Impact of variable egg incubation regimes on hatchling American alligator external genitalia

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Introduction

All crocodylians, including American alligators (*A. mississippiensis*), undergo temperature-dependent sex determination (TSD) where nest temperature during a specific time frame in egg incubation regulates gonadal differentiation. Based on this understanding, it has been established that American alligator eggs incubated at a constant 33°C produce exclusively testes and male hatchlings, while incubation at a constant 30°C results in ovary development and female hatchlings. These organs drive sexually-dimorphic hormonal milieus that generate secondary sex characteristics, such as genital morphology from a bi-potential anlagen. The presence of testicular androgens causes the previously undifferentiated genital tubercle to develop into a male penis, while the absence of these androgens results in the development of a female clitoris. While gonad differentiation occurs *in ovo*, the external genitalia diverge morphologically during the first several years of the organisms' development. However, the fine-scale details of this divergence that occur primarily around the time of hatching require further clarifications.

Figure 1. The influence of temperature-dependent sex determination on crocodylian genital differentiation schematic.

For this study, we investigated if multivariate analysis could better characterize the morphological divergence of alligator genitalia. Additionally, we investigated if other cliterophallic (CTP) morphological features could indicate gonadal sex soon after hatching.

Methods

- American alligator eggs were field collected from Tom Yawkey Wildlife Center (Georgetown, SC) and randomized into 5 incubation regimes:

Table 1. Treatment, incubation temperature, and fluctuating type for American alligator (*Alligator mississipiensis*) hatchlings collected at University of Georgia Savannah River Ecology Lab.

Treatment	Temperature	Fluctuating?	n=	Sex ratio (M/F)
MPT	33.0°C	No	16	16/0
IPTC	31.2°C	No	27	0/27
IPTF1	31.2 ± 0.6°C	Yes	21	0/21
IPTF2	31.2 ± 2.8°C	Yes	22	0/22
FPT	30.0°C	No	20	0/20

- CTP were dissected from 7 days post-hatch alligators and digitally photographed under a dissecting scope with a CCD camera and stage micrometer for scale.
- Landmarks were placed at the following points (Fig. 2).
- Landmark data was used to generate a Procrustes Superimposition, Principle Components Analysis, MANOVA, and Discriminant Function Analysis.
- Pigmentation variation was measured semi-quantitatively on a scale of 0 to 3.

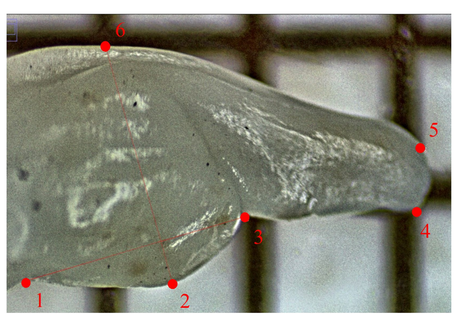

Figure 2. Landmark positions (red circles) and guides for calculations (red lines) on the CTP of an American alligator hatchling.

Results

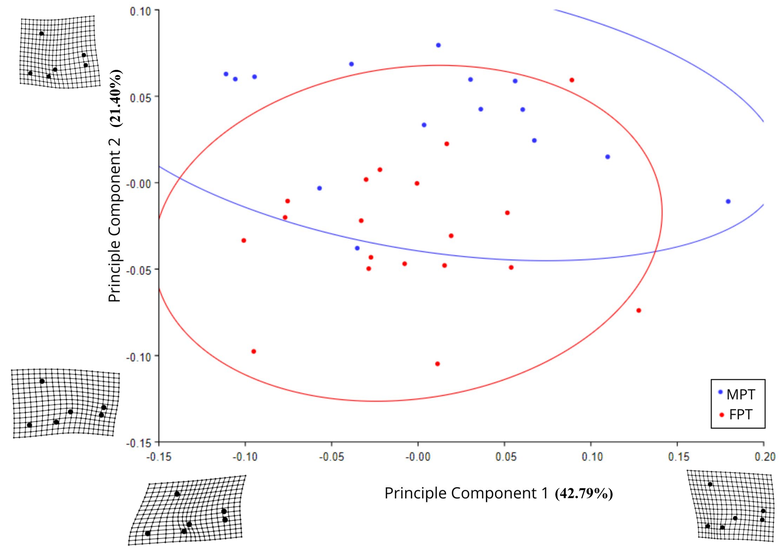

Figure 3. Principle Components Analysis with confidence ellipses for each treatment group (MPT,FPT: ANOVA p=0.007) and deformation grids for the positive and negative extremes of the corresponding PC. Eigenvalues for each PC are listed in parentheses.

Table 2. Results of the Discriminant Function Analysis demonstrating the level of significance in variation between treatment groups.

Pairwise Comparison	P-value
FPT v. MPT	0.0001
FPT v. IPTC	0.0001
FPT v. IPTF1	0.0041
FPT v. IPTF2	0.0004
MPT v. IPTC	0.0940
MPT v. IPTF1	0.0251
MPT v. IPTF2	0.1523
IPTC v. IPTF1	0.0876
IPTC v. IPTF2	0.1722
IPTF1 v. IPTF2	0.7239

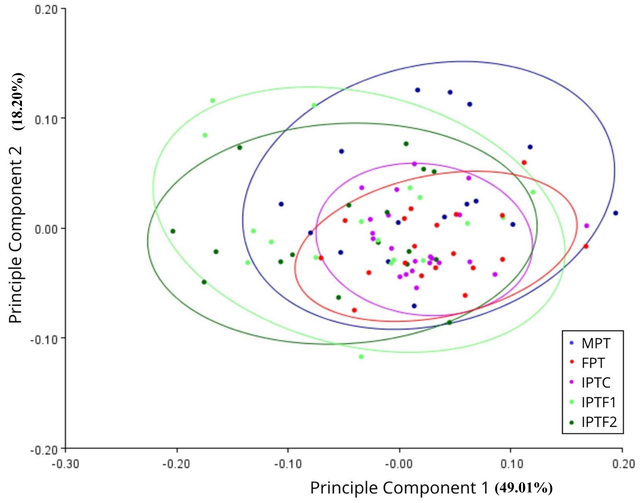

Figure 4. Principle Components Analysis of all treatment groups with confidence ellipses for each. Eigenvalues for each PC are listed in parentheses.

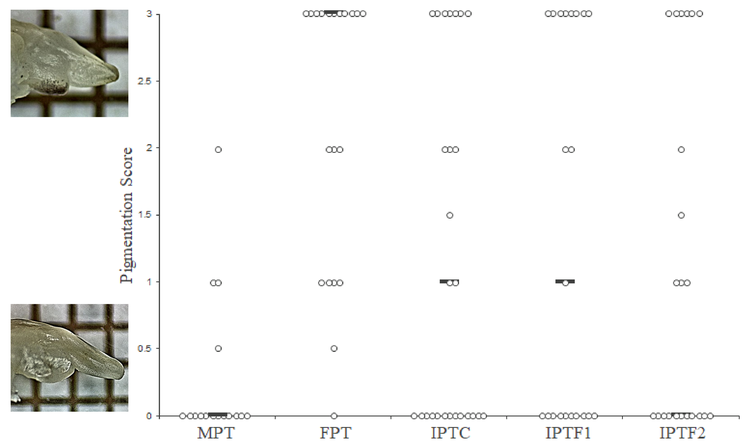

Figure 5. Semi-quantitative pigmentation univariate scattergram. Open circles represent individual specimen, and horizontal lines represent treatment modes.

Conclusion and Future Directions

- Genital development is semi-independent of gonadal sex in species with TSD.
- Intermediate incubation temperatures produce exclusively female specimen, but some hatchlings' genital morphology more closely resembled that of hatchlings incubated at male-producing temperatures.
- The genital morphological plasticity at this early life stage is due to the variations in egg incubation environment resulting from the three-dimensional structure of the alligator nest, fluctuations in the ambient temperature, and other environmental factors.
- Therefore, observation of the external genitalia *cannot* be used as a diagnostic for hatchling sex.
- Moving forward, we plan to analyze the effect of *in ovo* exposure to endocrine-disrupting pollutants on the patterns of genital morphologies across these stable and fluctuating temperature regimes.

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