The Role of Hedgehog Signaling During Hemipene Development in *Anolis sagrei*

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Abstract:
This project's goal was to identify the role of Hedgehog signaling during hemipene development in the lizards *Anolis sagrei*. Hemepenes are the paired genitalia of squamates, lizards and snakes. When compared to mammals, there little known about the details of lizard genital development. Based on prior observations in mammals, we hypothesized that Sonic hedgehog (Shh) contributes to the development of hemipenes. We knocked down Hedgehog signaling during hemipene patterning to assess the function of Shh during hemipene development. Preliminary data demonstrate that Shh is critical for hemipene patterning in lizards.

Introduction:
The external genitalia of vertebrates evolved once at the origin on amniotes. There is a wide array of variation in genitalia, from a single midline phallus to the paired hemipenes of squamates. In all species, the developmental process starts with the formation of paired genital swellings. In mammals, these expand and fuse along the midline. In Squamates the paired swellings don't fuse together, producing two paired hemipenes, (Sanger et al. 2015). Although the processes are similar, there have been no studies on the molecular patterning of squamate genitalia. The purpose of this research is to provide a wider understanding of the development of hemipenes in *A.sagrei.* Our focus for this investigation is how Shh affects hemipene development.

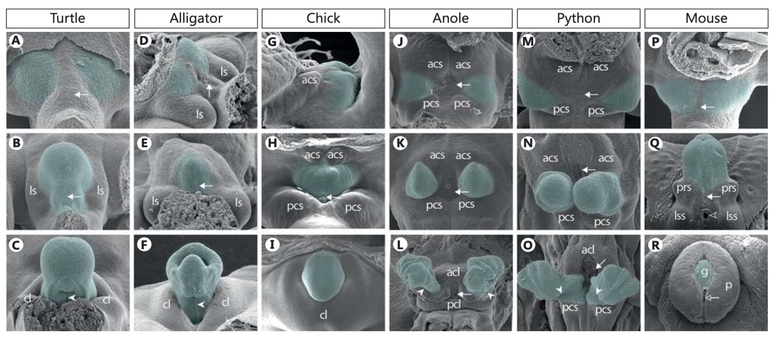

Figure 1:
Scanning electron micrographs of amniote external genitalia developmen at 3 approximately equivalent stages of development.Top row (**A, D, G, J, M, P**): Genital swelling stage, early in external genitalia development. Middle row (**B, E, H, K, N, Q**): Genital tubercle or hemiphallus buds present. Bottom row (**C, F, I, L, O, R**): Late genital tubercle or hemiphallus stage.

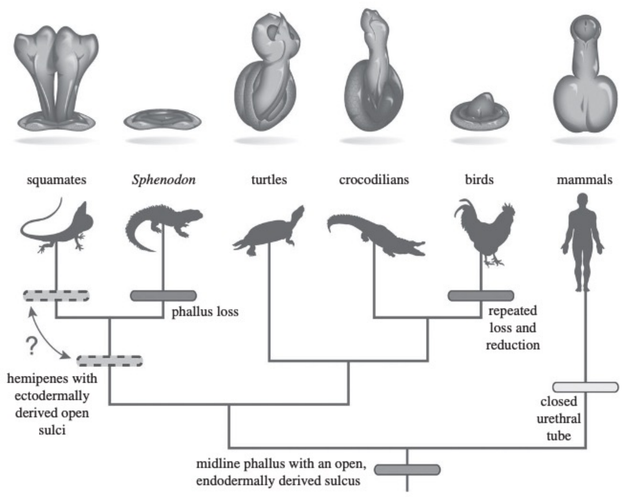

Figure 2:
A resolved hypothesis regarding the evolution of amniote external genitalia. Observations suggest that the phallus evolved once and diversified among amniote lineages.

Methods:
A. sagrei embryos were treated on Day 0 of development with 100µM of Vismodejib, a Hedgehog signaling antagonist and a control with equal part DMSO. Subsequently, they were dissected on the following days of development: day 8, 12, and 24. Afterwards, the embryos were photographed with Scanning Electron Microscopy (SEM) and their phenotypes were compared to observe how Hedgehog signaling affected Hemipene patterning in *A. sagrei*.

Results:

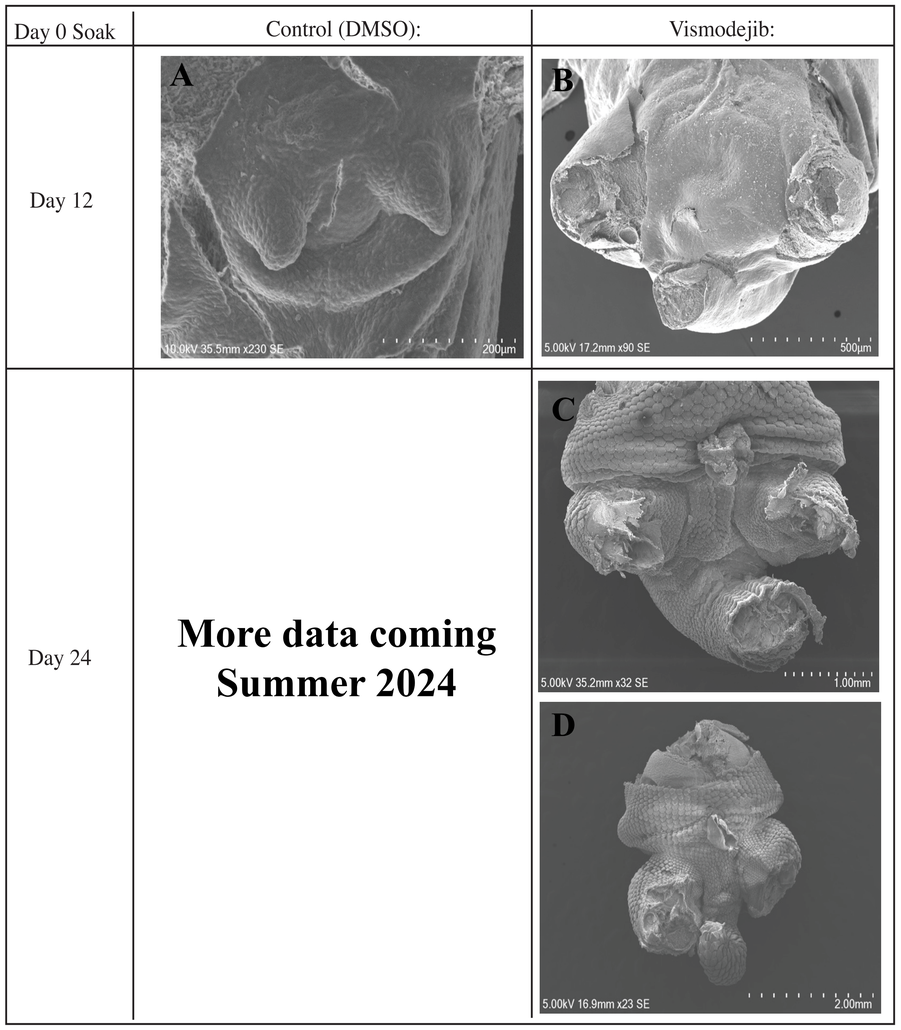

Figure 3:
Day 0 embryos divided into 100µL DMSO controls and 100µL of Vismodejib, a Hedgehog knockout. (A): Control hemiclitores developing. (B) No sulcus or protrusions observed. (C-D): Embryo was shorter and presented abnormal limb, no genital development present.

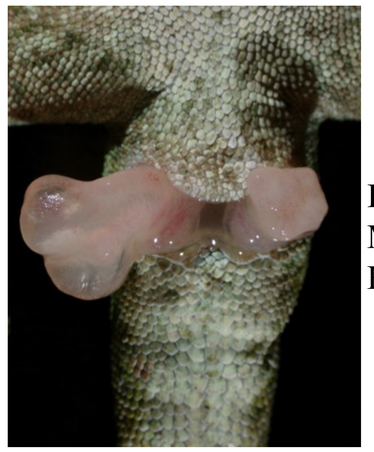

Figure 4:
Mature lizard Hemipenes

Conclusion:
Hedgehog signaling is present during the patterning stage of hemipene development due to the absence of hemipene development in the treated embryos.

Further research would be focused on PCR testing the embryos to determine their genetic sex and study the effect of Hedgehog knockout in the patterning, elongation and growth stage of the embryo to see the effect on hemipene and hemiclitore phenotypes.

Acknowledgements:
Thank you to the Biology Department for the use of the SEM and to Dr. Thomas Sanger for his guidance and support

References and citations:
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