Development of constructive taste bud expansion across Astyanax mexicanus morphotypes

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Abstract

Constructive trait evolution, including numerical expansions of taste buds, has been minimally examined. We approach this problem in Astyanax mexicanus, a fish species with two distinct morphotypes - a cave-dwelling and a surface-dwelling morph. For my project, I analyzed key developmental stages of taste bud development, focusing on the expansion of taste buds across the head and chin of adult cave-dwelling individuals, not seen in surface fish. Our approach involves comparisons of both morphs at monthly intervals of development, from 6 months post-fertilization to 1 year. We labeled taste buds using fluorescent immunohistological staining for an antibody against calretinin, a calcium-binding protein enriched in mature taste buds. Following staining, a fluorescent stereomicroscope was used to image the stained taste buds, which will be processed to count and identify positioning of taste buds, using an automated program (NIH ImageJ). Our preliminary evidence suggests that by six months of age, taste buds of the cave morph have not yet expanded beyond the lingual region (still matching the surface morph). By 9 months however, the expansion has begun and appears to be at an intermediate stage beyond the area seen in surface fish taste buds but have not reached the adult cave fish pattern of distribution. Future directions for this work include analyses of quantitative genetic features of this trait, to identify QTL and candidate genes that may mediate taste system expansion. In sum, this work provides tissue-level insights to a remarkable constructive phenotype evolving under the intense environmental pressures of the cave.

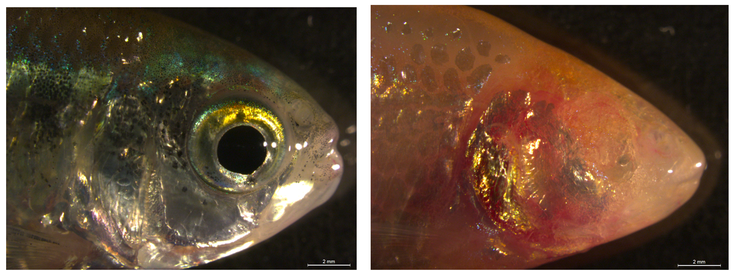

Figure 1. Images of the surface morphotype (left) and cave morphotype (right). The two morphotypes have several obvious phenotypic differences including color and eyesight. On the right, the cave fish have no eye whereas the surface fish on the left has an eye. The cave fish also has no pigmentation whereas the cave fish has a grey pigmentation.

Introduction

The species *Astyanax mexicanus* consists of two morphotypes, several cave fish populations and surface fish. These two morphotypes differ in many ways as seen in Figure 1 including their pigmentation, eyesight, and tastebuds [1]. The cave fish live in dark caves in the Sierra de El Abra region of Mexico [2]. These caves are harsh environments that exhibit a complete lack of sunlight and the cave fish have lost their sense of vision over time, unlike the surface morph. Another difference between the two morphotypes is that the cave fish have had an expansion of taste buds (Figure 2), possibly to make up for their loss of vision. The cave fish have taste buds expanded up dorsally and down ventrally, along with near their mouths. The surface fish only have taste buds along its lips [3]. These different traits may have been caused by the different environments these two fish were in. What is not known however is the precise developmental timing of this extraoral taste bud expansion seen in cave morphs. It is hypothesized that the taste bud expansion in the cave fish will occur at roughly six months of age.

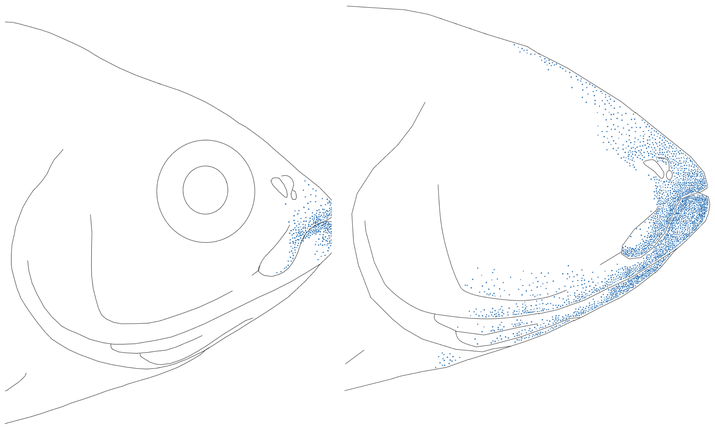

Figure 2. Outline of adult surface (left) and cave (right) fish taste buds (adapted from Schemmel 1967). These two images are outlines of the final development of taste buds in adult specimen. The cave fish shows more extraoral taste buds developed up and down its head whereas the surface fish only has them located around the lips. This is what is expected to be seen on adult specimen when stained.

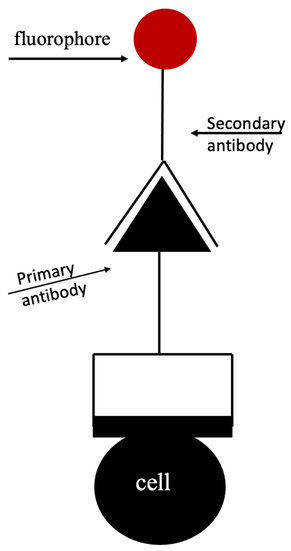

Figure 3. Primary and secondary antibodies attaching to a cell. The primary antibody first attached to the antigen on the cell, and then the secondary antibody attached to the antigen of the primary antibody that it is specific for. The fluorophore lights up when visualized under a specific wavelength to show a red circle, which is the stained taste buds.

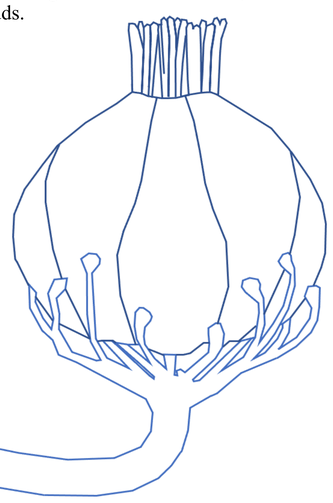

Figure 4. The outline of a taste bud. A taste bud has hair cells on the top that contain calcium which the primary antibody binds to. These hair cells are what originally detect the tastants found in the environment that they are exposed to. Directly underneath the hair cells is the bulb made up of structural, basal, and receptor cells. Receptor cells found in the bulb are what allow specific tastes to be detected, which is information relayed to an afferent nerve which carries this information away and allows the sense to be sent to the brain to be registered [4]. There are five different tastes- bitter, sweet, sour, salty, and umami.

Results

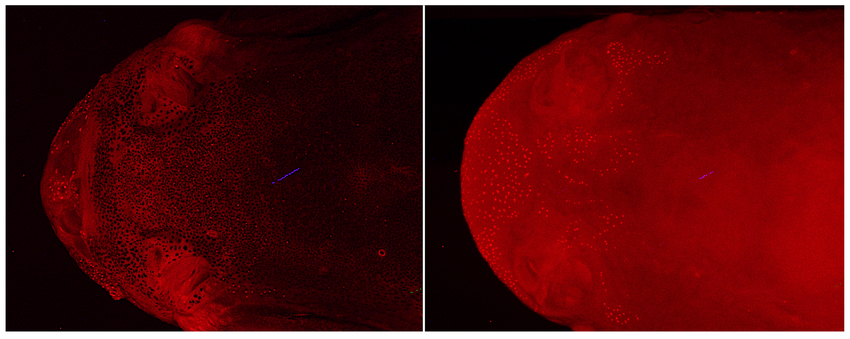

Figure 5. Adult surface (left) and cave (right) fish dorsal view taste buds stained. These images match what was expected in Figure 2. The surface fish show no extraoral taste bud expansion beyond the lips as an adult. The cave fish show taste buds expanded up dorsally.

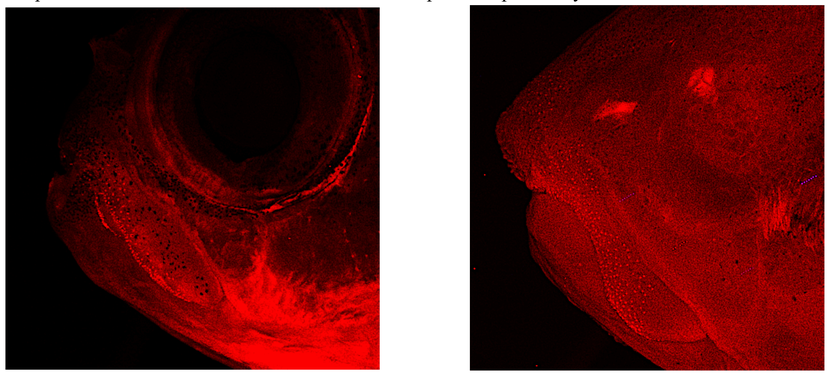

Figure 6. Side view of taste buds stained in surface (left) and cave (right) fish. These images also support Figure 2 in taste bud expansion as an adult. The surface fish only has taste buds located around its lips and mouth. The cave fish has taste buds expanding down its lips and jaw. These are the final taste buds formed on the fish.

Methods

Specimens of both the cave and surface morphotypes were taken from several monthly intervals between the ages of six months to a year and a half. These individuals were first stained with a primary antibody, anti-calretinin, which stains the calcium in the hair cells specifically found on taste buds. They were then exposed to a secondary antibody which binds to the anti-calretinin primary antibody. This secondary antibody contains fluorophores that, when visualized using a specific light wavelength on a stereoscope, fluoresce in red showing the tastebuds, which can be seen in figures 5-8. When visualizing the antibodies that mark the tastebuds, the wavelength used is 546nm.

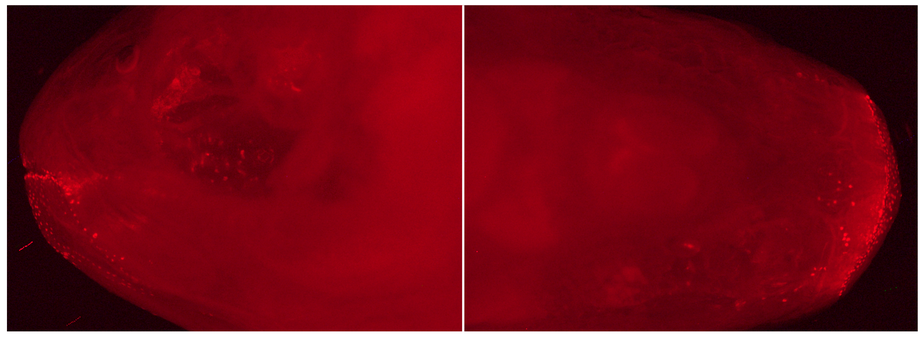

Figure 7. Side (left) and dorsal (right) view of six-month-old cave fish taste buds stained. These images both show that there has not been any taste bud expansion. The staining does not match the same degree of extraoral taste bud expansion as seen in Figures 5 and 6 of what is expected in the fully mature cave fish. Here we see the tastebuds primarily located near the lips still, appearing more like a surface fish than a cave fish still at this stage. This reveals that at six months of age, the expansion known to exist in adult cave fish has not yet completely developed

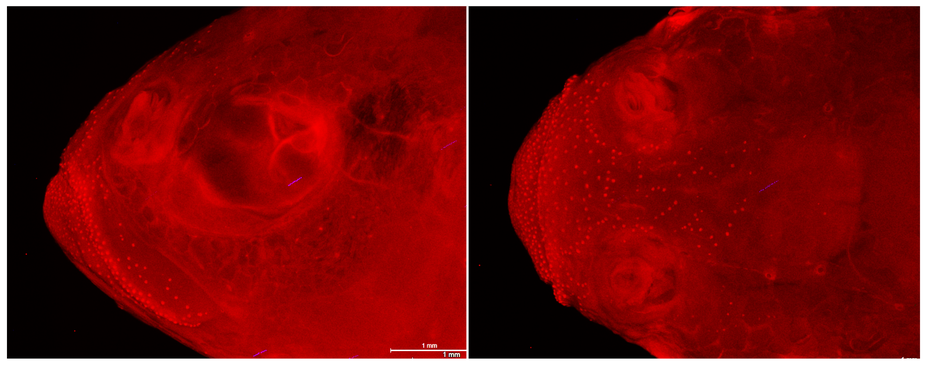

Figure 8. Side (left) and dorsal (right) view of nine-month-old cave fish taste buds stained. These images both show an intermediate stage of taste bud expansion in cave fish. They show that some regions of extraoral taste buds have expanded (unlike Figure 7), but not as fully expanded as to be expected based on what we see in Figures 5 and 6. The location and number has progressed further and they are shown further down the maxillary of the fish, as well as being located further up the cranium dorsally past the nares. This proves that at nine months expansion started but has yet to finish.

Discussion

The surface and cave fish imaged thus far have been six months of age, nine months of age, and adults (several years old). The adult cave fish showed taste bud expansion dorsally, ventrally, and along their maxillary as expected, whereas the adult surface fish showed zero expansion and only showed taste buds around their mouths which was also expected. The six-month-old cave fish showed no expansion, still appearing to have taste buds in the same location to be expected from an adult surface morphotype. The nine-month-old cave morphotype on the other hand showed an intermediate stage of taste bud expansion, with extraoral taste buds well beyond the anatomical regions seen in the six-month cave fish, but still not as expanded as what is seen in adult cave fish. These results show that the taste bud expansion begins after six months but ends sometime after nine months. Counts and spatial analyses still need to be performed, providing data that can show when this expansion in both location and number begins to be significantly different between the two morphs. Quantitative trait loci (QTL) analyses can be performed as well to show if these phenotypic differences can map to potential genes responsible for the extraoral taste bud expansion. Upon visually assessing our results, the hypothesis of taste bud expansion occurring at six months is rejected, and it appears as if taste bud expansion occurs after six months and continues even after nine months.

Acknowledgements

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Reference Short List

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