

Characterization of the transcriptome, nucleotide sequence polymorphism and selection in the desert adapted mouse *Peromyscus eremicus*

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1 Abstract

2 Introduction

3 For biologists interested in understanding the relationship between fitness, genotype, and pheno-
4 type, modern sequencing technologies provide for an unprecedented opportunity to gain a deep
5 understanding of genome level processes that together, underlie adaptation. One interesting ex-
6 ample of adaptation lies in animals ability to survive desert conditions. Here, heat *and* drought
7 provide for powerful selective forces, testing animals' ability to osmoregulate and thus to survive.

8

9 Specifically, the maintenance of water balance in animals is one of the most important phys-
10 iologic processes, and is critical to desert survival. Indeed, mammals are exquisitely sensitive to
11 changes in osmolality, with slight derangement eliciting physiologic compromise. When the loss
12 of water exceeds dietary intake, dehydration - and in extreme cases, death - can occur. Unlike
13 most mammals, animals living in desert habitats are subjected to long periods of extreme heat
14 and intense drought. As a result, desert animals have evolved mechanisms through which phys-
15 iologic homeostasis is maintained despite severe and prolonged dehydration.

16

17 One such desert-adapted rodent, a cricetid rodent endemic to the Southwest United states is
18 a novel model for the study of adaptation to desert environments. They have a lifespan typical
19 of small mammals, and therefore an individual may live it's entire life without ever drinking wa-
20 ter. These rodents have distinct advantage over other desert animals (e.g. *Dipodomys*) in that
21 they breed readily in captivity, which enables laboratory studies of the phenotype of interest.
22 In addition the focal species is positioned in a clade of well known animals (e.g. *P. californicus*,
23 *P. maniculatus* and *P. polionotus*) [?]. There are growing genetic and genomic resources avail-
24 able [1-3].

25

26 While the elucidation of the mechanisms underlying adaptation to desert survival is beyond
27 the scope of this manuscript, we aim here to lay the groundwork by characterizing the tran-
28 scriptome from four distinct tissues (brain, liver, kidney, testes). These data will be included in
29 current larger effort aimed at sequencing the entire genome. Further, via sequencing the renal
30 tissue of a total of 15 additional animals, we characterize nucleotide polymorphism and genome
31 wide patterns of natural selection. Together, these investigations will aid in our overarching goal
32 - to understand the genetic bases of adaptation in *P. eremicus*.

Materials and Methods

Animal Collection and Study Design

Animals

RNA extraction and Sequencing

Total RNA was extracted from each tissue using a TRIzol extraction (Invitrogen) following the manufacturers instructions. Because preparation of an RNA library suitable for sequencing is dependent on having high quality, intact RNA, a small aliquot of each total RNA extract was analyzed on a Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA). Following confirmation of sample quality, the reference sequencing libraries were made using the TruSeq stranded RNA prep kit (Illumina), while an unstranded TruSeq kit was used to construct the other sequencing libraries. A unique index was ligated to each sample to allow for multiplexed sequencing. Reference libraries (n=4 tissue types) were then pooled to contain equimolar quantities of each individual library and submitted for Illumina sequencing using two lanes of 150nt paired end sequencing using the rapid-mode of the HiSeq 2500 sequencer at The Hubbard Center for Genome Sciences (University of New Hampshire). The remaining 14 libraries were similarly multiplexed and sequenced in a mixture of 100nt paired and single end across two lanes of an Illumina HiSeq 2000 at the Vincent G. Coates Gnome Center (University of California, Berkeley).

Sequence Data Preprocessing and Assembly

Following recommendations from MacManes [4] and Mbandi [?], adapter sequence contamination was removed, and low quality nucleotides (defined as PHRED <2) were removed from the dataset using the program Trimmomatic version 0.32 [5]. We concatenated sequence data from each reference tissue type and assembled them using the Trinity beta version released 16 March 2014 [6]. We used flags indicating the stranded nature of sequencing reads and set maximum allowable physical distance between read pairs to 999nt. The assembly was conducted on the XSEDE computer resource Blacklight. To filter the raw sequence assembly, I estimated TPM for each assembled sequence using bwa-mem version 0.77 [7] and eXpress version 1.51 [8], removing all contigs whose expression was less than TPM=1 [6].

Assembled Sequence Annotation

From the filtered assembly, I extracted putative coding sequences using Transdecoder version 16Jan2014 (<http://transdecoder.sourceforge.net/>). These putative protein coding sequences were annotated using default settings of the blastx algorithm [9] against the SwissProt database downloaded on 1 March 2014. Because transcriptome assemblies typically contain non-coding elements (e.g. ncRNA) in addition to protein coding sequence, we annotated the entire filtered dataset using the NCBI nt dataset, downloaded on 1 March 2014. Lastly, because rapidly evolving nucleotide sequences may evade detection by blast algorithms, we used

69 HMMER3 [10] to search for conserved protein domains contained in the Pfam database [?].

70

71 To identify sequences unique to each tissue type, I mapped sequence reads from each tissue
72 type to the reference assembly using bwa-mem. We estimated expression individually for the
73 four tissues. Interesting patterns of expression, including instances where expression was limited
74 to a single tissue type were identified.

75

76 Population Genomics

77 In addition to the reference individual sequenced at four different tissue types, we sequenced
78 15 other conspecific individuals from the same population, located in Palm Desert, California.
79 Sequence data were mapped to the reference transcriptome using bwa-mem. The alignments
80 were sorted and converted to BAM format, then passed to the program ANGSD, which was used
81 for calculating the folded site frequency spectrum (SFS) and Tajima's D [?].

82

83 Results

84 RNA extraction, Sequencing, Assembly

85 Raw assembly 743314 and 418Mb Filtered Assembly: 130764 149Mb cds: 64355 and 60Mb 37960
86 complete 4203 3prime 16880 5prime 5312 internal

87 Subsection 2

88 Discussion

89 Acknowledgments

90 References

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115 **Figure Legends**

116 **Tables**

117 **Table 1**

118

DATASET	NUM. RAW READS
PEER360 TESTES	32M PE
PEER360 LIVER	53M PE
PEER360 KIDNEY	56M PE
PEER360 BRAIN	23M PE
PEER305	19M PE
PEER308	15M PE
PEER319	14M PE
PEER321	9M SE
PEER340	16M PE
PEER352	14M PE
PEER354	9M SE
PEER359	14M PE
PEER365	16M PE
PEER366	16M PE
PEER368	14M PE
PEER369	14M PE
PEER372	17M SE
PEER373	23M SE
PEER380	16M SE
PEER382	14M SE

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Table 1. The number of sequencing reads per sample