The Cellular Basis of Dermal Bone Evolution and Development in Threespine Stickleback Fish

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How Does Development Contribute to the Relationship Between Genotype and Phenotype?

-A main goal of evolutionary and developmental biology is to understand how an organism's genetic information is transformed into phenotypic traits
-Developmental processes translate the information encoded in our genes into the traits upon which natural selection can act
-Understanding how developmental processes evolve helps us gain insight into the complex relationship between genotype and phenotype

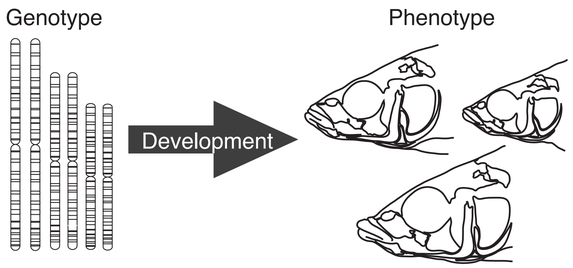

Figure 1. Developmental processes translate an organism's genetic information into phenotypic traits. Phenotypic traits are often controlled by multiple genes, which makes understanding this relationship incredibly complex.

Threespine Stickleback Fish as a Model

-Threespine stickleback are an excellent model because the genome is sequenced, they display phenotypic variation, and we can apply classic developmental techniques
-Threespine stickleback populations are found in marine and freshwater environments, the two populations are phenotypically and genetically distinct (1,2)

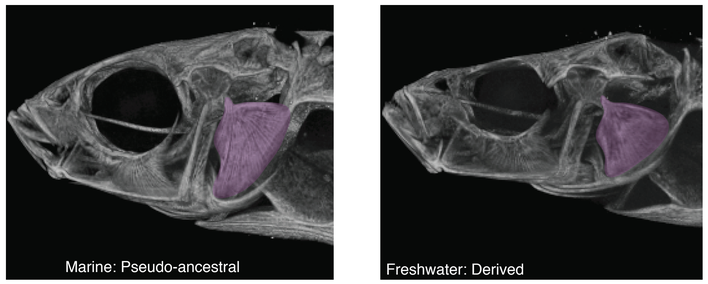

Figure 2. Images of adult marine (left) and freshwater (right) threespine stickleback heads. Note the differences in craniofacial skeletal structure, especially in the opercle bone (purple)

The Opercle Bone as a Model for Evo-Devo Research

-The opercle is the first dermal bone to develop in the head
-It differs in size and shape between marine and freshwater populations; shape is controlled by at least five genes (3)
-Research in zebrafish suggests that a difference in the number of proliferating cells near the growing edge of the opercle may contribute to size and shape differences (4)

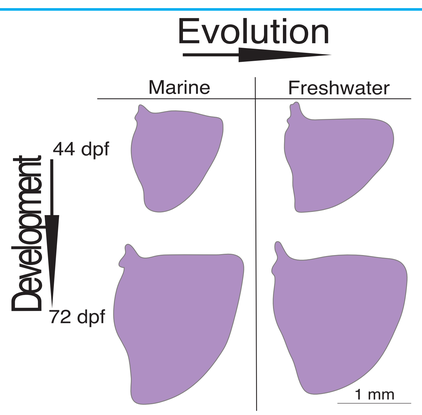

Figure 3. Adapted from Kimmel et al. 2007; marine and freshwater opercles during juvenile development. There are differences in opercle shape and size during development between the two populations.

How Does Cellular Behavior During Development Contribute to the Evolution of a Phenotype?

Hypothesis
In marine stickleback, a greater number of proliferating cells will contribute to the growing edge of the opercle bone.

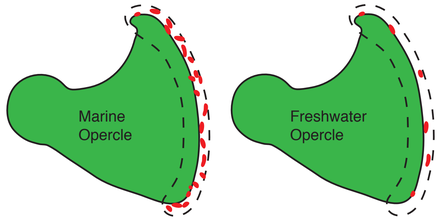

Figure 4. Illustration of opercle bones in 14dpf embryos. The opercle is illustrated in green and the EdU labeled cells are visualized in red. The black dotted line indicates the fan region of the opercle.

Opercles From Marine Populations Had More Proliferating Cells Near the Fan Than Freshwater Populations

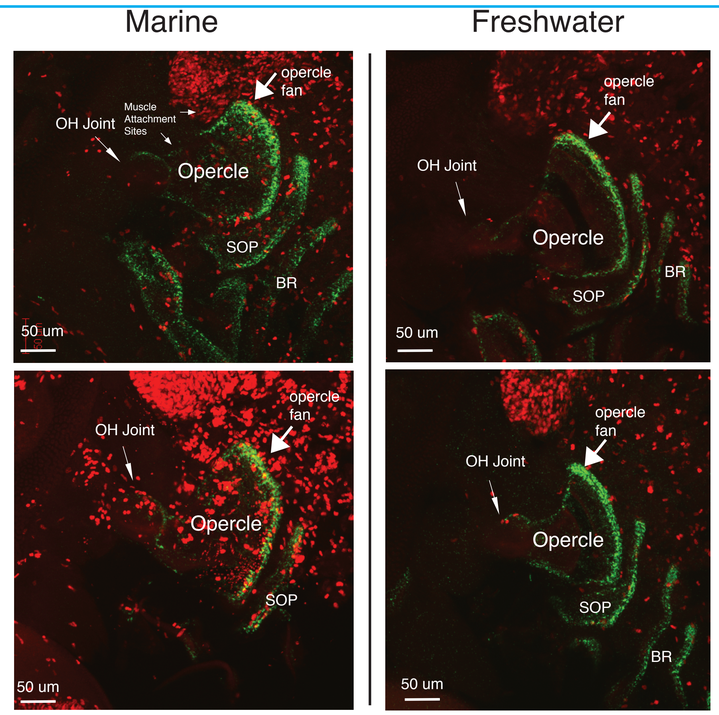

Figure 6. Z stack projections of opercle and surrounding bones at 14 dpf in marine (left) and freshwater (right) populations. *Col10a1 in situ* is visualized in green, showing the dermal bone development. Proliferating cells labeled with EdU are visualized in red. The subopercle (SOP), operculo-hyomandibular joint (OH joint), and branchiostegal rays (BR) are visible in the images as well, the musculus dialator and adductor operculi attach at the dorsal edge of the opercle. There are significantly more proliferating cells contributing to the opercle fan in the marine population than in the freshwater population.

Acknowledgements and References

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Significantly More Proliferating Cells Contribute to the Opercle Fan in Marine Populations Than in Freshwater Populations

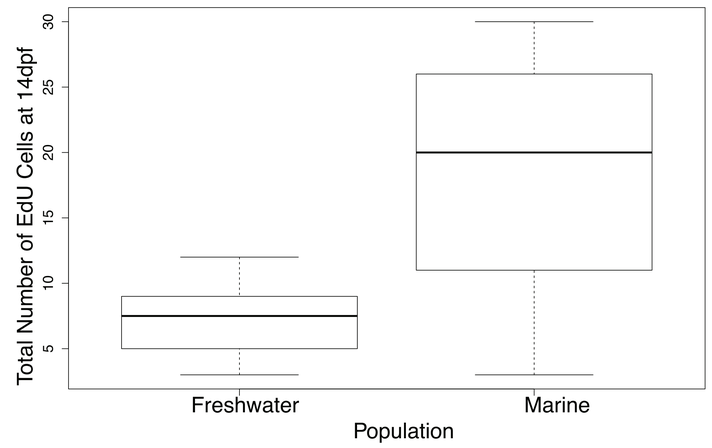

Figure 7. Boxplot of total number of EdU cells near the developing fan of the opercle in freshwater (n=10) and marine populations (n=11) at 14 dpf. There are significantly more proliferating cells contributing to the developing fan of the opercle in the marine population than in the freshwater population (p=0.0047).

Discussion and Conclusions

Hypothesis was supported: At 14 dpf, opercles from marine stickleback had significantly more proliferating cells at the growing fan than opercles from freshwater stickleback

-Differences in proliferation between populations at the growing opercle fan early may be one mechanism that accounts for adult opercle shape differences
-More proliferating cells near opercle fan in marine stickleback may lead to larger and differently shaped opercles in the adult. In further studies, I am analyzing this by comparing cell counts in the dorsal vs. ventral regions
-My study suggests taht developmental proliferation pathways might be a target of evolutionary processes in adaptations to new, freshwater environments
-This experiement is being continued on a series of stickleback developmental timepoints to assess if there is a critical window for proliferation differences
-Future studies will adress the role of apoptosis in shaping the opercle

Methods: Assessing Proliferation at the Opercle Fan

-The two populations used represent an ancestral marine population and a derived freshwater population
-At 14dpf, proliferating cells were labeled with 8hr of 1 mM EdU incubation.
-Fluorescent *in situ* hybridization was used to visualize the opercle (*Col10a1* probe)
-EdU positive cells and opercles were visualized with whole mount confocal microscopy and total cell numbers were counted (Figure 5).

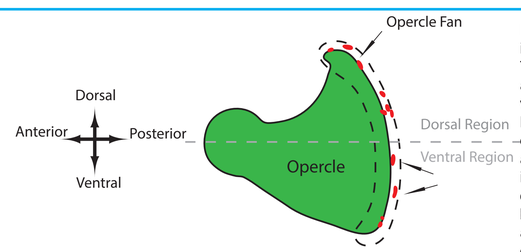

Figure 5. Illustration of an opercle in a 14dpf stickleback embryo. The opercle is visualized in green and the EdU labeled cells are visualized in red. The black dotted line indicates the fan region of the opercle. The opercle is divided along the A/P axis to separate it into dorsal and ventral regions for quantification (gray line). The black arrows indicate cells that are parallel to the edge of the opercle fan.