Hedgehog Signaling Contributes to Midline Facial Development and Facial Elongation in the Lizard *Anolis sagrei*

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Abstract

This project's intention is to understand the role of Hedgehog signaling in craniofacial development in the lizard Anolis sagrei. There has been extensive research on craniofacial development of avian and mammalian species, but there is a gap in knowledge regarding squamates, lizards and snakes. We hypothesized that the knockdown of Hedgehog in A. sagrei will result in disrupted formation to the midline facial structures, specifically the premaxilla. To investigate this question, stage matched embryos were collected and administered with differing concentrations, 50μm or 100μm, of the chemical cyclopamine to knock down Hedgehog signaling in the developing embryo at different stages of facial morphogenesis.

To assess the morphology of our experimental embryos, we μCT scanned and performed segmentations through an analytical software. In addition, we acquired microscopic images of the frontal and lateral view of late-stage embryos. We then recorded the range of malformations for each treatment: unaffected, mild, moderate, and severe.

Anole Craniofacial Diversity

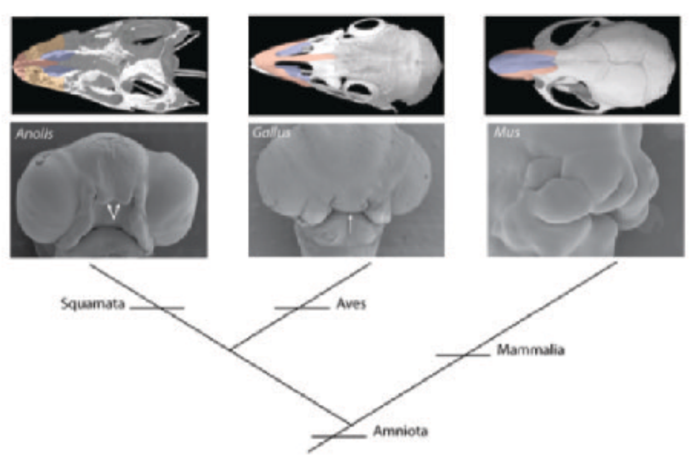

Figure 2. Phylogeny of the major amniote groups illustrating the differences in facial proportion (red = premaxilla, blue nasals, and orange maxilla). In each of these species one can see that the face forms from a series of facial prominences, yet the size, number, and proportion of those prominences varies among species.

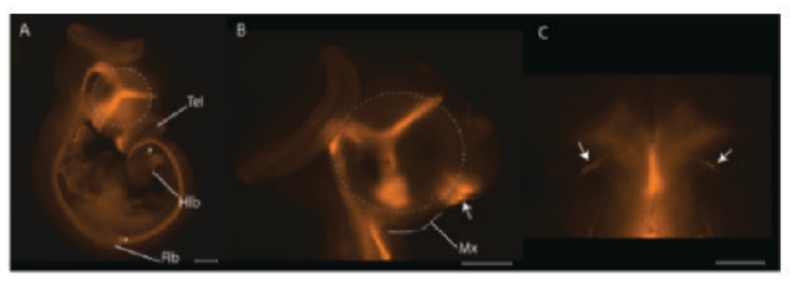

Figure 1. An A. sagrei embryo embryo at oviposition showing the expression of Shh (orange; Flb = forelimb bud, Hlb = hindlimb bud, Tel = telencephalon). B) Closeup of the A. sagrei head at oviposition highlighting the expression in the telencephalon (arrow). C) After 36-48 hours the expression moves into the oral ectodermal epithelium (arrows) as two paired expression domains.

Methods

Collection and treatment of specimens

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Photograph frontal and lateral images of specimens under microscope

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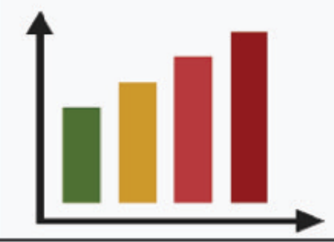

Sort specimens by malformation levels

Utilize micro-CT scanner to gather data

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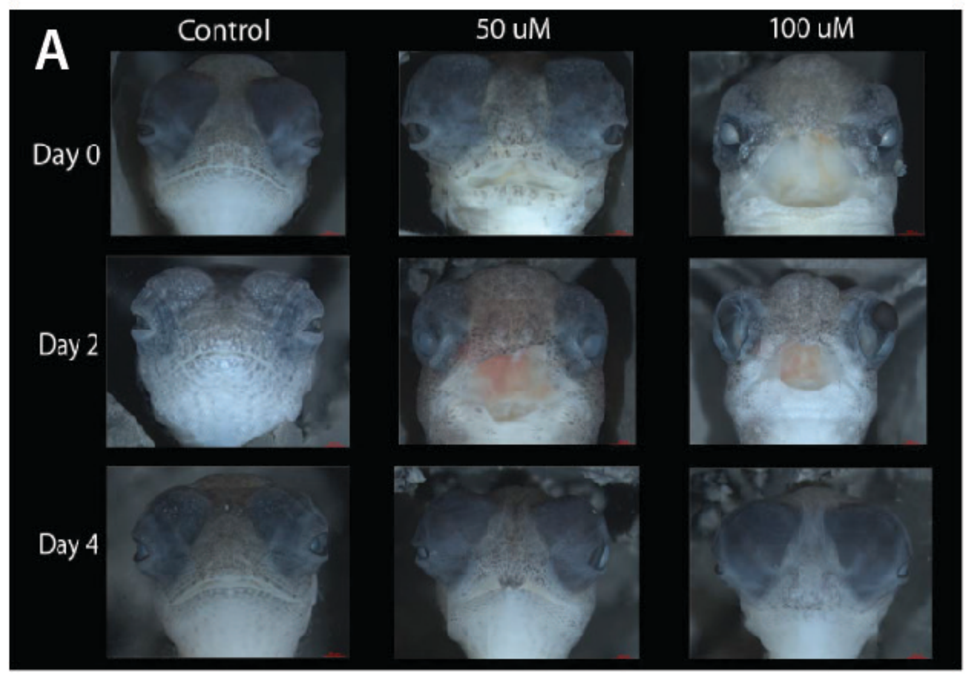
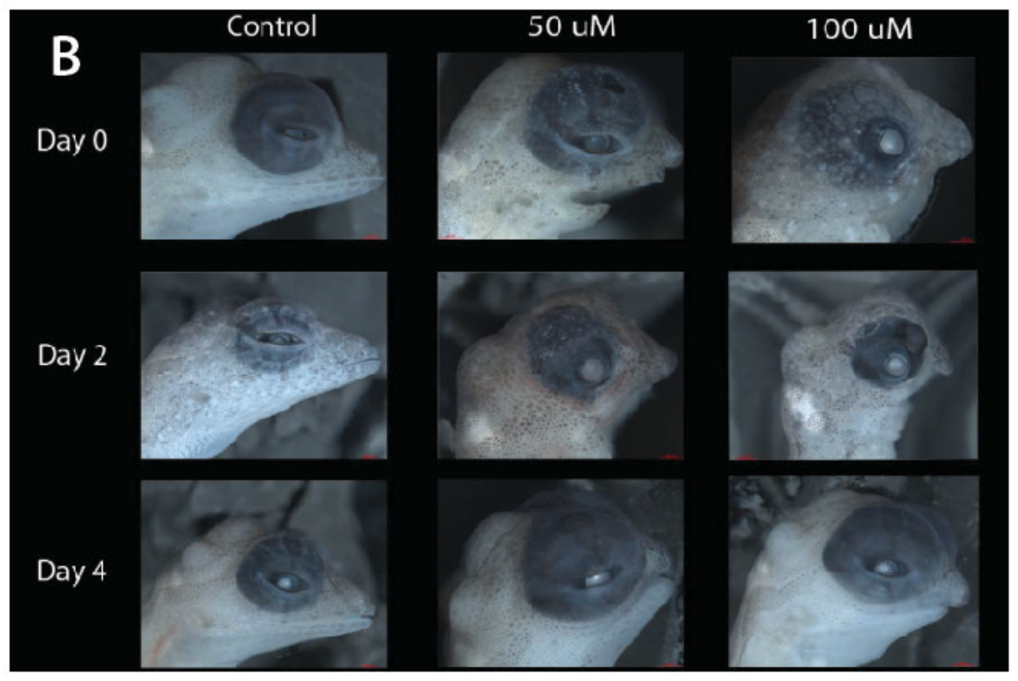
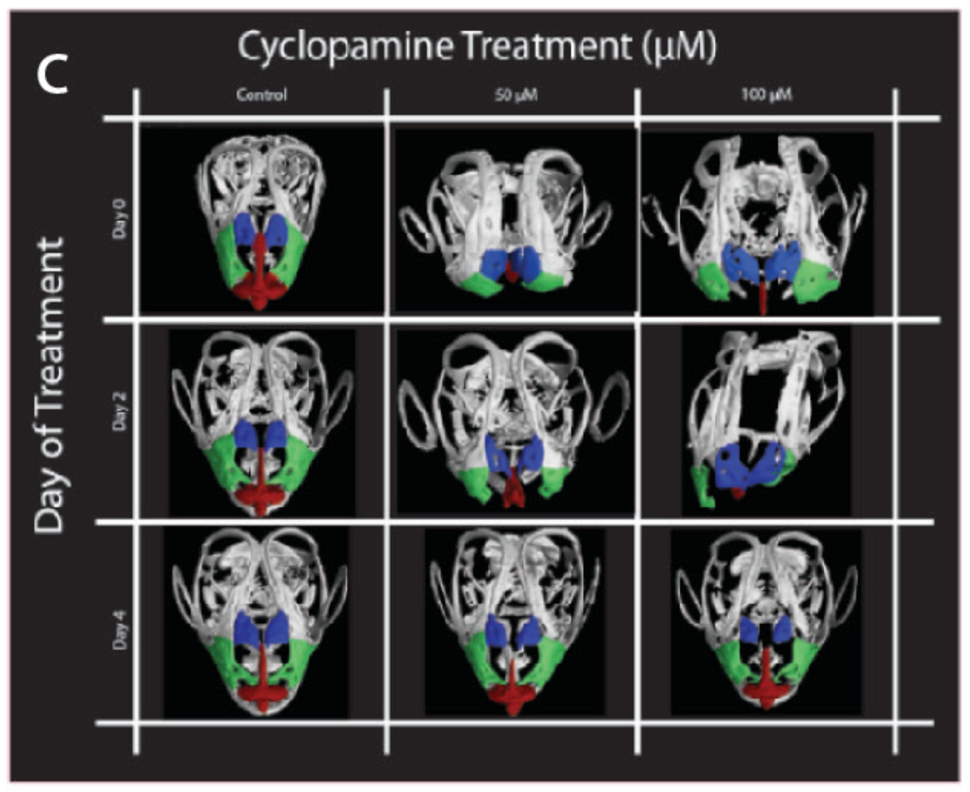
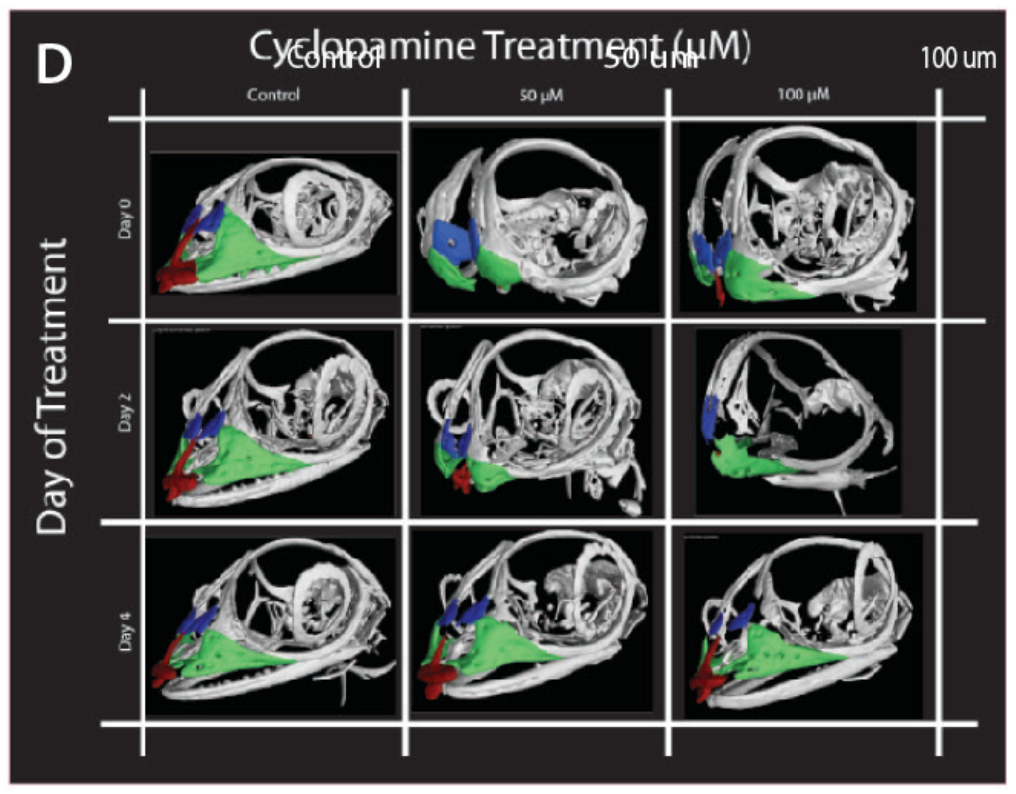
Create surface models and segment in VGStudio Max

Results









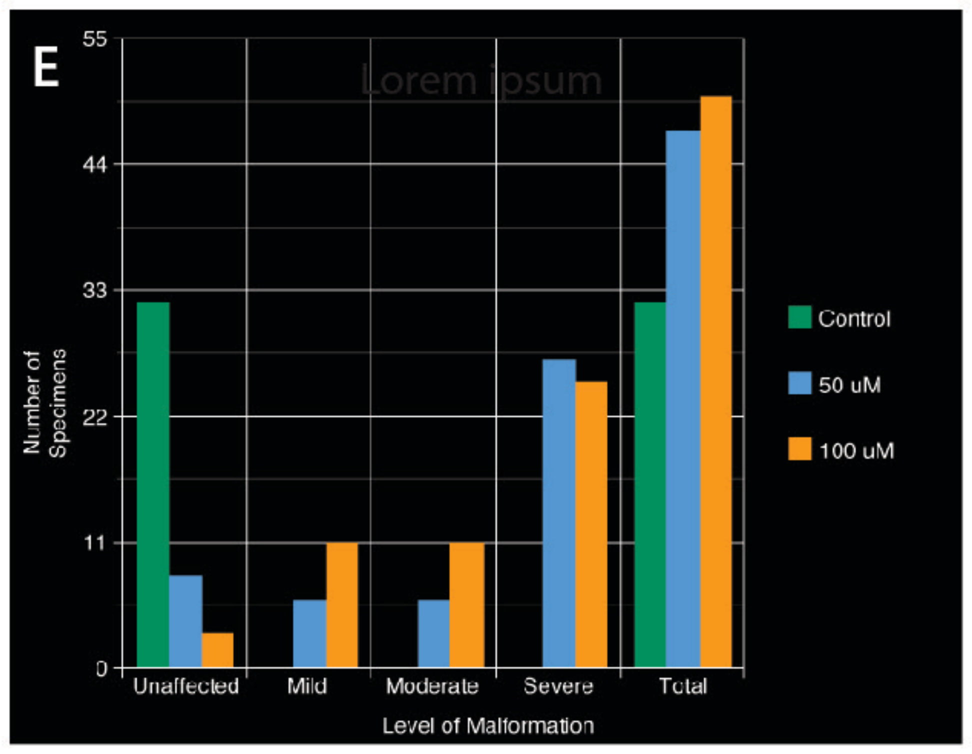


Figure 3. *Anolis sagrei* embryonic skulls influenced by varying treatments of cyclopamine on differing days of post-oviposition. (A) Frontal view of microscopic images of specimens with malformations. (B) Lateral view of microscopic images of specimens with malformations. (C) Frontal view of μCT scanned and segmented skulls (red = premaxilla, blue = nasal, green = maxilla). (D) Lateral view of μCT scanned and segmented skulls. (E) Observed levels of malformations in A. sagrei skulls ranging from unaffected to severe.

Conclusion

- Higher concentrations of chemical cyclopamine treatments result in more severe malformations due to higher levels of Hedgehog signaling knockdown
- Early ovi-position treatments ablate much of the midline skeleton while later treatments only mildly affect facial elongation
- We conclude that Hedgehog signaling plays a unique and critical role in lizard facial development compared to other amniotes.
- Especially noticeable is the unique way that Hedgehog signaling appears focused on the midline skeleton, while the more lateral maxillary derivatives appear to develop more normally.
- Further research should be conducted to understand the link between cell signaling and facial morphogenesis in lizards.

Acknowledgements

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