NATURE FOUNDATION SA INC

Scholarship Grant 2016 Report

1. Applicant/s

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2. Abstract

The Australian pygmy bluetongue lizard is an endangered species endemic to South Australia, found in highly fragmented habitat patches of native grassland in the state's mid-North. It is our goal to generate genomic tools which may be used as a reference for future studies into functional genes in this species. This study uses transcriptomic techniques to Identify functional genes which may play a role in surviving dry periods. This is achieved by comparing gene expression data from six kidney samples taken from both males and females, at the beginning and end of the dry seasons, between 2014 and 2016. Additional tissue samples will be added to the dataset to generate a comprehensive map of the *T. adelaidensis* transcriptome, and to identify functional genes of interest such as reproductive and immune genes.

3. Introduction

Understanding functional genes and how much adaptive potential a species of interest has, at both the individual and population level, has becoming increasingly critical in informing appropriate conservation methods (Ouborg et al. 2010). Neutral genetic markers such as microsatellites are frequently used to give indications of population dynamics; however functional data is needed to generate an accurate picture of population health (Kohn et al. 2006). A greater understanding of functional genes allows relationships between genetic observations and ecological processes, mating systems, pathogens, and other selection pressures, all of which allow a greater understanding of a species' ecology.

Population wide studies have been conducted to evaluate breeding processes in *Tiliqua adelaidensis* (Smith et al. 2009; Schofield et al. 2013), however there is currently very little data on functional genes. Further habitat restrictions caused by anthropogenic and climactic processes are of high risk to this species (Fenner et al. 2008) and may result in translocation becoming one of the main conservation measures considered (Fordham et al. 2012; Delean et al. 2013). In order to accurately predict how this (and other) species will respond to changing conditions within their range, functional genetic data and a measure of the species' ability to adapt to these changes is needed (Boyle et al. 2016). Functional genetic data also allows a different metric with which to gauge levels of local adaptation, and are crucial when considering suitability of translocation options (Weeks et al. 2011).

When functional areas of the genome are expressed, these areas are transcribed into RNA for use in the cell to make proteins. Transcriptomics uses RNAseq technology and allows us to analyse these RNA transcripts to identify functional,

expressed genes (Wang et al. 2009). As RNA can only be sampled at the time of expression, comparisons of gene expression can be made between samples collected at different times and under different environmental conditions (Xu et al. 2015; Gugger et al. 2016). This allows for both a genomic and epigenetic picture of a species ability to adapt to these changes (Vijay et al. 2013).

The objective of this study, as stated in the original 2016 application is to generate a reference, commonly called a transcriptome map, for the entire pygmy bluetongue lizard genome. While this is mainly an exploratory study, the main hypothesis under test is that different genes which may have adaptive significance will be expressed between groups of samples collected at the beginning and end of the dry season. This will provide a reference upon which we can identify and target important adaptive genes in the following years of this project. While the transcriptome map of the six sampled Kidneys is not yet assembled, these data have been collected and it is nearing completion.

4. Materials and Methods

Date of project commencement: 29/03/2016

Sample Collection

Tissue samples were collected from eight individuals from the NFSA Tiliqua reserve near Burra, SA. Due to high morphological overlap between sexes, three males and five females were collected between 2014 and 2016; four at the beginning of the dry season and four at the end. The tissues are stored frozen at the South Australian Regional Facility for Molecular Ecology and Evolution (SARFMEE). The bodies of the specimens have gone into the SA Museum collection as important voucher specimens.

RNA Isolation and sequencing

The preserved kidney tissue was extracted for total RNA from six individuals using a Qiagen RNeasy mini plus kit and the standard protocol. We also conducted quality controls of the RNA yield at this stage. Using a Clontech SMARTer cDNA synthesis kit and the Advantage2 PCR kits, and their standard protocols, the RNA was translated into cDNA “library” using reverse transcription PCR methods. Library preparation from these samples and sequencing was completed by the Australian Genomics Research Facility (AGRF) using Illumina HiSeq next generation sequencing system technology.

Data analysis

Sequencing data quality checks were conducted using FastQC on raw data, as well as after running the reads through Trimmomatic, to ensure sequences are cleaned and paired to as high a quality as possible. Trimmed reads will be put through the program TRINITY for de-novo assembly, using the NeCTAR Research Cloud computing facilities.

5. Expenditure Breakdown:

Amount of 2016 grant: $3,000.00
RNA Isolation and cDNA synthesis were completed using funds from Flinders University, and the Holsworth Wildlife Research Endowment.
Funds from the Nature foundation SA and the Field Naturalists Society SA were used for library preparation and sequencing costs.

6. Results

Sequencing results output a total of 97,309,600 paired reads and 29.19 Gb of Data, with a read depth of over 15,000,000 paired reads for each of the six kidney samples. These outputs were checked using FastQC and all samples showed an overall quality score greater than 30. Overall the quality of data obtained is promising. However, overrepresented sequences in the dataset indicate a proliferation of poly-a RNA tails due to the cDNA synthesis method chosen. These will be eliminated at the trimming stage using the program Trimmomatic. Optimisation of parameters at this step is where data analysis currently stands. The final trimmed reads will be checked again using FastQC and then run through the TRINITY de-novo assembly program to obtain transcripts which can be compared to existing transcriptomes and genomes of related species to identify functional genes.

7. Discussion

Samples will be run through analysis steps multiple times to allow for comparison of different groups which may have differential expression. Samples from the beginning of the dry season will be compared to the end of the season. Samples from males and females will be compared, and when additional data is added, different tissues will also be compared. The cDNA synthesis step above was chosen due to the RNA yields obtained at the isolation stage. The data obtained will fulfil the goals of this study, however (based on future RNA yields), subsequent cDNA preparation will be outsourced to AGRF to enable a different method of cDNA synthesis which may not then require the additional trimming steps.

As evidenced by the sampling in this study, there is a large amount of morphological overlap in sexes and visual identification is not always possible. Analysis of reproductive tissues using the methods in this study will allow the opportunity to identify and develop target primers for reproductive genes, and potentially develop a genetic test for the sex of individuals. This will benefit future studies into population dynamics and mating systems, as well as provide an additional parameter of reference for historical studies, for which blood samples are still available for re-testing. The genomic tools we develop will also allow for the evaluation of accuracy of primers for related species which have been shown to work in *T. adelaidensis* (Ansari et al. 2015).

This study's analysis will be continued in tandem with the project goals outlined in the 2017 application. Additional tissues will ensure a greater likelihood of capturing genes which may be regulated differently in different organs, and all tissues will still be compared across the seasonal collection periods. The continued analysis of these data generated in this study, coupled with future aims will all contribute to completing the three year aim of this project to obtain base line data on the adaptive areas of the genome of the Australian pygmy blue tongue lizard.

8. References

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9. Relevant Photos

Figure 2: Pygmy bluetongue lizard (*T. adelaidensis*) adult, NFSA Tiliqua Reserve, Feb 2016. -Carmel Maher *

Figure 3: Pygmy bluetongue lizard (*T. adelaidensis*) juvenile, NFSA Tiliqua Reserve, Feb 2017. -Carmel Maher*

*Figures 2 and 3 were taken during field work volunteering for other student's studies. The animals depicted are not a part of this study and no further sampling for this study is required.