Collection and analysis of soil environmental DNA for the detection of the Texas kangaroo rat *Dipodomys elator*

Hannah G. Belinne[1], Matthew A. Barnes[1], and Richard D. Stevens[1,2]

1 – Department of Natural Resources Management, 2 – Natural Science Research Laboratory

hbelinne@gmail.com

Introduction

The Texas kangaroo rat *Dipodomys elator* warrants careful monitoring and management due to its endangered species status.

Camera trapping and live trapping will struggle to detect *D. elator* in areas of low density or with *D. elator* that are neophobic and therefore trap-shy.

Environmental DNA (eDNA), genetic material organisms shed into their environment, can be extracted from soils to give an idea of species present without trapping.

The use of eDNA in *D. elator* monitoring can result in a more accurate depiction of habitat range, allowing for better management practices for this endangered species in the future.

Dipodomys elator

Objective

To determine if eDNA extracted from the soil can be used to detect *D. elator* in the environment.

Methods

Soil was collected from the entrance of burrows suspected of being *D. elator* homes or confirmed via camera trapping.

Genetic data from Genbank was used to develop primers based on CytB sequences of *D. elator* and similar *Dipodomys* species.

After several rounds of qPCR utilizing various primers and temperatures proved unsuccessful in routinely obtaining specific and efficient amplification of the target species, a probe-based assay was ordered.

Probe-based assay was tested with extracted DNA from the target species and its three closest genetic relatives:

D. Merriami *D. Phillipsi* *D. Ordii*

After decently promising results, eDNA was extracted from the previously collected samples and used to test the efficiency of the primer in detecting eDNA.

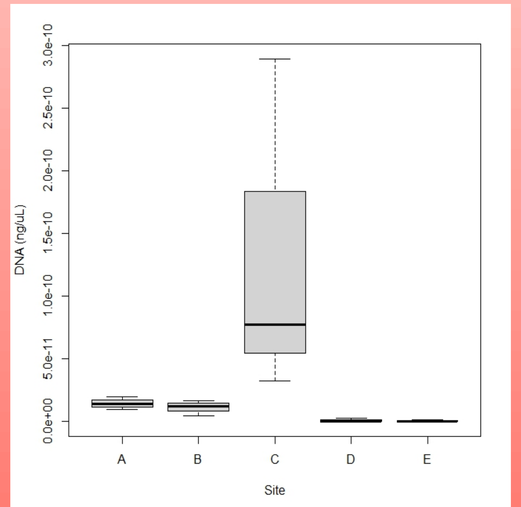


Fig. 2 This box plot shows the amount of eDNA present at each site according to the standard curve plot created by known quantities.

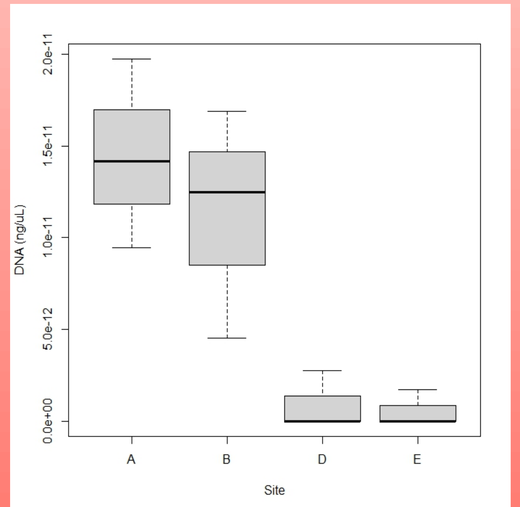
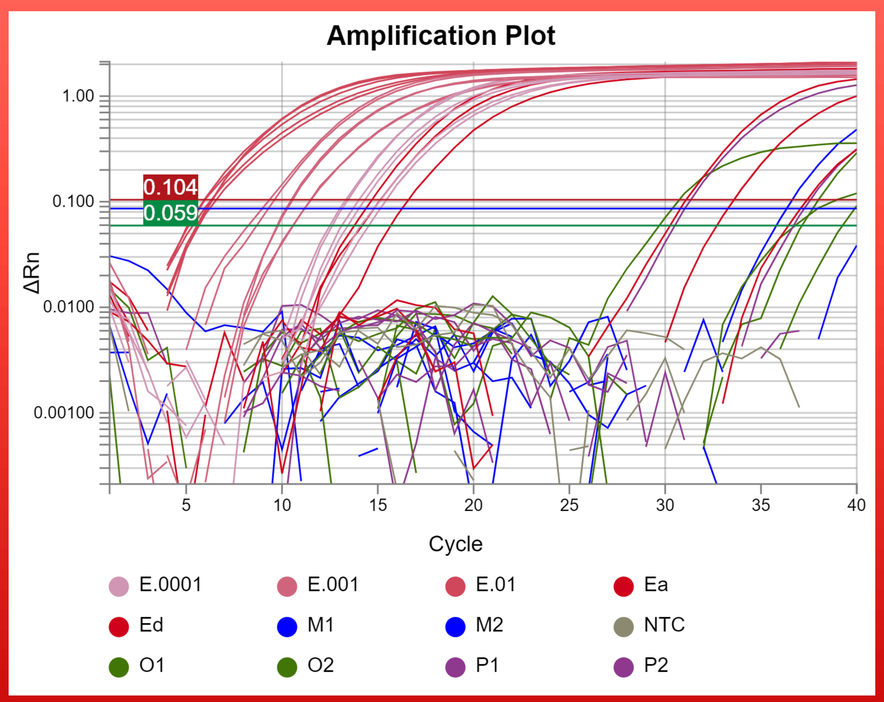
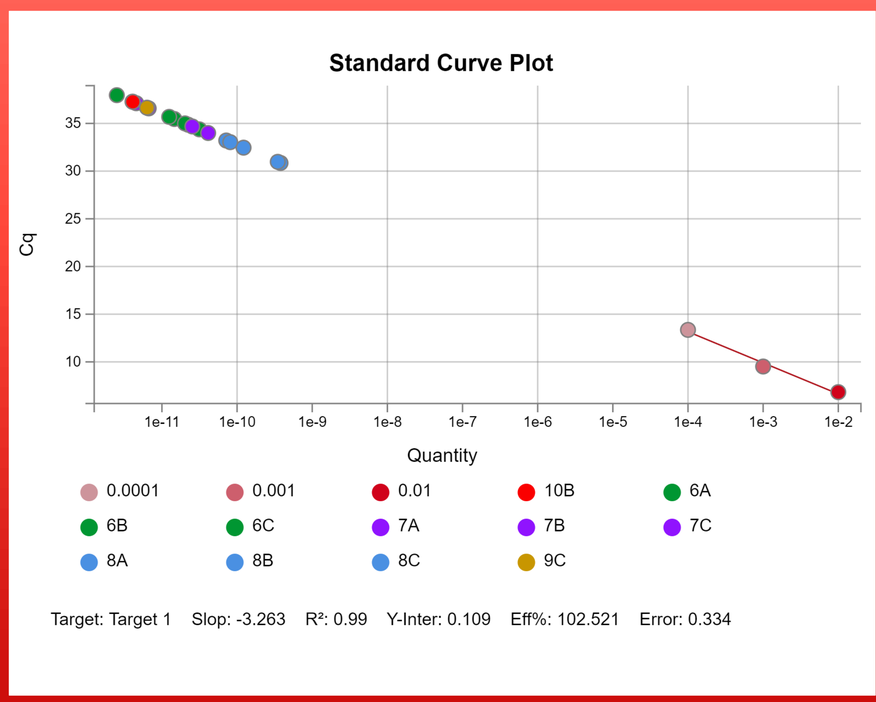


Fig. 3 This box plot shows the amount of eDNA present at each site excluding an abnormally concentrated sample (site C).





Results

The probe-based PCR is successful in amplifying *D. elator* eDNA (Standard Curve Plot) and can confidently quantify the DNA present (Eff%:102.520, R=0.99).

The probe-based assay can confidently distinguish *Dipodomys* DNA. However, further specification is necessary to reliably confirm that amplification is due to the presence of target species eDNA.

Some non-target species were amplified in the trial runs, but with less efficiency.

Conclusions

In combination with gene sequencing, this assay can confidently be used to determine the presence of *D. elator* at a burrow.

Next Steps

Use the primer to detect 5m, 10m, and 15m from the burrow entrances to determine how easily data can be collected from a plot of land while remaining accurate.

Collect airborne eDNA to determine if this assay can be used to detect *Dipodomys* using airborne samples.

Adjust the assay to be more species-specific so that post-PCR sequencing is unnecessary.

Sequence the eDNA amplified in samples to confirm the species being detected.

Complete a sequencing run without NTC contamination to confirm the results.

Acknowledgments

I would like to thank the Honors College Undergraduate Research Scholars Program supported by The CH Foundation and the Helen Jones Foundation, Inc. Many thanks to Dr. J. Geotze of Laredo Community College for assistance with sample collection.