Molecular genotyping the Texas horned lizard (Phrynosoma cornutum) - a goal for inquiry-based learning in biochemistry

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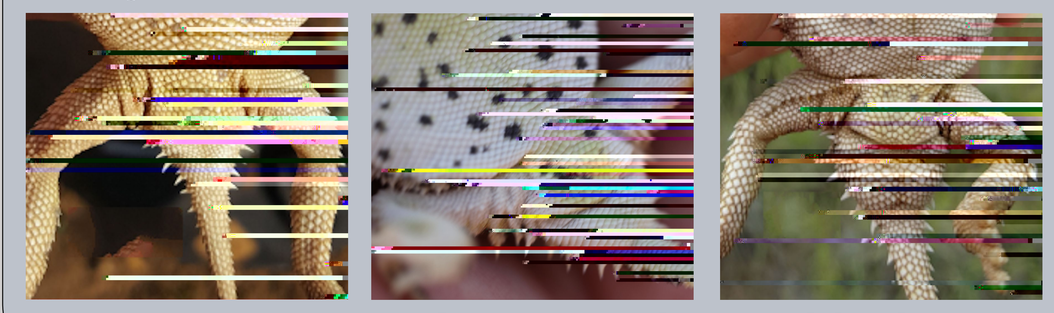
Can you tell the sex of our Texas Horned Lizards?



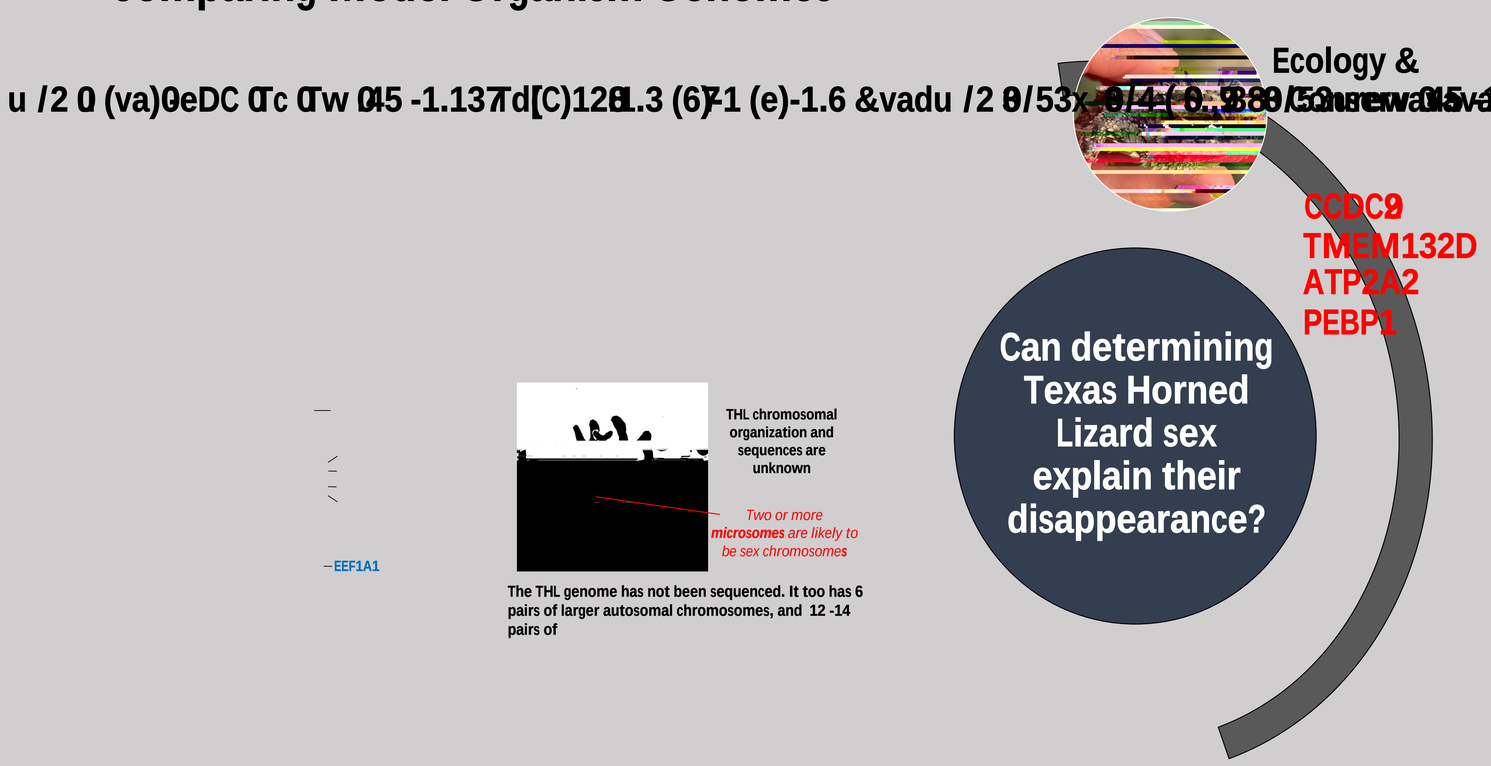
(We can't always either)

- **THL populations drastically declined over the last five decades.**
- **Parity in their sex ratio is uncommon.**
- **Physical identification of sex is not possible in juveniles.**
- **The molecular mechanism for sex determination is unknown.**

Abstract:

Texas horned lizard (THL; *Phrynosoma cornutum*) populations have declined throughout North America, and an unexplained skew in sex ratio could be the cause. Improved conservation efforts are needed for this threatened species, and students in the classroom have an opportunity to cultivate familiarity with biochemistry by inquiring into mitigating this decline. THL lack easily visualized sex chromosomes which impedes sexing them by karyotype. Instead, molecular genotyping is needed to study THL reproduction, but its genome is not sequenced. Quantitative Polymerase Chain Reaction (qPCR) strategies based on the green anole (*Anolis carolinensis*) have measured genetic variation across a diverse array of reptiles. If sexual determination in THLs is governed genetically then similar qPCR strategies could resolve THL sex. Application of this knowledge of genetic variation lends itself to further research questions in a classroom or lab setting. This study evaluated qPCR assays for 6 different loci predicted to be in THLs: two autosomal (*EF1A* and *ADARB2*), and four X-linked (*TMEM132D*, *ATP2A2, CCDC92,* and *PEBP1*). In anole, X-linked loci amplify with half the signal in males. Primer fidelity was established using male anole gDNA. Amplification efficiency (*E*: 0.92-1.01; R2≥0.94) was optimized per MIQE guidelines. THL DNA was collected from cloacal swabs of adult males and females (n=5) captured by hand (Hale County, TX). *CCDC92* and *PEBP1* amplified anole gDNA with reduced efficiency compared to other assays, possibly due to increased GC-content. These assays also failed to amplify THL samples consistently. In contrast, all other assays [illegible] the predicted X-linked signals. These findings suggest that qPCR strategies derived from the anole can be employed to study the THL, but assay conditions need further optimization for this specific species. Surprisingly, this data could not confirm the predicted XX/XY system of sex determination, thus other mechanisms cannot be ruled out. An adaptable genotyping assay used by multiple classrooms would provide an exciting platform to encourage insight into the problem through teaching and learning of biochemistry.

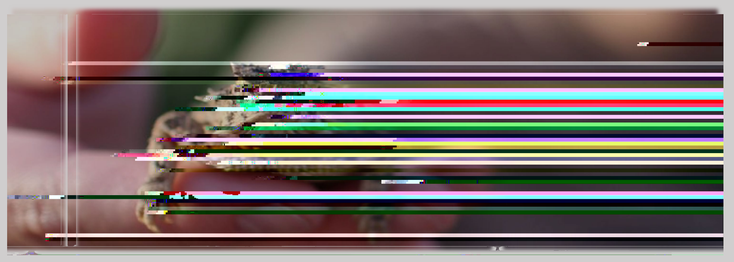
Comparing Model Organism Genomes



Why Texas Horned Lizards?

The Texas horned lizard (THL; *Phrynosoma cornutum*) is known by many as the "horny toad," our Texas state reptile. As stated in the abstract, the THL populations have declined throughout North America. There is a need for improved conservation efforts for this threatened species, and students in the classroom starting from as early as Jr. high have an opportunity to incorporate biochemistry and education techniques to analyze this decline in our prized state reptile.

Quantitative Polymerase Chain Reaction (qPCR) strategies are a great way to incorporate inquiry-based learning. With a qPCR machine available, all things are accessible for students to conduct this graduate level project in their secondary school education systems.



Amplification Curve (all assays)- Anole

Amplification Curve (all assays)- THL

Quantitation of the THL gDNA

Primary findings: what's next?

Standard curves are used to determine the efficiency, linear range, and reproducibility of a qPCR assay. Using the fully sequenced Anole genome as a template, we successfully created a standard curve that we then applied to our studies in the THL.

Our PCR approach detected DNA in THL! Our methods created an assay sensitive enough to detect DNA obtained from juvenile THL swabs. These findings suggest that qPCR strategies derived from the anole can be employed to further study the THL.

Understanding how sex determination occurs in THL is fundamental to explaining their survival. Our preliminary data could not confirm the predicted XX/XY system of sex determination.

Inquiry-based research excels at incorporating biochemistry principles. An adaptable genotyping assay used by multiple classrooms would provide an exciting platform to encourage insight into teaching and learning biochemistry.

Acknowledgements:

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