatly to our knowledge of both process and pattern in vertebrate evolution (Billett *et al.*, 1985).

In this review I describe how experimental techniques have contributed to our knowledge of turtle development, and how this knowledge can be used to understand the evolution of novel forms. I will also point out how experimental data from two widely divergent tetrapod embryos, turtles and birds, can bring many of our implicit assumptions about homology into question while it expands our knowledge of variation.

HISTORICAL OVERVIEW

The known history of the order Chelonia begins abruptly in the Triassic with the fossil *Proganochelys*. This animal has the highly derived trunk morphology by which we recognize the order. Both pre- and post-Darwinian morphologists have sought affinities or ancestors for the Chelonia among various fossil groups, including the plesiosaurs (Owen, 1849; Baur, 1887), the placodonts (Jaekel, 1902; Broom, 1924), the pariesaurs (Gregory, 1946), and even the labyrinthodont *Gerrothorax* (Vallén, 1942).

Because of the extreme morphological leap between turtles and other tetrapods, much of the conjecture on turtle ancestry has resorted to the construction of hypothetical taxa. The hypothetical ancestor is often constructed in light of assumptions about the selective advantage of the shell as a protective adaptation. Cope (1871) suggested that the leatherback, *Dermochelys*, is the lone survivor of the earliest turtles, the "Atheca," and that all other turtles, the "Thecophora," are more recent offshoots whose excessive ossification can be explained by the rigors of the terrestrial environment. This distinction influenced ideas of turtle relationships for many years (Gaffney, 1984).

Deraniyagala (1930), again using *Dermochelys* as representative of the primitive condition, proposed the taxon "Saurotestudinata." He imagined the carapace and plastron to have evolved from the habit of hunching the shoulders around the head and rolling up "armadillo fashion" for protection. This habit, he felt, led to the migration of the scapula over and around the anterior ribs into the chelonian position.

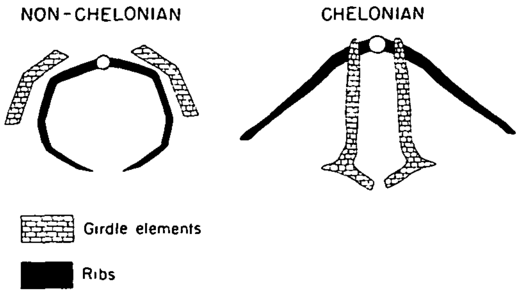

FIG. 1. Schematic diagram illustrating the different relationships between the axial elements and the pectoral girdle in chelonian and non-chelonian tetrapods. Reprinted with permission from Burke (1989*b*).

Eunotosaurus africanus Seeley from the middle Permian of South Africa was presented by D. M. S. Watson (1914) as a "missing link" between the Chelonia and more primitive cotylosaurs. This animal is known from only five specimens with little well-preserved cranial material. The single headed thoracic ribs are expanded along their shafts into broad plates that make contact with one another. Watson considered this character of the ribs to be intermediate between normal tetrapods and the carapace of turtles, and that *Eunotosaurus* closely approaches a hypothetical chelonian ancestor he called "Archichelone." He proposed that the pectoral girdle had migrated posteriorly in "Archichelone," under an already well-developed shell. Many authors adopted *Eunotosaurus* as a representative of the chelonian line (Ruckes, 1929; Deraniyagala, 1930; Gregory, 1946). No dermal bones are associated with the axial skeleton, however, and the pectoral girdle takes the standard tetrapod position, external to the ribs. In Romer's words, "the inclusion of *Eunotosaurus* in the Chelonia does violence to any definition of that group" (1956, p. 517). *Eunotosaurus* has now been placed on its own as an order *incertae sedis* within the subclass Anapsida (Carroll, 1988).

Proganochelys has a full carapace and plastron but its cranial anatomy links it to *Eucaptorhinus*, a Permian captorhinomorph (Gaffney and McKenna, 1979; Gaffney and Meeker, 1983). The captorhinids have been identified as the sister group of turtles, indicating the chelonian form evolved from a tetrapod with typical (nonchelonian) postcranial anatomy. No intermediate morphologies are represented in the fossil record between *Proganochelys* and the Captorhinomorpha. It is, in fact, very difficult to imagine any functional intermediates, and the evolutionary history of the turtle body plan has remained relatively obscure.

Having settled on an ancestor, hypothetical or fossil, or a sister group, few authors have been willing to tackle what I see as the most crucial and interesting aspect of chelonian evolution—the aberrant positioning of the ribs and pectoral girdle. Because of their highly derived trunk morphology, Carroll (1988) comments that turtles might well be placed in their own subclass of the Amniota.

MORPHOGENESIS OF THE CARAPACE

A good deal of detailed descriptive work has been done on turtle embryology, especially in the 19th century (*e.g.*, Agassiz, 1857; Rathke, 1848; see review by Ewert, 1985), but little that investigated the unusual relationship between the ribs and the pectoral girdle. An important exception is the work of Ruckes (1929), who examined the stages of carapace formation in embryos of a wide range of turtles. His observations of histological material and embryo wholemounts led him to conclude that two related phenomena are responsible for the positioning of the ribs relative to the girdles. First, an exaggerated lateral growth of the dorsal dermis greatly exceeds dorso-ventral growth and causes the apparent flattening of the body. Second, and most important, the rib primordia are "ensnared" within this specialized dermis. This later phenomenon, Ruckes felt, was responsible for the "deflection" of the ribs resulting in their position dorsal to the limb girdles. He also disagreed with Watson's idea about the migration of the pectoral girdle. Based on his observations the girdles form *in situ* at the level of the eighth cervical vertebra and do not migrate posteriorly along the axis from the position in which they first appear.

In addition to Ruckes' work, turtles have been the subject of other developmental studies (see Ewert, 1985, for review), some of which have provided more detail on the regionalization of somitic derivatives along the axis. For example, Vasse (1973, 1974, 1977) studied early limb development in embryos of *Emys orbicularis* and *Testudo graeca*. His studies localized the forelimb bud at the level of somites 6–13 in both these species. Yntema worked extensively with turtle embryos and performed a wide variety of experiments on *Chelydra serpentina* and other species. He created a series of normal stages for *Chelydra* (1968) and published a detailed protocol for collecting and working with their eggs (1964). Most pertinent to the subject of this paper were his somite extirpations (Yntema, 1970) that confirmed the somitic origin of the ribs and dermal carapace in *Chelydra*, as well as the somite level of their origin.

I have reinvestigated the problem of carapace formation and scapular position using techniques of immunohistochemistry as well as classical surgical perturbations. These techniques have identified one developmental mechanism of carapace outgrowth and brought the problem of the scapula into sharper focus.

Normal development

The species used in this study was *Chelydra serpentina*, the common snapping turtle. This species is hypothesized to be the most primitive living member of the suborder Cryptodira (Gaffney and Meylan, 1988). A single species study is admittedly typological. The chelonian body plan, however, defined by the relationship between the axial and appendicular elements, is essentially uniform in all turtles. This uniformity legitimizes generalization for the order regardless of the variation in the extent of ossification, size and shape of the shell. Approximately 200 eggs, representing 17 different clutches from at least 14 different *Chelydra* females, were used in various aspects of this study. Observations were also

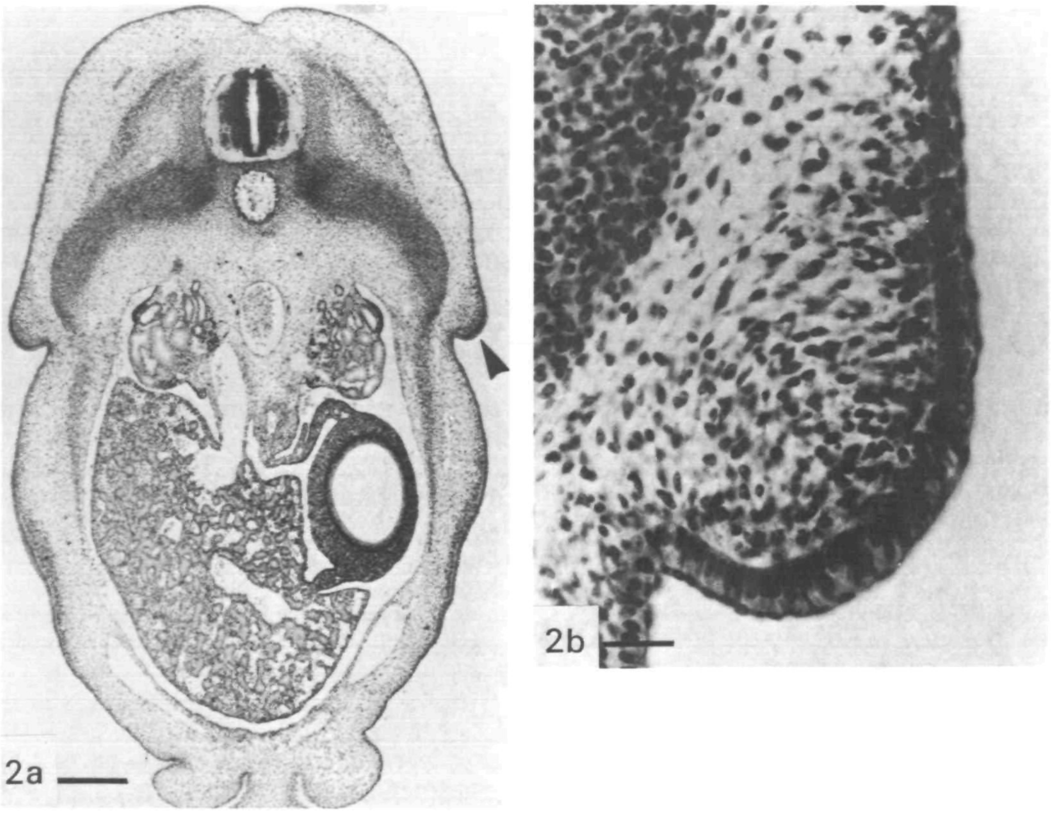

FIG. 2. a) Mid-trunk cross section of an Yntema (1968) stage 15 *Chelydra serpentina* embryo showing the carapacial ridge (arrow). Bar = 300 μm. b) Higher magnification of the carapacial ridge. Bar = 28 μm. Reprinted with permission from Burke (1989*b*).

made on histological material of *Chrysemys picta* and *Trionyx* sp.

For wider comparative purposes I use the chick embryo as a representative non-chelonian species. Obviously, this choice is for practical rather than phylogenetic reasons. The chick has been the experimental vertebrate of choice for decades and a great deal of developmental data are available in the literature. The interpretive problems that arise as a result of the phylogenetic distance between birds and turtles is discussed in the final section of this paper.

The *Chelydra* embryo at Yntema's (1968) stage 14 resembles the chick embryo at Hamburger and Hamilton's (1951) stage 24; it is a typical tetrapod embryo at the "pharyngula" stage of development (Ballard, 1981). There is nothing about its morphology that would reveal its identity as a turtle. The first sign of departure from typical tetrapod development is the appearance of a ridge along the flank dorsal to the limbs. In subsequent stages this ridge extends anteriorly and posteriorly to become continuous over the base of the tail and cervical region to form the complete margin of the carapace. A cross-sectional view of this carapacial ridge reveals that it is formed by a thickening in the ectoderm underlain by a condensation of mesenchymal mesoderm (Fig. 2). This morphology is typical of areas of epithelial–mesenchymal interactions.

Epithelial–mesenchymal interactions are very common inductive interactions in vertebrate development. A great deal of research has addressed the mechanisms of these inductions (see papers in Sawyer and Fallon, 1983). The data generated by these studies provide a wealth of information on the temporal and spatial distribution of various molecules during the development of various structures (*cf.* Edelman, 1986). These data can then be used as characters to identify epithelial–mesenchymal interactions in other systems.

I have shown that the distribution of two "morphogenetic molecules," fibronectin and the neural cell adhesion molecule (N-CAM), in the carapacial ridge is similar to the distribution of these molecules in the equivalent stages of feather formation and early limb outgrowth, both well known sites of epithelial–mesenchymal interactions (Burke, 1989*b*). Based on the histological morphology and this molecular data, I have identified the carapacial ridge as the site of an epithelial–mesenchymal interaction. Further, I have hypothesized that this interaction in the early body wall influences rib outgrowth and positioning, and is the causal factor in what Ruckes called the "ensnarement" of the ribs. This mechanism is seen as analogous to the interactions that initiate early limb bud outgrowth and morphogenesis of many integumental features (Burke, 1989*a*, *b*).

EXPERIMENTAL MANIPULATIONS

The hypothesis that the carapacial ridge is the site of an epithelial–mesenchymal interaction invites experimental investigation. The certain identification of an inductive interaction requires experimental verification to prove its causal role in the morphogenesis of a structure.

In order to further investigate this hypothesis, a series of experiments were designed to determine the inductive role of the carapacial ridge and the migratory behavior of somitic cell populations during body wall formation. Working from an analogy with other structures initiated by epithelial–mesenchymal interactions, especially the limb, two surgical perturbations were used to test the causal role of the carapacial ridge in rib positioning. The surgeries were performed *in ovo,* the eggs having been windowed within 12 hours of oviposition (see Yntema, 1964, and Burke, 1989*b*). The embryos were stained with 0.2% neutral red to enhance visualization of structure, and manipulated with sharpened tungsten needles.

Testing the inductive role of the carapacial ridge (CR)

In the first set of experiments, the ectoderm and immediately subjacent mesoderm in the area of the incipient carpacial ridge (CR) were surgically removed on embryos from stage 13 through stage 16 (Fig. 3a). These stages bracket the period between the first appearance of the CR and its maturity. No attempt was made to completely separate the ectoderm from the mesoderm. These experiments test the effect of removal of the CR on the formation of the dermal carapace and the placement of the ribs within it.

The results from these surgeries demonstrate a remarkable degree of regeneration and regulation on the part of the embryo. However, several trends can be noted. Embryos perturbed at stage 14 showed a higher percentage (93%) of effects to the carapace than embryos perturbed at earlier (36%) or later stages (26%). Embryos that fail to regenerate show definite gaps in the dermal margin of the carapace. When the gaps correspond to levels of normal rib placement, the ribs are deflected and crowd with a neighboring rib into the next available marginal scute (Fig. 4).

The source of embryonic cells that produce the ribs of tetrapods has been extensively investigated in the chick. Sweeney and Watterson (1969), using tantalum foil barriers to block the migration of somitic cells into the lateral plate mesoderm, demonstrated that the vertebral and sternal ribs of chicks are somitic in origin. The quail-chick chimera method of heterospecific transplantations has verified these conclusions and showed that the lateral and ventral dermis, in which the distal ends of the ribs differentiate, is formed exclusively from lateral plate mesoderm (Gumpel-Pinot, 1984). Yntema's (1970) somite-removal experiments on embryos of *Chelydra* demonstrate that chelonian ribs and dorsal dermis also arise from somitic mesoderm.

I placed barriers between the somitic and lateral plate mesoderm in *Chelydra* embryos with 6 to 19 somites. Tantalum foil, 0.005 mm in thickness, was cut into rectangular pieces measuring approximately 0.5 by 1.0

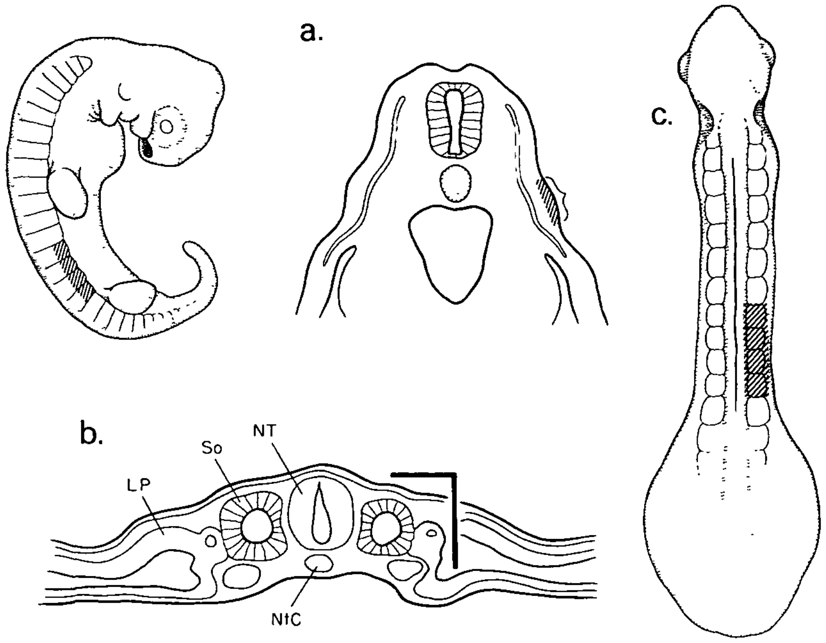

FIG. 3. Three surgical manipulations discussed in the text: a) Tissue removed in carapacial ridge extirpations. b) Placement of tantalum foil barriers. Foil thickness = 0.005 mm. c) Extirpation of somitic mesoderm. Drawings are not to scale.

mm, and folded along the long axis to form an "L." One flange of the "L" was inserted into an incision made lateral to the somites, and the other lay medially over the ectoderm (Fig. 3b). These experiments have two purposes. First, placing a physical barrier at the border between somitic and lateral plate should interfere with or inhibit the formation of the carapacial ridge and provide additional data on the relationship between the ridge and the ribs. Secondly, barriers will evaluate the extent of migration of somitic cells into the lateral plate, and the role of these somitic cells in ventral body wall formation.

Barrier placement is highly disruptive to development and survivorship is low (38%). Of the survivors, slightly more than half rejected the barrier and are normal or show only superficial scute disruptions. The rest of the embryos (44%) retained the barrier and show disruption of the body wall. In these cases, the carapace margin is discontinuous and entire quadrants of the dermal carapace are absent. The ribs associated with these gaps can be seen to interdigitate with the bones of the plastron, which are of dermal origin. Even in the most extreme cases of carapace disruption, all of the bones of the plastron are present.

These results show that development of the carapacial ridge can be inhibited resulting in severely disrupted axial morphology. Further, they show that while somitic cells are necessary for the normal development of the carapace, the plastron has some degree of developmental independence (Burke, 1987). The interdigitation of the ribs with the plastral elements indicates that the ribs have entered the lateral plate as they do in avian embryos.

The following conclusions have been drawn from the results of these two experimental perturbations: (1) The carpacial ridge is the site of an epithelial–mesenchymal interaction that is responsible for the outgrowth of the carapacial dermis. (2) The normal placement of the ribs is dependent on the integrity of the carapacial ridge (Burke 1989*c*). This system is likely analogous to

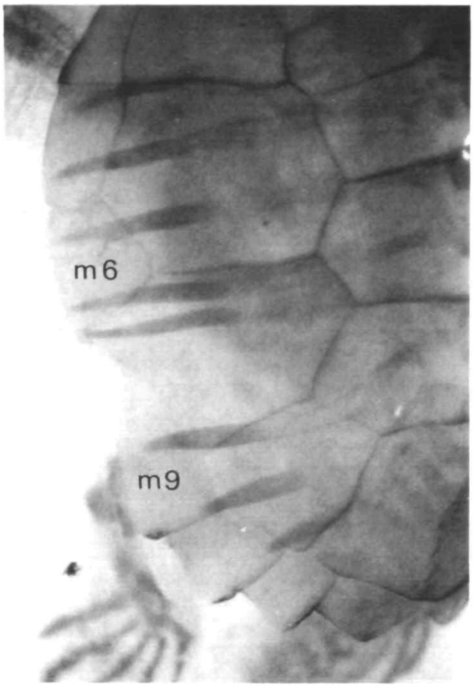

FIG. 4. Left rear quadrant of the carapace of a specimen in which the carapacial ridge was removed at Yntema's (1968) stage 14. Marginal scute 7 is reduced and 8 is missing; m = marginal.

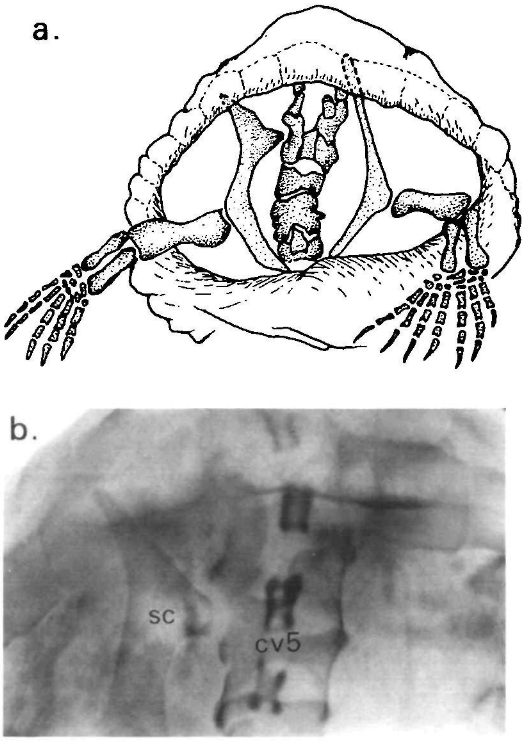

FIG. 5. a) Illustration showing anterior view of a specimen in which the somitic mesoderm at the level of somites 8–12 was removed from the right side of the embryo at Yntema's (1968) stage 6. The head has been removed for clarity, the neck hangs ventrally. b) Photograph of scapula and cervical vertebrae of the specimen drawn in (a); sc = scapula; cv = cervical vertebrae.

the role of the apical ectodermal ridge in normal limb development (*cf.* Saunders, 1948).

Homology of the scapula

A developmental segregation parallels the functional distinction between the axial and appendicular skeletons of tetrapods. The axial elements arise from somitic mesoderm, the appendicular elements from lateral plate mesoderm. Recent data generated by experimental embryologists, however, have shown that in at least one group of higher tetrapods the scapula does not conform to this neat developmental segregation of functional skeletal systems. Chevallier (1977), using quail-chick chimeras, found the chick scapula to be formed at least in part by somitic cells. Grafting blocks of quail somites into the same level of a chick host resulted in quail cells in the scapular cartilage in segmental fashion, more anterior somites contributing to more proximal regions of the scapula. Therefore this series of somites (15–24) contributes both to the axial and appendicular skeletons. The same methods demonstrate that the rest of the limb skeleton, the coracoids, the entire pelvis as well as the distal limb bones, all arise from lateral plate mesoderm.

The cervical vertebrae in primitive tetrapods are equipped with ribs. As axial elements (the serial homologues of thoracic ribs) they arise from somitic mesoderm. Cervical ribs of amniotes are reduced or absent, presumably reflecting specializations for mobility. The fossil *Proganochelys* has quite well-developed cervical ribs for an amniote though they are small relative to the ribs associated with the carapace. In all living turtles cervical ribs are present only as occasional, atavistic rudiments (Wil-

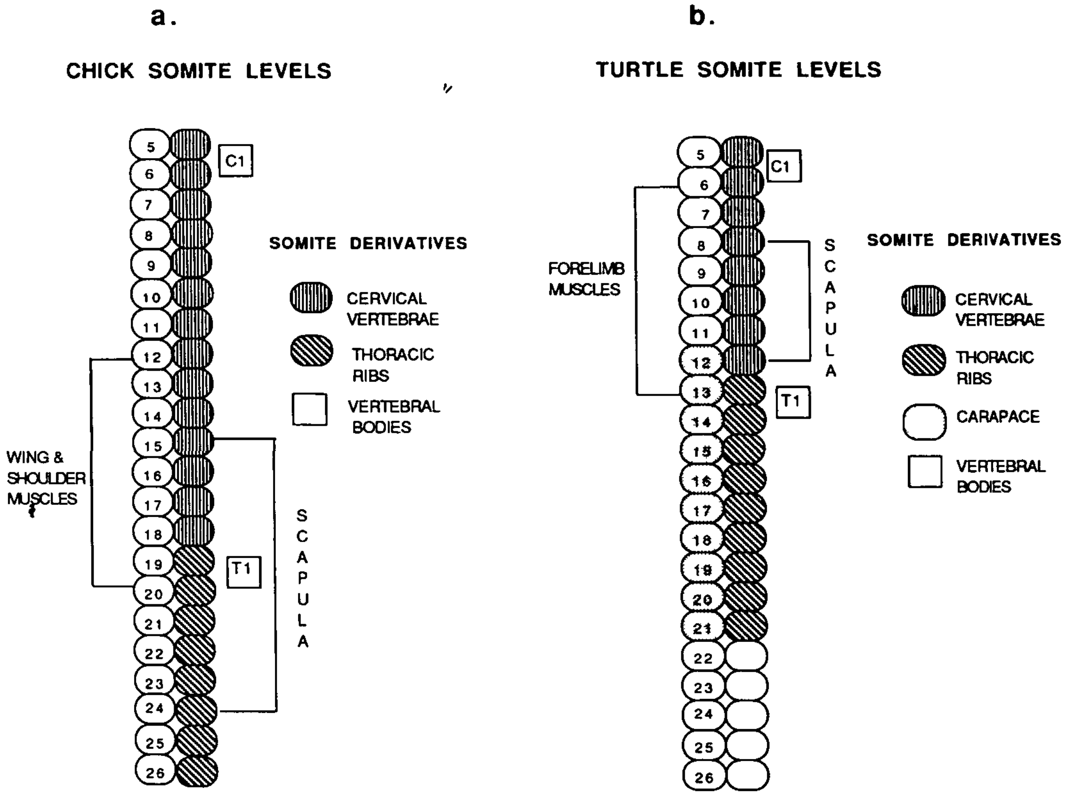

FIG. 6. a) Schematic fate map of the skeletal somitic derivatives in the chick compiled from the literature. b) Schematic fate map of the somitic derivatives in *Chelydra serpentina*.

liams, 1959). This latter case is also the situation seen in birds.

A fate map compiled from the literature on chick development shows that the cervical–thoracic transition lies at somites 18–19 (Fig. 6; see Gumpel-Pinot, 1984, for review of mapping). The first trunk somite in chicks is somite 5, and chicks have 14 cervical vertebrae. According to Chevallier (1977), the scapula arises from a series of somites that overlap the cervical–thoracic transition. Somites 19–24, contributing cells to the middle and distal shaft of the scapular blade, also provide cells that form thoracic vertebrae and ribs. This requires a degree of cell segregation and skeletal pattern formation not usually expected of trunk somites.

Given the unusual position of the scapula in turtles, the embryonic origin of the scapula *vis-à-vis* the thoracic ribs is critical to understanding the evolution of chelonian anatomy. To this end, I performed somite removal experiments on embryos of *Chelydra serpentina*. The levels chosen for extirpation were based on the results of Yntema (1970). His experiments demonstrated that the thoracic vertebrae and ribs arise from somites 13–21, and the dermal carapace from somites 12–21. He reported no damage to the scapula or cervical vertebrae, but removed no somites anterior to somite 12. Yntema's data place the cervical–thoracic transition at somites 12–13. I removed somites 8–12 and 12–16 in an attempt to bracket the cervical–thoracic boundary (Fig. 3c).

The number of embryos that survived the surgery without total regeneration are too few to determine with certainty the effects of somite removal (57.7% survivorship, 34% of these with skeletal effects). The only specimens showing disruption of the scapula are those perturbed at somite levels 8–12. The extent of the effect varies from minor malformations of the shaft to total absence of a recognizable scapula. In 5 of 7 cases, the disruption is limited to the dorsal scapular shaft; the acromion and glenoid are relatively normal. Scapular defects are always associated with malformations of the cartilagenous arches of cervical vertebrae 4–7, and in three specimens the distal forking of the scapula makes contact with the malformed arches of cervicals 5–7 (Fig. 5a, b). Three embryos have cervical disruptions and normal scapulae. In every case with scapular and cervical disruption, the anterior thoracic vertebrae and ribs are completely normal. The coracoid is also normal, though in two cases the distal limb shows abnormalities. The contralateral limb shows additional digits in one specimen (illustrated in Fig. 5a).

This mixture of results does not allow an interpretation free from the effect of artifact, but a working model can be proposed that will be further tested over future nesting seasons. In the majority of cases (71%) the limb skeleton, including the coracoid, is normal, indicating that the lateral plate was unaffected by the surgery. The defects in the cervical vertebrae indicate successful perturbation of the somitic mesoderm. The correlation of scapular and cervical defects, and the close association of scapular and cervical cartilages in the affected specimens (Fig. 5), allow the tentative conclusion that the chelonian scapula is formed in part by somitic cells from the levels of somites 8–12 (Fig. 6b). The somitic contribution appears to be limited to the suprascapular cartilage and the dorsal extent of the scapular shaft.

It is interesting to note that the "scapular" somites (8–12) form cervical vertebrae 4–7. In normal development the scapula differentiates *in situ* at the level of the eighth cervical. This indicates a posterior migration of scapular cells along the axis from their point of origin to their site of differentiation. This ontogenetic migration echoes the phylogenetic migration of the scapula proposed by Watson (1914) that was undetected by Ruckes (1929) in his analysis of static developmental stages.

EVOLUTIONARY INFERENCES

The experimental data reviewed above give insight into the developmental patterns responsible for the ontogeny of the turtle body plan. In conjunction with experimental data generated from non-chelonian embryos, inferences can be made about the developmental changes involved in the evolution of the chelonian morphology from a non-chelonian ancestor. Furthermore, these comparative data increase our knowledge of the nature of morphological variation, and the role of development in generating this variation.

The primary difference between chelonian and non-chelonian body wall formation is the migratory route followed by the presumptive costal cells of the somitic mesoderm. I have proposed that the epithelial–mesenchymal interaction in the body wall, the carapacial ridge, has a causal connection to this migration and therefore to placement of the ribs (Burke, 1989*b*). Based on this interpretation of the ontogeny of the chelonian trunk, I further proposed that this novel interaction in the body wall was instrumental in the evolutionary transition from the typical tetrapod arrangement of the trunk to that of the chelonian. I hypothesize that the origin of the carapace involved the progressive elaboration of a primitive integumental interaction in the proto-chelonian.

The differentiation of the integument is generally a late stage phenomenon, occurring well after the morphogenesis of the skeleton. The precocious initiation of an inductive interaction would change the developmental context of its influence. In essence, the inductive effect would act upon a smaller embryo and could influence deep layers of as yet undifferentiated mesoderm—in this case the costal sclerotome.

The detailed mechanisms of epithelial–mesenchymal interactions remain fairly mysterious, but their wide distribution in vertebrate development indicates that they are a primitive phenomenon. Their initial invariant morphology and subsequent generative versatility indicate they are sources of developmental potential and, as such, vehicles for evolutionary novelties (Maderson, 1983). A temporal shift of an originally integumental interaction may well have rapidly produced the highly modified chelonian body plan from a non-chelonian ancestor, and set the stage for all the subsequent modifications in turtle evolution.

This theory is analogous both conceptually and mechanistically to the lateral fin fold theory of the evolution of the paired appendages (Thacher, 1877; Balfour, 1881). This theory, based on observations of median fin development, proposes that an originally integumental lateral fin fold was elaborated by the invasion of somitic muscle buds that provoked the *in situ* formation of endochondral skeletal structures. It seems reasonable to propose that an epithelial–mesenchymal interaction was involved in the formation of the early integumental fin fold, and evolved in concert with the increasing complexity of the limb.

In a "systems" approach to vertebrate morphology, an implicit assumption persists that the functional segregation of axial and appendicular systems is paralleled by some degree of developmental segregation. This is also implicitly if not explicitly assumed in the theory of the origin of the paired appendages mentioned above. The girdle elements are seen to arise as extensions of the primary radials into the body wall to anchor the fin (see Goodrich, 1906, and Jarvik, 1965, for discussion). The scapula in chicks, however, is known to arise from somitic mesoderm, in contrast to the rest of the appendicular skeleton. The somite removal experiments on turtles outlined above indicate that the chelonian scapula also has a somitic component. (The somitic nature of the scapula is very interesting considering Owen's [1849] interpretation of its identity. He described the scapula as the homologue of a haemal arch of an archetypal occipital vertebra, in other words, an axial element. Thus, to his own satisfaction, he explained the positioning of the scapula in turtles by its axial nature.)

In contrast to the chick embryo, the somites that contribute to the turtle scapula do not overlap with those that form the thoracic ribs, but arise from cervical levels only (Fig. 6). The most straightforward explanation for this fact requires only that fewer somites are involved in the formation of the scapula in the turtle than in the chick. The chick and turtle scapulae are therefore developmentally incongruent. While few would argue that they are not homologous elements, clearly the difference between them—a matter of somites—reflects their divergent phylogenetic history.

Knowledge of the primitive developmental pattern of the tetrapod scapula is necessary in order to determine the evolutionary polarity of this developmental character. This information would clarify the possible intermediate morphologies involved in the transition of the chelonian from the non-chelonian body plan. For example, if one were to accept that the situation in chicks is primitive, the proto-chelonian scapula would have been formed from a series of somites which, as in the chick, overlap the cervical–thoracic transition. One could hypothesize that a decoupling of costal and scapular somites may have been one of the developmental modifications leading to the new position of the scapula in turtles. There is no reason to suppose, however, that chicks represent a primitive tetrapod pattern of development. It is more likely that the situation in chicks is a uniquely derived character of birds related to the evolution of flight. The participation of the somitic mesoderm, with the capacity to behave as a mosaic, should allow for developmental and therefore evolutionary plasticity in the pectoral girdle.

Unfortunately, no data exist on the embryonic origin of the pectoral girdle in any other vertebrate. With the available developmental data and the variation of adult structure seen in phylogeny, predictions can be made that can be tested by further experimentation on the appropriate embryos. As a current working hypothesis, I would make the following predictions:

1) Primitively in vertebrates the primary pectoral girdle (scapulocoracoid) was derived from lateral plate mesoderm consistent with its appendicular identity and with the current theories on the origin of the paired appendages in vertebrates.
2) At some point during the modification of the limbs for terrestrial locomotion, somitic cells were recruited to augment the pectoral girdle. Therefore, a somitic

contribution to the scapula is a derived condition among tetrapods, perhaps at the level of the amniotes.

3) The extent of the somitic contribution has been shown to vary between avian and chelonian embryos. A variable contribution by the somites reflects different locomotor adaptations among different groups of tetrapods. The overlap of costal and scapular somites (as seen in chicks) is derived among the amniotes.

In order to test these predictions, experimental work is now in progress to determine the embryonic origin of the scapulocoracoid in several species of urodeles and anurans.

CONCLUSIONS

This study is an example of how observations of development lead to hypotheses of both ontogeny and phylogeny. Ontogenetic hypotheses are accessible to the techniques of experimental embryology and the understanding of mechanism gained by these methods can be used to form predictions about variation in phylogeny. That these predictions can be tested using classical methods of experimental embryology, testifies to the utility of this approach to the study of the origin of form in phylogeny. Variation in developmental pattern provides information about intrinsic aspects of the evolutionary process. Comparative work is needed in order to fully understand the constraints which lead to the invariant aspects of form, and the plasticity of mechanisms which generate novelties. A problematic consequence of the lack of comparative developmental data is that one is forced to make inappropriate phylogenetic comparisons. But, by using the framework of independently derived phylogenies, hypotheses can be tested, and gaps in our knowledge filled, by experimentation on the appropriate embryos.

ACKNOWLEDGMENTS

This work was done at the Museum of Comparative Zoology and is distilled from parts of a dissertation submitted to Harvard University in partial fulfillment of the requirements for a Ph.D. degree. I would like to thank P. Alberch in general, and B. K. Hall, P. M. Mabee and J. E. A. Bertram as well as two anonymous reviewers for helpful comments on the manuscript. Figures 1 and 2 are reprinted from Journal of Morphology, 1989, 199:363–378, and I thank Alan R. Liss for permission. L. Meszoly (MCZ) drew Figures 1 and 3, T. Miyake adapted Figure 5a from a color plate by K. Wing-Brown. M. Primrose printed the photographs. Financial support for this research came from NSF grant BSR-84-07430 (to P. Alberch) and from Sigma Xi (to A.C.B.). Symposium support was provided by a National Science Foundation grant (BSR-8904370) to J. Hanken and M. H. Wake.

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