Morphogenesis: Pharyngeal Arch Development in *Ambystoma tigrinum*

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Introduction

A population of eastern tiger salamanders (*Ambystoma tigrinum)* inhabits a stable water reservoir in WI. Most populations of eastern tiger salamanders transform into terrestrial adults (metamorphose). However, members of this specific population do not transform, but instead become a fully reproductive aquatic adult (neotene; Figure 1). In some instances, these neotenes experience a late metamorphosis. For unknown reasons, neotenes are not very successful at completing metamorphosis, and die during or shortly after they metamorphose. In order to evaluate a potential cause of death during metamorphosis, remodeling of the pharyngeal arch (PA) is being examined.

The PA is a series of six arches which gives structure to the gills (Figure 2). It is also essential in feeding and moving water across the pharyngeal and branchial chambers. To ensure proper feeding and respiratory function in terrestrial salamanders, the PA needs to be properly remodeled during metamorphosis. To discover whether the PA was improperly remodeled during metamorphosis, ossification of the arches were examined. Based on current findings, we hypothesize that abnormal ossification may be in part due to an altered morphogenesis process.

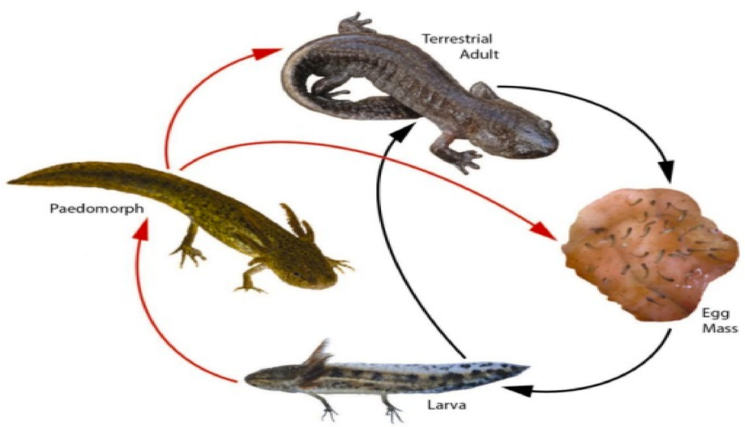

Figure 1: The common developmental pathway for larval tiger salamander is to metamorphose into a terrestrial adult. Some populations of tiger salamanders will diverge from this developmental pathway. Instead, a larval salamander matures into an adult neotene, where they remain aquatic and retain their larval features. These neotenes may later transform (rather unsuccessfully) into a terrestrial.

Methods

- Salamander carcasses were obtained from salamanders that died before, during, or after transformation
- The salamanders were dissected and skinned
- The salamanders were cleared using KOH, and stained using alcian blue (cartilage identification) and alizarin red (bone identification)
- The mandible of each salamander was dissected
- Ossification of the arches were examined and quantified

Results

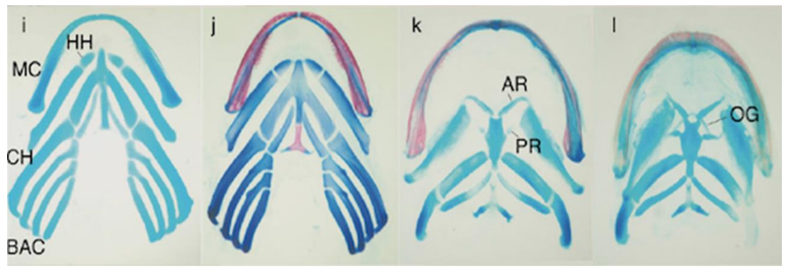

Figure 2: Ventral view of pharyngeal arch skeleton from *Ambystoma maculatum*. From left to right: early larval, late larval, mid-metamorphic, and post-metamorphic (1). The first arch is the mandible. This is made of Meckel's cartilage (MC) which will be covered by bone (stained red). The second arch is the hyoid, made of ceratohyal cartilage and is responsible for manipulating the jaw up and down. Arches 3-6 all support the gills and are branchial arch cartilage (BACs).

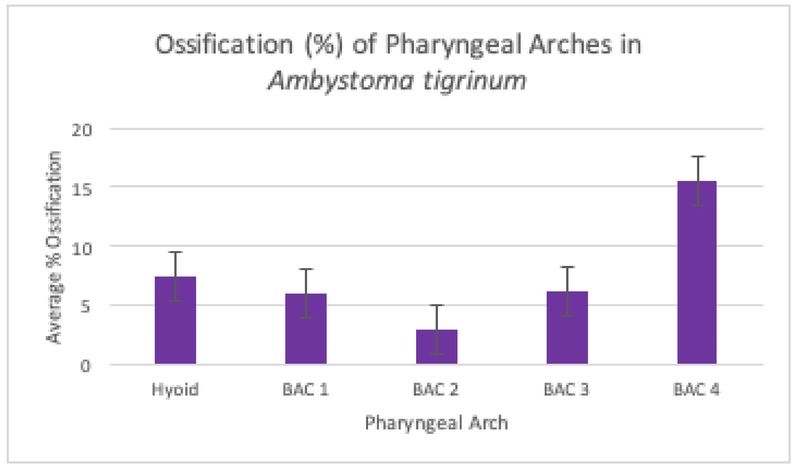

Figure 3: Observed ossification (%) of PAs in *Ambystoma tigrinum.*

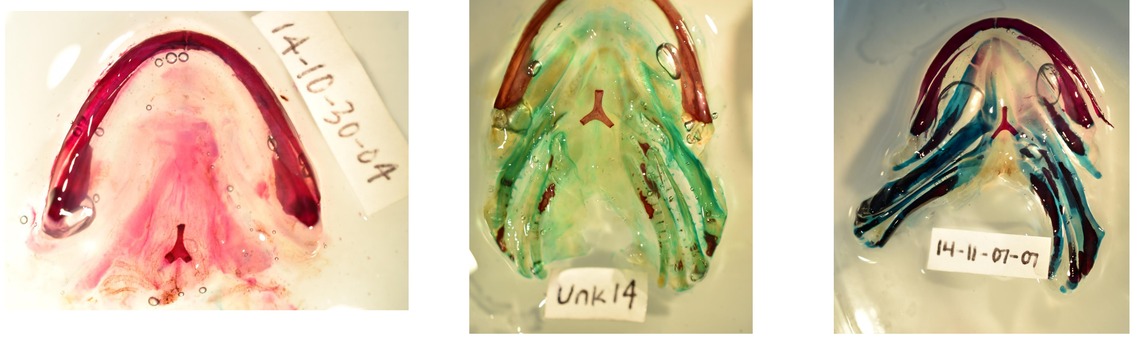

Figure 4: Cleared and stained PAs from our population of neotenic tiger salamanders. Left to Right- Normal absorbed arches, moderate ossification and extreme ossification.

Discussion and Future Work

Ambystoma tigrinum undergoes a morphogenesis process, which includes remodeling of the pharyngeal arches (PA). During the metamorphic process, there should be complete cartilage and bone resorptions within the cranial skeleton, which includes the PA. Specifically, throughout metamorphosis, cartilage shape decreases and becomes thinner (2). However, progressive metamorphic processes are delayed and therefore, more prone to abnormalities in morphogenesis (3).

Without proper remodeling of the PA, this species of salamanders may struggle to survive as they transform. The PA is important in many functions including feeding, breathing, moving, protection, heat diffusion, sound production, sensory production, as well as gas exchange for respiration in terrestrials (5). Based on previous studies, it is accepted that the thyroid hormone (TH) is an essential signaling molecule to induce this process. TH concentration dependencies increase from larvae (minimal) to metamorphic stages (~100 fold increase), which suggests its importance as metamorphosis is initiated (4).

This study examined the PAs of deceased salamanders in different metamorphic stages, and an abnormal ossification of the PAs were discovered. The unexpected ossification of the PA did not seem to follow a specific pattern, as seen in Figure 3. It is noted, however, Branchial Arch 4 appeared to possess the highest average ossification with 15.56% compared to the others. These results suggest that there may be a disruption in the normal morphogenesis process. While these results cannot conclude that this was the cause of death, it is to be considered.

Future work includes continuing to gather and quantify the amount of ossification on the PA of deceased salamanders. Also, and more importantly, identifying a pattern of abnormal ossification depending on the morphogenic stage of the salamander. This work could be furthered by analyzing the presence of the TH due to the fact that it is a key molecule in the induction of remodeling of cartilage and bone in a metamorphosing salamander.

Acknowledgements

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