Staging of Cranial Ossification Patterns in the Tiger Salamander, *Ambystoma tigrinum*

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Introduction

The skulls of many species of salamanders have been described in the literature for the last 100 years. Early contributions include the morphological descriptions of W.K. Parker (1877, 1882), and E. Emerson (1905) on the general anatomy of *Typhlomolge rathbuni*, and E. Francis (1934) on the anatomy of *Salamandra salamandra*. Goodrich (1930) made a large contribution to our understanding with his studies of development and the structures associated with development in vertebrates. This was one of the first complete works to account for the structure and evolutionary history of different vertebrates—from fish to higher mammals. Studies like these, that analyze the course of development in vertebrates, have contributed greatly to our understanding of form and function in the vertebrate skull.

Studies of development in vertebrates, such as the volume written on vertebrate skull development by deBeer (1937) and the work on the structure and development of vertebrates by Goodrich (1930), have provided numerous opportunities for phylogenetic analysis. In addition, these studies have greatly increased our understanding of the developmental pathways that result in the formation of the skull. Further examples of this experimentation are the work done by Hall (1984a,b) on developmental and evolutionary mechanisms underlying skull and bone formation. Alberch and Alberch (1981) also looked at evolutionary mechanisms and diversification of the skull in the neotropical

While these ontogenetic analyses of skull development have contributed to our understanding of evolutionary patterns in vertebrates, there are relatively few studies of taxa that undergo a relatively discrete and rapid change in morphology during development. Hanken and Hall (1993), in their three-volume landmark publication of vertebrate skull development and diversity, summarized the previous work and filled in some of the gaps in this area of developmental biology. These works are essential for understanding the dynamic changes that occur during metamorphosis.

Some of the changes in anuran and urodele cranial morphology during their metamorphosis from an aquatic to a terrestrial anatomy have been examined since the 1800’s. These changes have been and continue to be studied in order to collect evidence for and against various theories of tetrapod origins and relationships and of the basic organization and structure of the vertebrate head (Pusey, 1938; vanEeden, 1951). There are still many gaps to be filled, though, in this increasingly important area of study.

Some of the most recent studies relating to salamander skull development have focused only on particular parts of the skull: the inner ear (Lombard, 1977), the nose (Jurgens, 1977), and the sound conducting apparatus (Monath, 1965). There have been others that have looked specifically at evolutionary patterns in specific parts of the skull, like tongue evolution and projection (Reilly and Lauder, 1989, Ozeti and Wake, 1969). Other studies have focused primarily on specific aspects of behavior related to skull
development, namely, feeding behavior (Lauder and Shaffer, 1985, 1986; Lauder and Reilly, 1988; Larsen and Guthrie, 1975; and Reilly, Lauder, and Collins, 1992).

In looking specifically at metamorphic salamander skull development, there are relatively few studies that give a detailed developmental sequence. However, other amphibians have been studied during metamorphosis, and some of the concepts from these may extend to this current study. For example, Hanken and Hall (1984, 1988b) studied the cranial ossification sequence during anuran metamorphosis using Bombina orientalis, the Oriental Fire-Bellied toad. They studied variation and timing of skull development (1984) since carefully timed and precise sequences underlie the formation of both bony and cartilaginous skeletal elements in most vertebrates. Changes in the timing of developmental events may inhibit or retard normal skull development in many vertebrate lineages. They also outlined the early development of the first three bones to form during anuran skull development, these being the exoccipital, the parasphenoid, and the frontoparietal (Hanken and Hall, 1988). A further study by Hanken and Summers (1988) investigated the effects of thyroid hormone on anuran cranial metamorphosis. They found that cartilage transformation in during anuran metamorphosis entails profound changes in the cranial morphology. Through these changes, virtually the entire cranium is repatterned. The larval skull, which is well suited for feeding and respiration in an aquatic environment, is converted to an adult skull which is adapted to a terrestrial existence. Through these studies, the development of the anuran skull was outlined in detail, and while anuran skulls are not directly parallel to that of the tiger salamander, this information can be used in making some inferences to tiger salamander skull development.
Lauder and Shaffer (1988) reported on the conservation of motor patterns in *Ambystoma tigrinum* during metamorphosis, with an emphasis on cranial muscle activity. However, Reilly and Lauder’s (1990) study on the development of the entire skull of *Ambystoma tigrinum* has been one of very few detailed examinations on the metamorphosis of cranial design. They recorded the morphological changes at metamorphosis in the tiger salamander using a morphometric analysis; this process consisted of detailed measurements at different landmarks within the skull using statistical analysis. From this study, they concluded that most aspects of general head size were not significantly different in larval and transformed salamanders, but internasal distance and head length did differ. In addition, they found that the hyobranchial apparatus shows a dramatic change at metamorphosis: the snout becomes wider, and there is an overall decrease in skull length. These and other changes in the functional design of the skull accompany and help facilitate the transition of the animal from an aquatic lifestyle to a terrestrial one.

Pedersen (1993) compared skull growth in cannibal versus non-cannibal tiger salamanders. He found that *Ambystoma* larvae display intraspecific aggression at all body sizes. He also noted that the cannibalistic tiger salamanders have a distinctive hypertrophy of the head and vomerine teeth. Pierce et. al. (1983) did a similar study on cannibalistic and non-cannibalistic Tiger Salamanders from West Texas. They found that some larval individuals display a striking morphological differentiation associated with cannibalistic activity. These characteristics include a disproportionally large head, a wide mouth, and slit-like eyes. Reilly, Lauder, and Collins (1992) in their study on performance consequences of cannibalism in *Ambystoma tigrinum* wrote that the cannibal larvae have
been distinguished morphologically by possessing a larger body size, wider heads, and
hypertrophied vomer bones and vomerine teeth. Further, they hypothesized that cannibal
morphs, through their richer diet or perhaps hormonal effects, achieve higher growth rates
and maybe earlier metamorphosis than typical morphs. According to Pedersen (1993) the
differences in morphology between cannibalistic and non-cannibalistic larvae begin to show
when osteogenesis replaces chondrogenesis during development. It is at this stage that the
skull is the most plastic and can be shaped to meet the structural demands of cannibalism.
However, further study needs to be done on this topic before one can classify specific A.
tigrinum larvae as "cannibal morphs".

In a study similar to mine, Bonebrake and Brandon (1971) have staged the sequence
of cranial ossification of a different species, the small-mouthed salamander, Ambystoma
texanum. They identified nine stages, which are recognizable by discernible events during
metamorphosis. By comparing complete sequences of other groups of salamanders, these
stages may prove useful for better understanding the relationships within the genus,
Ambystoma. Similarly, Theron (1952) published an article outlining the cranial
morphology of Ambystoma maculatum (Shaw). He investigated the skull of this species in
great detail and subsequently summarized his findings in twenty-four statements about the
distinctive qualities of the A. maculatum (Shaw) skull.

Through a related investigation, Hoheisel (1931) presented a comparison of
distinctive skull characteristics among five different species of the genus Ambystoma,
including A. tigrinum. He found that the tiger salamander skull was not directly
comparable to any of the other five species due to a variety of specific differences. These
differences not only involve many particular bones but also the gross contours of bones within the skull.

Many amphibian species, including the tiger salamander, exhibit metamorphosis, or changes that accompany their transition from an aquatic to a terrestrial lifestyle. Species that metamorphose are helpful in research because the rapidity with which some of the changes occur allows us to study major changes in the functional design of the organism. The basic physiological, biochemical, and behavioral transformations have received extensive study and are summarized by Dodd and Dodd (1976), Duellman and Trueb (1986), Etkin and Gilbert (1968), Fox (1984), and Gilbert and Frieden (1981).

Salamanders that metamorphose are especially useful in experiments, for they exhibit a less radical metamorphosis than anurans, thus allowing homologous bones, muscles, and landmarks to be identified across metamorphosis. Also, the morphological changes are great enough in salamanders to challenge our attempts to understand the biological development of functional design (Reilly and Lauder, 1990).

An interesting evolutionary characteristic of salamander metamorphosis is the phenomenon of paedomorphosis. Paedomorphosis is the retention of embryonic and/or larval characteristics in sexually mature adults. This phenomenon has received considerable attention recently as a mechanism for producing evolutionary changes in animal morphology (Gould, 1977). Paedomorphosis is of particular importance when considering salamander evolution since all nine families of salamanders have paedomorphic species and four of these families are entirely paedomorphic (Duellman and Trueb, 1986).

The genus *Ambystoma* is of particular interest when studying salamander
paedomorphosis. There are several Mexican species that are facultative paedomorphs (Thomas Williams, pers. com.), one species that may breed once as a larva and then transform into sexually mature adults (Thomas Williams, pers. com.), and two species, *A. tigrinum* and *A. gracile*, that have some populations where some or all individuals are larval breeders (Duellman and Trueb, 1986).

The tiger salamander, *A. tigrinum*, is the most widespread species of *Ambystoma*. It ranges from the Atlantic and Gulf Coasts of the USA, west to the Pacific Coast and, excepting New England and the Appalachians, from southern Canada south through parts of the northern Mexican Plateau (Gehlbach, 1967). Various populations of the tiger salamander have been studied by Dunn (1940), in his study on the different races of *Ambystoma tigrinum*. He knew that this species of *Ambystoma* was made up of a number of "races", which differed in their physiological makeup, and he wanted to divide them into geographical races. Dunn came up with seven primary races within this species but concluded that this study would need some more information before being fully completed. Collins (1980) also studied the species and described some general characteristics of the subspecies. He also concluded that more research needs to be done to fully classify and understand *Ambystoma tigrinum*. Many of the variations in *Ambystoma tigrinum*, including cannibalism and color, were attributed to environmental conditions in the studies done by Dunn and Collins.

The morphology of the tiger salamander is of special interest because it may represent the ancestral morphological condition for the genus (Williams, 1984). This, combined with the fact that there are many paedomorphic populations of tiger
salamanders, means that the developmental process of this species has the potential for providing insights into the role of paedomorphosis within the entire genus. It also may increase our understanding of the role of this phenomenon in other vertebrates, including man.

In order for us to understand paedomorphosis in tiger salamanders it is necessary to carefully describe the development of transforming individuals from the time of hatching until metamorphosis is complete, a technique termed staging (Gosner, 1960). The process of metamorphosis in salamanders is a complex one with changes in nearly every aspect of morphology taking place in less than one month (Duellman and Trueb, 1986; Latimer and Roote, 1964). This study will focus on the morphological changes of the skull occurring throughout development in *Ambystoma tigrinum*. Provided in this study is experimental staging of the ossification sequence of the skull in the tiger salamander before, during and after metamorphosis using the staging technique.

**Methods and Materials**

Approximately 200 larval specimens of *Ambystoma tigrinum* were collected from a Naperville breeding pond in early June 1994. These salamanders were fed natural food and black worms and they were kept in an aqueous environment of deionized water which was changed daily. The animals were to be sacrificed at intervals of one week until all of the animals had transformed. In early July, however, all the remaining specimens died
following a feeding. The worms are presumed to be the cause of death of these salamanders since no other variables within the lab had been varied. At this point, it was too late to collect new larval salamanders for the study, so we obtained preserved specimens of *A. tigrinum* from Dr. David Sever at St. Mary's College, Notre Dame, IN. These specimens had been preserved in 10% buffered formalin and were labeled with the date of capture. The specimens that were now available to me came in a wide variety of sizes and were in different stages of development. The dates of capture and preservation of the specimens can be found in Appendix A.

The first step after obtaining the salamanders was to prepare them for staining. I took measurements of the body length, head width, and head length of fifty animals. I measured body length as the distance from the tip of the snout to the end of the tail, head width at the widest point of the skull, and head length after the head was severed, from the tip of the snout to just posterior to the gill area. As mentioned, when I measured the salamander, its head would be cut off, usually just past the gill area. After taking measurements, it was necessary to clean the skull; to remove the eyes, skin, and any excess tissue that would interfere with clearly viewing the bones and cartilage of the developing skull. This was a delicate, learning process as it was not always clear what could and could not be removed. As the project progressed, it became easier for me to distinguish superficial tissue from skull elements.

After the skulls were cleared of excess tissue, they were stained to show bone and cartilage using the "bone-cartilage double-stain" technique outlined by Hanken and Wassersug (1981). This technique consists of four stages: fixation, cartilage stain,
maceration with bone staining, and clearing. This process will reveal even slight amounts of cartilage and mineralized tissue. During fixation, the specimens are preserved in 10% buffered formalin for at least 24 hours. Next, the specimens are washed in several changes of deionized water for at least 48 hours. The cartilage staining process follows, and during this stage, the specimen is placed in a solution of 20 mg alcian blue 8GX, 70 mL absolute ethanol, and 30 mL glacial acetic acid for 12-48 hours. The specimen should not be left in this solution any longer than necessary since ossified tissues may begin to decalcify; however, I found that the specimens stained best if left in the solution for the maximum 48 hours. Following cartilage staining, the specimens are placed in at least two successive baths of absolute alcohol for at least 24 hours in each.

In the maceration stage, specimens are put through a graded, decreasing series of ethanol solutions (e.g., 75%, 50%, 25%) and then into two changes of distilled water, at least two hours in each step. Specimens are then transferred to an enzyme solution consisting of 1 g trypsin, 30 mL saturated aqueous sodium borate, and 70 mL distilled water. This solution clears the surrounding tissues, and specimens should be left here until soft tissues become transparent and fully destained. This step requires an average of 3 days to complete.

Bone staining follows maceration and during this step the specimens are placed in a solution of 0.5% KOH to which enough alizarin red S stock solution has been added to turn the solution deep purple. This step requires 24 hours and when completed, the bone will appear red to purple.

The last step in the "double-stain" technique is clearing. In this stage, the specimen
is transferred to 100% glycerin via a graded series of glycerin-KOH solutions (e.g. 25%, 50%, 75%), 24 hours in each solution. The specimens can then be stored in a fresh solution of 100% glycerin until they are needed for analysis.

Metamorphic changes in the skull of *A. tigrinum* were examined by observation of the fully stained specimens under 10X magnification with a dissecting scope. Since the exact ages of the specimens could no longer be discerned, as would have been possible with our own collection, the focus and exact goal of the observations had to be modified slightly to accommodate the use of "obtained" specimens. Instead of a focus on when specific bones ossified during metamorphosis in the tiger salamander, the goal was to determine the specific order in which the bones developed. This is consistent with other ossification studies done in which the ages of the salamanders are not generally given. Body size and stage of development in salamanders are related more to thermal history and food consumption than to chronological age.

Results

*** For purposes of discussion and comparison, eight arbitrary stages have been designated (table I).

The first detectable cranial ossification in *Ambystoma tigrinum* is present in larvae of a very small size, with a body length of 3.05 to 3.95 cm. In these, the smallest specimens that I examined, several cranial elements could be observed. I have designated this phase as Stage I. At this stage, the parasphenoid has begun to develop, along with the palatines,
vomers, coronoids, dentaries, prearticulars, and premaxillas (See Figure I). Excepting the parasphenoid and prearticular bones, all of the earliest ossifications are tooth-bearing bones. Descriptions of these bones and their locations follow in the discussion. Unfortunately, the cartilaginous elements did not stain in most of my specimens, so I was unable to identify the places where a cartilage precursor was present.

The next developmental stage that can be distinguished consists of the presence of all of the previously mentioned bones with the addition of the squamosal, which first appears as a thin sliver of bone posterior to the eyes. Also during this stage we see a small part of the maxilla beginning to ossify, along with the appearance of the pterygoid (See Figure II).

In stage III of the cranial development of *A. tigrinum* we can see the trace beginnings of the frontal and parietal bones, along with the early development of the exoccipital and quadrate bones (See Figure III). Subsequently, in stage IV, there is a broadening of the exoccipitals, and they eventually fuse with the prootic and the opisthotic. Specimens in this stage show a clear view of the otic processes, which house the hearing apparatus of the salamander (See Figure IV).

Next, in stage V, the opisthotic and prefrontal bones begin to develop (See Figure V). Also in this stage there is the appearance of ossified material near the median of the skull within the parietal bones (See Figure VI). This has been identified as calcified endolymph and it usually disappears by the completion of metamorphosis.

In stage VI, we see a more complete otic capsule, as it is formed by the developing opisthotics, along with the prootic bones. The prootics begin to ossify in this stage,
although they do not yet fuse with the opisthotics. Also during this phase, the prefrontals spread toward the maxilla and frontal bones, and in some specimens they begin to overlap the frontals (See Figure VII).

Stage VII is distinguished by the early development of the nasal and septomaxilla bones. At this stage, the prefrontals are well developed, and the prootic and opisthotic bones are fused to form the otic capsule (See Figure VIII and IX).

The last stage (Stage VIII) in the cranial development of *Ambystoma tigrinum* consists of ossification of the articular bones at the posterior end of the prearticulars. Also in this stage, there is further development of the nasal, septomaxilla and prefrontals, along with modification of previously ossified bones. These changes are discussed in the following dialogue.

*Summary of the Ossification sequence:* In summary, it was found that no single ossification sequence is followed by all specimens, but the distribution of bones in most specimens is consistent with the following sequence of appearance: dentaries, coronoids, vomers, palatines, prearticulars, parasphenoid, premaxillae, squamosals, pterygoids, maxillae, quadrates, exoccipitals, frontals, parietals, otic processes, opisthotics, prefrontals, prootics, nasals, septomaxillae, and articulars.
Descriptions and Discussion

Cranial bones ossify in a specific and predictable sequence. All bones of the skull are paired except the parasphenoid, and bilateral symmetry in the *Ambystoma tigrinum* skull structure is pronounced in all larval and postmetamorphic specimens that I have examined. Slight variations occur, however, in shape, size, and degree of ossification between the paired bones in the developing skull.

The first ossifications of the *Ambystoma tigrinum* skull are tooth-bearing bones, including the dentaries, coronoids, vomers, and palatines. The early emergence of these bones indicates their importance in the capture of planktonic food (Bonebrake and Brandon, 1971). A short discussion and description of each of these bones follows.

**Dentary.** The dentaries are long conspicuous bones in the larval stage. They are found ventrally to the vomers and palatines, and the paired dentaries are separated by an unossified gap which grows smaller with time. The dentaries grow in length and girth throughout metamorphosis, along with the head in general.

**Coronoid.** The coronoids are difficult to distinguish at first with a dissecting scope, but can be seen just medial to the dentaries. The coronoids bear more teeth than any other bone.

**Vomer.** The vomer appears as an oval-shaped bone lying in the same plane as the parasphenoid. In latter stages it enlarges and extends posteriorly toward the palatine, and in some specimens the vomer and palatine fuse to form the vomero-palatines (Hoheisel, 1931). The combination vomero-palatine stretches backward on the cranium to form a...
Palatine. The palatines have a roughly diamond-like shape and are the base of many teeth. A rapid increase in size of the palatines and vomers in the early stages of development reflects the increase in the number of ossifying teeth. Additionally, the palatine fuses with the developing pterygoid to form the palatopterygoid.

Along with the early ossification of tooth-bearing elements, there is also the appearance of the premaxilla, prearticular, and parasphenoid bones.

Premaxilla. At the beginning of their ossification in Stage I, the paired premaxillae resemble an inverted "T", with a horizontal portion that bears teeth, and a vertical, non-tooth bearing portion. Through development, the vertical elements in the premaxillae meet at the midline and contact the frontal bones, while their dorsal portion expands to articulate with the maxillae. As Hoheisel (1931) has noted, Ambystoma tigrinum is unique within the species due to the almost circular arc that is formed by the premaxillae and maxillae. In other species within the genus, this arc is usually elliptical or angular.

Prearticular. The prearticular first appears in Stage I as a small sliver of bone medial to the end of the dentary. It expands in width and length throughout metamorphosis until it contacts the dorsal edge of the dentary (Bonebrake and Brandon, 1971).

Parasphenoid. The parasphenoid is a large, thick bone forming practically the entire ventral floor of the cranium. It begins to ossify in Stage I as a single sheet of bone posterior to the palatines and on the same plane as them. It is the only unpaired bone in the skull. The parasphenoid increases in length and level of ossification throughout metamorphosis, but it does not undergo any major changes in configuration.
In Stage II, the ossification of the squamosal, the pterygoid and the maxilla occur.

**Squamosal.** The paired squamosals first appear as thin slivers of bone behind the eyes. The squamosal thickens and widens to form dense, strong plates and they ultimately form part of the otic processes and overlap the otic capsule. The squamosals articulate laterally on the skull with the prootics and ventrally with the quadrates (Hoheisel, 1931).

**Maxilla.** The maxillae are tooth-bearing elements located posterolateral to the premaxilla and lateral to the vomer. While the premaxillae form the anterior portion of the arch of the upper jaw, the maxillae complete the jaw arch laterally. Near their junction with the premaxillae, the maxillae also articulate laterally, via an ascending extension, with the prefrontals.

**Pterygoid.** The pterygoid is roughly triangular in shape and bears a distinctive laterally hooked spine. From a ventral view, its shape is primarily convex and the contours of the bone suggest smooth-flowing lines. The pterygoid is generally fused with the palatine in *Ambystoma* to form the palatopterygoid (Bonebrake and Brandon, 1971). The pterygoid continues to extend posteriorly and eventually articulates posteriorly and inferiorly with the quadrates and anteriorly and medially with the prootics.

In Stage III, the frontal and parietal bones begin to ossify, along with the exoccipital and quadrate. The otic processes are also clearly developed at this time. It is difficult to clearly discern the exact time that the frontal and parietal bones begin to develop from my specimens. However, it is during Stage III that they are clearly beginning to ossify.

**Frontal.** The frontal bones are irregularly shaped, long, flat bones that articulate with each other at the midline of the skull. When fully developed, they overlap the
parietals posteriorly and laterally they are overlapped by the prefrontals. Additionally, the premaxillae partially cover the anterior portion of the frontals.

**Parietal.** The parietals are more broad and irregular than the frontals and their borders extend further ventrally. As in the frontals, the parietal bones fuse with one another medially. The parietals and frontals are the primary bones involved in forming the dorsal brain case.

**Exoccipital.** The exoccipitals begin to ossify as little knob-like structures posterior to the squamosals. They begin to broaden ventrally and begin to form a disc-shaped process on the anterior end. Further development of these discs reveal them to be part of the opisthotic, which in *Ambystoma* is continuous with the exoccipital. The dual nature of the fused exoccipital and opisthotic has not been clearly identified (Bonebrake and Brandon, 1971)

**Quadrate.** The quadrates are small angular bones that are located just ventral to the squamosals. The quadrates articulate with the squamosals and in some specimens the also articulate with the pterygoid.

**Otic processes.** Although this is not an "official" bony element in other studies, the otic processes clearly showed the characteristic red coloration of ossification in my study. As mentioned above, the squamosals help to form the otic processes. They appear in the specimens as dark, circular pits, surrounded by concentric ossification of a lighter coloration.

In Stage V, the opisthotic and prefrontal bones begin to show clear signs of ossification. There is also the appearance of the ossified material within the parietal bones.
that was mentioned in the results. This material is calcified endolymph and it typically disappears by the completion of metamorphosis. Its function has not been clearly identified.

**Opisthotic.** As mentioned above, the opisthotics form as a part of the exoccipital bones. The opisthotic has a double wall and it usually spreads over the otic capsule, along with the squamosal. Toward the end of development, the opisthotics spread anteriorly beneath the otic processes of the squamosal and parietal and fuse with the prootic bones (Bonebrake and Brandon, 1971).

**Prefrontal.** The prefrontals ossify concurrently or immediately following the appearance of the opisthotics. The prefrontals are irregular and they arise between the maxilla and frontal bones. The prefrontals eventually overlap the frontals and articulate with the nasals. In *A. tigrinum*, the prefrontal forms a straight, lateral border and effects a sharp angle with the frontal, which is a distinguishing mark of the species.

In Stage VI, the prootics begin to ossify, although they do not yet fuse with the opisthotics.

**Prootic.** The prootics first appear as semicircular bones just ventral to the otic capsule. They expand posteriorly and dorsally over the sides of the capsule and fuse with the opisthotics in stage VII.

In Stage VII, the nasal and septomaxilla bones begin to ossify. The prootics and opisthotics fuse in this stage, as mentioned previously.

**Nasal.** The nasals are irregularly shaped, thin plates of bone. They begin as very small circular ossifications, and expand in all directions. The nasals develop to form a
straight marginal border toward the nasal processes of the premaxilla and an articulation with the prefrontals. In some specimens the nasals overlap the frontals (Hoheisel, 1931). I had very few specimens that showed full nasal development, indicating that this is indeed one of the very last bones to form during metamorphosis.

**Septomaxilla.** The septomaxilla appears as a ring of bone between the prefrontal and nasal bones, encircling the nasolacrimal duct (Bonebrake and Brandon, 1971). The septomaxillae increase in size, but little other change occurs in their development. Again, as with the nasals, there were very few specimens that showed full septomaxillae development.

In the last stage, Stage VIII, there is the development of the articular bone, along with further development and modification of previously discussed bones.

**Articular.** The articular bones ossify at the posterior end of the larger prearticular bone. They are present in very few specimens that I observed and are difficult to distinguish from the prearticulars. Bonebrake and Brandon (1971) observed some specimens of *A. texanum* in which the articular bones articulated with the quadrates.

As mentioned previously, *Ambystoma tigrinum* is not directly comparable to the other species of *Ambystoma* that were studied by Hoheisel (1931). *A. tigrinum* has such a large distribution that the cranial descriptions used by Hoheisel, and those that I have used in describing my specimens, might possibly represent a geographical type only. Furthermore, *A. tigrinum* represents a dimorphic species, and this also complicates a generalized study of its morphology (Hoheisel, 1931).

Despite these limitations, there are several observable changes that occur in the skull
of *A. tigrinum* during development and metamorphosis. The most obvious change is the ossification of new bones as described in the sequence above. In order to accompany the new ossifications, however, the size and shape of the skull undergo some changes. The snout of the animal becomes shorter and wider to adapt to terrestrial feeding. Also, the otic compartment becomes wider and longer, contributing to the skull itself shortening in length. Additionally, changes in the hyobranchial apparatus at metamorphosis allow for tongue development and tongue projection mechanisms (Reilly and Lauder, 1990).

There are also conspicuous changes in the external morphology of the gills and tail fin at metamorphosis. Since several populations of *A. tigrinum* are facultative paedomorphs and can retain larval characteristics as adults, one cannot judge their metamorphic condition solely on the basis of external features. Thus, the presence of external larval characteristics may not coincide with the internal changes in *A. tigrinum*, so it is necessary to check more than external factors in judging the metamorphic condition of this species (Reilly and Lauder, 1990). The level osteocranial development outlined here may be a guideline in determining the metamorphic condition of *Ambystoma tigrinum*.

The exact cranial structure of salamanders has been used to help demonstrate evolutionary relationships; however, fusions and losses of bones have occurred independently in many species and cannot always indicate a definite phylogenetic relationship. The problem in the genus *Ambystoma* seems to be tracing and properly classifying the biological development of such forms as *A. tigrinum*, and then considering the evolutionary significance of others within the genus (Hoheisel, 1931). These results lend some support to the idea that *Ambystoma tigrinum* is the ancestral morphological
condition of the genus, however, further investigation must be done not only to classify and understand the species, but also to gather evidence for and against various theories of tetrapod origins and evolutionary relationships.
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### Appendix

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### KEY to FIGURES

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<tr>
<td>Septomaxilla</td>
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### FIGURE I

**Stage I Ossification**

Visible bones:
- Parasphenoid (Ps)
- Vomer (V)
- Palatine (P)
- Dentary (D)
- Premaxilla (Pm)

Coronoid and Prearticular are present, but not visible in picture.
FIGURE II
Stage II Ossification

Visible bones:
Ps, V, P, D, Pm
Squamosal (S)
Pterygoid (Pt)-faint
Maxilla (M)

FIGURE III
Stage III Ossification

Visible bones:
Ps, V, P, D, Pm, S, Pt, M
Exoccipital (E)
Quadrate (Q)
Parietal (Pa)
Frontal (F)
FIGURE IV
Stage IV Ossification

Visible bones:
Ps, V, P, Pm, S, Pt, M
E, Q, Pa, F
Otic Processes (OP)

FIGURE V
Stage V Ossification

Visible bones:
Ps, V, P, Pm, S, Pt, M,
E, Q, Pa, F, OP
Opisthotic (Op)
FIGURE VI
Calcified Endolymph (CE)

FIGURE VII
Stage VI Ossification

Visible bones:
Ps, V, P, Pm, S, Pt, M, E, Q, Pa, F, OP, Op
Prootic (Pr)
Prefrontal (Pf)
FIGURE VIII
Stage VII Ossification

Visible bones:
All previous bones
Nasal (N)
Septomaxilla (Sm)
Articular is present, but not visible in the figure

FIGURE IX
Stage VIII Ossification

All previous bones
Further nasal and septomaxilla development

Nearly complete ossification


