Genital Development in an All-Female Lizard Species, *Lepidodactylus lugubris*

Ryan P. Bonate, Elyse D. Laps, Thomas J. Sanger

Loyola University Chicago, Department of Biology, Chicago, IL 60048

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Abstract

Little is known about reptilian embryonic and sexual development. While lizards display characteristics quite different from other classes of vertebrates, scientists often ascribe mammalian processes to reptiles. The purpose of this study is to examine genital development in a parthenogenetic, all-female lizard species, *Lepidodactylus lugubris*. Although this species is fully female as an adult, all embryos examined possess well-developed male genitalia. We characterize the extent of cell proliferation in the genitalia at different points in development. This data will be the first step towards telling us whether mammalian processes of differentiation hold significance across biological classes.

Introduction

- Sexual development among reptiles is a chronically understudied area of vertebrate biology
- Researchers assume that the same biological processes that drive mammals through sexual differentiation would occur in other classes
- The presence of male genitalia in an all-female species during early life challenges this assumption
- By closely examining how these genitalia develop in relation to the rest of the body, we can begin to evince the importance of this unexplained and temporary embryonic sexual differentiation

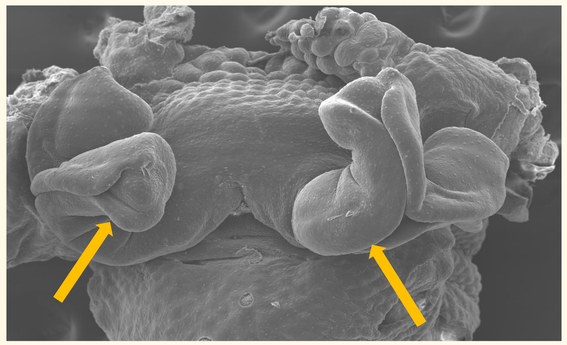

Figure 1: Developed hemipenes, marked by yellow arrows, in a late-stage (~18 day) *Lepidodactylus lugubris* embryo

- Our experimental design studied the patterns of cellular proliferation and growth during the course of embryonic development, with special interest paid to the gonadal region

Methodology

- *Lepidodactylus lugubris* eggs were incubated for periods of time needed to collect all stages of hemipenis development via dissection, which occurred as according to Figure 2

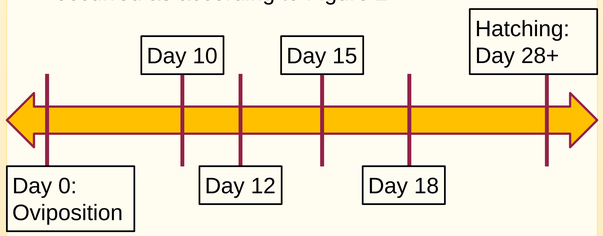

Figure 2: A short timeline of *Lepidodactylus lugubris* development; marked days represent the dissection times of different study groups.

- The embryos were dissected once they reached their desired time of incubation, and the head, legs, and tail were removed, leaving just the abdomen and genitals to examine
- These specimens were preserved in PBS with sodium azide, which prepared them for staining
- The specimens were then processed using a Click-iT™ EdU kit
  - The staining protocol followed was a close adaptation of the procedure that comes with the kit; the only change was to ignore the "preparation" steps because our samples were already ready to receive the stains
- This protocol produces images much like Figure 3
  - Nuclear content, stained with DAPI, is visualized as blue coloration
  - Cell proliferation, stained with EdU, is visualized as orange coloration
- By comparing the relative brightness of the signal across the specimen, we can determine where cell growth is occurring (EdU) and how the cells are organized (DAPI)

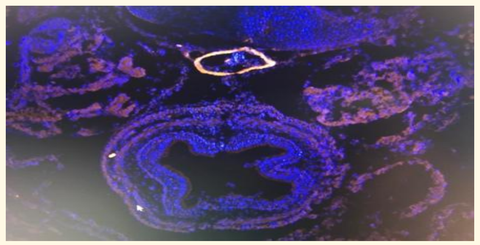

Figure 3: an example immunohistochemistry (IHC) stain of an embryonic androgen receptor, marked by strong orange signal

Results

- The experimental data are not yet complete, but there are a number of preliminary indications
  - One reason for this delay in data retrieval is the pandemic and its related shutdowns
  - The second reason for delays is that both stains used interact with Thymine-rich DNA, so exposure times to each stain needed to be carefully monitored and adjusted
    - Exposure to EdU for 30 minutes and DAPI for 20 minutes produced the clearest images
- First, it appears that male sexual differentiation is one of the most cell-intensive process occurring during the early growth period
  - This indication is seen by comparing the orange coloration in the hemipene of Figure 4 with the rest of the body

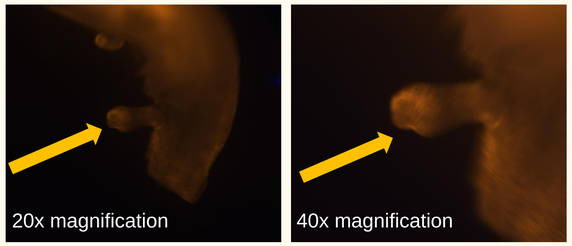

Figure 4: An EdU stain showing the localization of cell proliferation, with hemipenes indicated by arrow in yellow

- It also appears that male sexual differentiation slows down to match the rest of the body by the end of the early growth period (~day 15)
  - This is seen in Figure 5 by comparing the orange coloration in the hemipene with the rest of the body

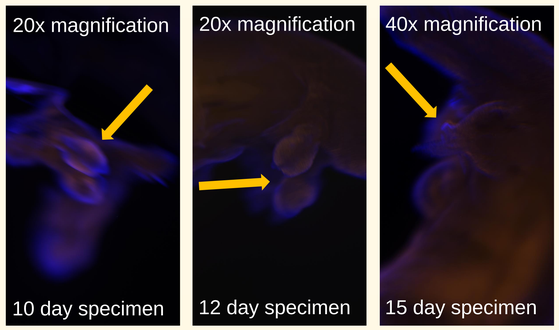

Figure 5: an IHC stain with hemipenes indicated by yellow arrows. Note the relaxation of the orange signal in the hemipenes as the lizard develops

Conclusion

- The male sexual organs are not pronounced nor functional after hatching, which suggests that any advantage of undergoing male sexual differentiation lies elsewhere
- Because the process of male sexual differentiation occurs so early in the growth period, it is reasonable to suspect that this pathway is important to other development pathways during later embryogenesis
- While mammals are considered "innately female" due to their early development of feminine features, *L. lugubris* could be considered "innately male" due to its rapid development of male genitalia
- Our monitoring of *L. lugubris's* sexual development has revealed that mammalian sexual development is not analogous to lizard sexual development, despite the widely held assumptions otherwise

Further Directions

- Identification of the specific molecular pathways that drive sexual development in *L. lugubris* and other lizards
- Effects of hormonal silencing on sexual development in *L. lugubris* and other lizards
- Effects of male sexual development on phenotype, survival, and reproductive capabilities
  - the parthenogenetic nature of *L. lugubris* may be tied to male sexual differentiation as an embryo
- Analogous studies in other clades to determine whether male development is limited to certain species or related groups of lizards

Literature Cited

- Gredler, Sanger, Cohn., et al. 2014. Evolution of External Genitalia: Insights from Reptilian Development. *Sexual Development* 8:5, 311–326., doi:10.1159/000365771.

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