Transcriptomic Evidence for Reproductive Suppression in Male *Peromyscus eremicus* (Cactus Mouse) Subjected to Acute Dehydration

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Abstract

Understanding how organisms adapt to extreme environments is an outstanding question facing evolutionary biologists. Research related to a specific example of adaptation, mammals in desert environments, has focused on survival, while questions related to the reproductive effects of dehydration have been largely ignored. Here, we explore the reproductive consequences of acute dehydration by utilizing RNAseq data in the desert-specialized rodent, *Peromyscus eremicus*. Nine genes were consistently differentially expressed between hydrated and dehydrated mice, a low number which aligns with current perceptions of this species' extreme desert specialization. However, these differentially expressed genes include Insulin-like 3 (Insl3), a regulator of male fertility, as well as Slc45a3 and Slc38a5, both of which interact with genes with important roles in reproductive function. Together, these findings suggest that acute dehydration is linked to reproductive mitigation, a result which is unexpected in an animal capable of surviving and successfully reproducing without available external water sources.

Introduction

Populations native to particular environments are believed to possess physiological, behavioral and ecological adaptations that enable their long term persistence. For decades, evolutionary biologists have successfully described examples where natural selection has resulted in the exquisite match between organism and environment (*e.g.* Salinity adaptations in three-spine sticklebacks: Hohenlohe et al., 2010; Jones et al. 2012; high-altitude adaptations for hemoglobin: Storz et al., 2010, Lorenzo et al., 2015; and *Peromyscus* adaptations for multiple environments: Hoekstra et al., 2006; Bedford & Hoekstra, 2015; Munshi-South & Richardson, 2016). The match between organism and environment has largely been studied in the context of one component of fitness – survival – while the other component, reproductive success, has received negligible attention in the context of environmental adaptations (but see Kordonowy & MacManes, 2016). However, this frequently neglected aspect of selection is critical to long term persistence in a given environment. While substantial research has been done on the effects of various types of stress on reproduction (*e.g.* Wingfield & Sapolsky, 2003; Ahmed et al., 2015; Nargund, 2015; Wingfield, 2013), the impacts of dehydration stress on reproduction in extreme environmental specialists have not been studied. Habitat specialists must possess phenotypes enabling survival and successful reproduction; therefore, cases where environmental selective pressures result in reduced reproductive success (*e.g.* Martin & Wiebe, 2004; Bolger, Patten & Bostock, 2005; Evans et al., 2010; Wingfield, Kelley & Angelier, 2011), but not survival, demand attention. Species occupying extreme environments are likely more vulnerable to the bifurcation of these two components of fitness. Moreover, long-term events like global climate change are predicted to increase climate variability and may enhance the challenges faced by species living on the fringes of habitable environments (Martin & Wiebe, 2004; Somero, 2010; Wingfield, Kelley & Angelier, 2011; Wingfield, 2013; Asres & Amha, 2014).

Deserts present extraordinary environmental impediments for habitation, including extreme heat, aridity, and solar radiation. Examples of well-described desert mammal behavioral adaptations are seasonal torpor (reviewed in Kalabukhov 1960; Geiser, 2010), nocturnality (*e.g.* Stephens & Tello, 2009; Fuller et al., 2014) and burrowing (reviewed in Vorhies, 1945; Kelt, 2011) to avoid high temperatures and sun exposure. Desert mammals also exhibit a wide range of morphological adaptations, including large ears for effective heat dissipation (*e.g.* Schmidt-Nieslen, 1964; Hill & Veghte, 1976), metabolic water production (*e.g.* MacMillen & Hinds, 1983; reviewed in Walsberg 2000), and renal adaptations to minimize water-loss (*e.g.* Schmidt-Nielsen et al., 1948; Dantzler, 1982; Diaz, Ojeda & Rezenda, 2006).

Although desert rodents must possess adaptations conferring survival *and* reproductive benefits, researchers have focused exclusively on their adaptations for survival. For example, renal adaptations in species of Kangaroo rats (*Dipodomys* species) have been described and explored for over 60 years (Schmidt-Nielsen et al., 1948; Schmidt-Nielsen and Schmidt-Nielsen, 1952; Marra et al., 2012; Urity et al., 2012). While early research determined the renal physiology for Kangaroo rats (Schmidt-Nielsen et al., 1948; Schmidt-Nielsen and Schmidt-Nielsen, 1952; Vimtrup and Schmidt-Nielsen), recent research has focused on the genetic underpinnings of this phenotype (Marra et al., 2012; Urity et al., 2012; Marra, Romero & DeWoody, 2014; Marra et al., 2014), which is indicative of a larger methodological shift in the approach for examining adaptation.

Research in another desert-adapted rodent, *Peromyscus eremicus* (cactus mouse), has followed a somewhat different trajectory; however, it too has only pursued survival oriented mechanisms (but see Kordonowy and MacManes, 2016). The ecology, physiology and behaviors of the cactus mouse in comparison with other *Peromyscus* species were summarized in 1968 (King, ed.), and the relationships between basal metabolic rate, body mass, and evaporative water loss were reviewed several decades later (MacMillen and Garland, 1989). Known desert adaptations for cactus mouse include nocturnality and torpor (reviewed in Veal and Caire, 1979); however, the cactus mouse does not possess the same elaborate kidney structures responsible for renal adaptations in kangaroo rats (Dewey, Elias & Appel, 1966; MacManes 2016, *unpublished data*). The physiological renal adaptations in *P. eremicus* have not been described in detail, despite considerable explorations of other aspects of this species' biology (reviewed in Veal and Caire, 1979). In order to initially characterize renal function of the cactus mouse, water consumption measurements and electrophysical dehydration effects for this species have also recently been documented (Kordonowy et al., 2016). Because the renal mechanisms for mitigating renal water-loss in *P. eremicus* have not been determined, a comparative genetic approach may be instrumental for characterizing this species' adaptive kidney phenotype. To this end, MacManes and Eisen (2014) conducted a comparative analysis to find genes expressed in the kidney tissue of cactus mouse that were under positive selection relative to other mammals. Furthermore, the transcriptomic resources available for this species extend considerably beyond renal tissue; transcripts from cactus mouse (as well as numerous other *Peromyscus* species) have been heavily utilized to pursue questions related to multiple aspects of evolutionary biology (reviewed in Bedford and Hoekstra, 2015; Munshi-South and Richardson, 2016). Current investigations into cactus mouse desert-adaptive physiology include transcriptomic

93 analyses; however, we extend this genetic approach by shifting the focus from adaptions for survival to
94 include adaptations for reproductive success.

95 In nature, wild cactus mice are subjected to both acute and chronic dehydration, and understanding
96 the reproductive effects of dehydration stress is an initial step for fully characterizing the suite of
97 phenotypes enabling successful reproduction. Given that this species has evolved in southwestern United
98 States deserts, we predicted that neither acute nor chronic water stress, while physiologically demanding,
99 would be associated with reproductive suppression. To test these predictions, we leveraged previous
100 research that characterized the transcriptome of male *P. eremicus* reproductive tissues from functional and
101 comparative perspectives (Kordonowy and MacManes, 2016). We extend upon this work by performing
102 an RNAseq experiment to identify differentially expressed genes in testes between male *P. eremicus*
103 subjected to acute dehydration versus control (fully hydrated) animals in order to determine the impacts,
104 if any, on male fertility. We pursue this line of research on the effects of dehydration on reproduction in
105 cactus mouse in order to begin to address the need for studies focusing on adaptation related to
106 reproductive success in animals living in extreme, and changing, environments.

107

108 **Methods**

109 *Treatment Groups, Sample Preparation and mRNA Sequencing*

110 The *cactus mice* used for this study include only captive born individuals purchased from the
111 *Peromyscus* Genetic Stock Center (Columbia, South Carolina). The animals, originally collected from a
112 hot-desert location in Arizona, have been housed for several generations, at the University of New
113 Hampshire in conditions that mimic temperature and humidity levels in southwestern US deserts, as
114 described previously (Kordonowy & MacManes, 2016). Males and females are housed together, which
115 provides olfactory cues to support reproductive maturation. Males do not undergo seasonal testicular
116 atrophy, as indicated by successful reproduction throughout the year. The individuals used in this study
117 were deemed reproductively mature once they became scrotal.

118 Males that were provided with water *ad libidum* had free access to water prior to euthanasia, and
119 these individuals are labeled as WET mice in our analyses. Mice that were water deprived, which we refer
120 to as DRY mice, were weighed and then water deprived for ~72 hours directly prior to euthanasia. All

121 mice were weighed prior to sacrifice, and DRY mice were evaluated for weight loss during dehydration.
122 Individuals in the study were collected between September 2014 – April 2016.

123 Cactus mice were sacrificed via isoflurane overdose and decapitation in accordance with
124 University of New Hampshire Animal Care and Use Committee guidelines (protocol number 130902) and
125 guidelines established by the American Society of Mammalogists (Sikes et al., 2016). Trunk blood
126 samples were collected following decapitation for serum electrolyte analyses with an Abaxis Vetscan VS2
127 using critical care cartridges (Abaxis). The complete methodology and results of the electrolyte study, as
128 well as the reported measures of water consumption and weight loss due to dehydration are described fully
129 elsewhere (Kordonowy et al., 2016). Rather, this study focused on differential gene expression between
130 the testes of 11 WET and 11 DRY mice. Testes were harvested within ten minutes of euthanasia, placed
131 in RNAlater (Ambion Life Technologies), flash-frozen in liquid nitrogen, and stored at -80° degree
132 Celsius. A TRIzol, chloroform protocol was implemented for RNA extraction (Ambion Life
133 Technologies). Finally, the quantity and quality of the RNA product was evaluated with both a Qubit 2.0
134 Fluorometer (Invitrogen) and a Tapestation 2200 (Agilent Technologies, Palo Alto, USA).

135 Libraries were made with a TruSeq Stranded mRNA Sample Prep LT Kit (Illumina), and the
136 quality and quantity of the resultant sequencing libraries were confirmed with the Qubit and Tapestation.
137 Each sample was ligated with a unique adapter for identification in multiplex single lane sequencing. We
138 submitted the multiplexed samples of the libraries for processing on lanes at the New York Genome Center
139 Sequencing Facility (NY, New York). Paired end sequencing reads of length 125bp were generated on an
140 Illumina 2500 platform. Reads were parsed by individual samples according to their unique hexamer IDs
141 in preparation for analysis.

142 *Assembly of Testes Transcriptome*

143 We assembled a testes transcriptome from a single reproductively mature male using the *de novo*
144 transcriptome protocol described previously (MacManes, 2016). The testes transcripts were assembled
145 with alternative methodologies utilizing several optimization procedures to produce a high-quality
146 transcriptome; however, the permutations of this assembly process are described extensively elsewhere
147 (MacManes, 2016; Kordonowy and MacManes, 2016). The testes transcriptome we selected was
148 constructed as described below. The raw reads were error corrected using Rcorrector version 1.0.1 (Song
149 & Florea, 2015), then subjected to quality trimming (using a threshold of PHRED <2, as per MacManes
150 2014) and adapter removal using Skewer version 0.1.127 (Jiang et al, 2014). These reads were then

151 assembled in the *de novo* transcriptome assembler BinPacker version 1.0 (Liu et al., 2016). We also
152 reduced sequence redundancy to improve the assembly using the sequence clustering software CD-HIT-
153 EST version 4.6 (Li & Godzik, 2006; Fu et al., 2012). We further optimized the assembly with Transrate
154 version 1.0.1 (Smith-Unna et al., 2015) by retaining only highly supported contigs. We then evaluated the
155 assembly's structural integrity with Transrate and assessed completeness using the vertebrata database in
156 BUSCO version 1.1b1 (Simão et al., 2015). We quasimapped the raw reads to the assembly with Salmon
157 version 0.7.2 (Patro, Duggal & Kingsford, 2015) to confirm that mapping rates were high. Finally, the
158 assembly was also annotated in dammit version 0.3.2, which finds open reading frames with TransDecoder
159 and uses five databases (Rfam, Pfam, OrthoDB, BUSCO, and Uniref90) to thoroughly annotate transcripts
160 (https://github.com/camillescott/dammit).

161 *Differential Gene and Transcript Expression Analyses*

162 Several recent studies have critically evaluated alternative methodologies for differential transcript
163 and gene expression to determine the relative merits of these approaches (Gierlinski et al., 2015; Schurch
164 et al., 2016; Soneson, Love & Robinson, 2016; Froussios et al., 2016). Soneson and colleagues (2016)
165 demonstrated that differential gene expression (DGE) analyses produce more accurate results than
166 differential transcript expression (DTE) analyses. Furthermore, the differential gene expression approach
167 is more appropriate than differential transcript expression for the scope of our research question, which is
168 true of many evolutionary genomic studies (Soneson et al., 2016). However, because both DTE and DGE
169 approaches are widespread in current literature, we deemed it important to confirm that these
170 methodologies yielded concordant results in the current study.

171 We utilized edgeR (Robinson, McCarthy & Smith, 2010; McCarthy, Chen & Smith, 2012) as our
172 primary statistical software because Schurch and colleagues (2016) rigorously tested various packages for
173 analyzing DGE, and edgeR performed optimally within our sample size range. While edgeR is a widely
174 used statistical package for evaluating differential expression, we also confirmed our results with another
175 popular package, DESeq2 (Love, Huber & Anders, 2014), in order to validate our findings.

176 We performed differential expression analyses with three alternative methodologies. Two analyses
177 were conducted in R version 3.3.1 (R Core Team, 2016) using edgeR version 3.16.1, a Bioconductor
178 package (release 3.4) that evaluates statistical differences in count data between treatment groups
179 (Robinson, McCarthy & Smith, 2010; McCarthy, Chen & Smith, 2012). Our first method utilized
180 tximport, an R package developed by Soneson and colleagues (2016), which incorporates transcriptome

181 mapping-rate estimates with a gene count matrix to enable downstream DGE analysis. The authors assert
182 that such transcriptome mapping can generate more accurate estimates of DGE than traditional pipelines
183 (Soneson et al, 2016). While our first methodology evaluated differential gene expression, our second
184 analysis used the transcriptome mapped read sets to perform differential transcript expression and identify
185 the corresponding gene matches. The purpose of this second analysis was to evaluate whether the
186 transcript expression results coincided with the gene expression results produced by the same program,
187 edgeR. Finally, our third methodology determined differential gene expression with tximport in
188 conjunction with DESeq2 version 1.14.0 (Love, Huber & Anders, 2014), a Bioconductor package (release
189 3.4) which also evaluates statistical differences in expression. We performed this alternative DGE analysis
190 with DESeq2 in order to corroborate our DGE results from edgeR. Thus, the results for all three
191 differential expression analyses were evaluated to determine the coincidence among the genes identified
192 as significantly different between the WET and DRY groups. These alternative differential expression
193 methods are described in detail below.

194 We quasimapped each of the 11 WET and 11 DRY sample read sets to the testes transcriptome
195 with Salmon version 0.7.2 to generate transcript count data. To perform the gene-level analysis in edgeR,
196 we constructed a gene ID to transcript ID mapping file, which was generated by a BLASTn (Altschul et
197 al., 1990; Madden, 2002) search for matches in the *Mus musculus* transcriptome (ensembl.org) version
198 7/11/16 release-85. We then imported the Salmon-generated count data and the gene ID to transcript ID
199 mapping file into R using the tximport package (Soneson et al. 2016) to convert the transcript count data
200 into gene counts. This gene count data was imported into edgeR for differential gene expression analysis
201 (Robinson, McCarthy & Smith, 2010; McCarthy, Chen & Smith, 2012). We applied TMM normalization
202 to the data, calculated common and tagwise dispersions, and performed exact tests ($p < 0.05$) adjusting
203 for multiple comparisons with the Benjamini-Hochburg correction (Benjamini & Hochburg, 1995) to find
204 differentially expressed genes, which we identified in Ensembl (ensemble.org).

205 Next, we performed a transcript-level analysis using edgeR. To accomplish this, the Salmon-
206 generated count data was imported into R and analyzed as was described above for the gene-level analysis
207 in edgeR. After determining which transcript IDs were differentially expressed, we identified the
208 corresponding genes using the gene ID to transcript ID matrix described previously. The significantly
209 expressed transcripts without corresponding gene matches were selected for an additional BLASTn search
210 in the NCBI non-redundant nucleotide database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). However, these

211 results were not subjected to any additional analyses, because these matches were not consistent across all
212 three differential expression analyses. This list of BLASTn search matches is provided in supplementary
213 materials (DTEno-matchBLASTnSequences.md).

214 The third analysis used DESeq2 to conduct an additional gene-level test, using the same methods
215 as described for the previous gene-level analysis, with the exception that data were imported into an
216 alternative software package. We determined the significantly differentially expressed genes ($p < 0.05$)
217 based on normalized counts and using the Benjamini-Hochburg correction (Benjamini & Hochburg, 1995)
218 for multiple comparisons. We only retained genes with a $-1 < \log_2$ fold change > 1 in order to filter genes
219 at a conservative threshold for differential expression based on our sample size (Schurch et al., 2016).
220 This filtering was not necessary for either of the edgeR analyses because $\log_2$ fold changes exceeded this
221 threshold for the differentially expressed genes and transcripts ($-1.3 < \log_2$ fold change > 1.4, in all cases).

222 We also compared the $\log_2$ fold change values (of treatment differences by mapped count) for each
223 gene from the edgeR and DESeq2 gene-level analyses in a linear regression. This statistical test was
224 performed in order to evaluate the degree of concordance between the two DGE analyses. Furthermore,
225 we constructed a list of genes identified as differentially expressed by all three analyses, which were
226 further evaluated for function as well as chromosomal location. These genes were also explored in
227 STRING version 10.0 (string-db.org) to determine their protein-protein interactions (Snel et al., 2000;
228 Szklarczyk et al., 2015).

229

230 **Results**

231 *Data and Code Availability*

232 The testes transcriptome was assembled from a 45.8 million paired read data set. Additionally,
233 there were 9-20 million paired reads for each of the 22 testes data sets used for the differential expression
234 analysis **(Supplemental 1)**, yielding 304,466,486 reads total for this analysis. The raw reads are available
235 at the European Nucleotide Archive under study accession number PRJEB18655. All data files, including
236 the testes un-annotated transcriptome, the dammit annotated transcriptome, and the data generated by the
237 differential gene expression analysis (described below) are available on DropBox
238 (https://www.dropbox.com/sh/ffr9xrmjxj9md1m/AACpxjQNn-Jlf25qNdslfRSCa?dl=0). These files will

239 be posted to Dryad upon manuscript acceptance. All code for these analyses is posted on GitHub
240 (https://github.com/macmanes-lab/testesDGE).

241 *Assembly of Testes Transcriptome*

242 The performance of multiple transcriptome assemblies was evaluated thoroughly, and the selected
243 optimized testes assembly met high quality and completeness standards, and it also contains relatively few
244 contigs and has high read mapping rates (**Table 1**). Therefore, this transcriptome was used for our
245 differential expression analyses. The transcriptome was also annotated, and the complete statistics for this
246 dammit annotation are provided in **Table 2**.

247 *Differential Gene and Transcript Expression Analyses*

248 Salmon quasimapping rates of all read datasets to the assembly were sufficiently high (range:
249 81.46% - 87.02%; **Supplemental 1**), indicating the successful generation of transcript count data for our
250 differential expression analyses. The exact test performed for our gene-level analysis in edgeR indicated
251 that fifteen genes reached statistical significance (after adjusting for multiple comparisons) for DGE
252 between the WET and DRY treatment groups **(Figure 1)**. Specifically, seven genes were more highly
253 expressed in WET individuals, and eight genes were more highly expressed in DRY individuals **(Table**
254 **3).**

255 We also performed an alternative transcript-level analysis using the referenced transcriptome
256 mapped reads exclusively with edgeR. The exact test found 66 differentially expressed transcripts **(Figure**
257 **2)**, 45 of which were more highly expressed in the WET group, and 21 were more highly expressed in the
258 DRY group (**Table 4).** 10 of these differentially expressed transcripts were consistent with differentially
259 expressed genes from the edgeR DGE analysis. In addition, the significantly expressed transcripts without
260 an Ensembl ID match (nine WET and nine DRY) were retrieved for performing an nt all species BLASTn
261 search (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and these results are in the supplementary materials.

262 The gene-level analysis conducted in DESeq2 yielded 215 significantly differentially expressed
263 genes **(Figure 3),** 67 of which were more highly expressed in the WET group, while 148 were highly
264 expressed in the DRY group. However, only 20 of these genes remained when we filtered them with a -1
265 $< \log_2$ fold change > 1 to retain genes with a conservative threshold difference between treatment groups.
266 This list of 20 genes yielded 16 genes more highly expressed in WET mice and four genes highly

267 expressed in DRY mice **(Table 5**). Nine of these genes overlapped with those found to be significant in
268 the previous two edgeR analyses.

269 To evaluate the correlation of $\log_2$ fold change results for each gene (Ensembl ID) from the two
270 DGE analyses (EdgeR and DESeq2), we performed a regression of these log values, and they were
271 significantly correlated (**Figure 4:** Adj-$R^2 = 0.6596$; $F(1,14214) = 2.754 \times 10^4$; $p < 2.2 \times 10^{-16}$). This further
272 demonstrates the concordance of the DGE analyses in these two software packages.

273 To evaluate the degree to which the three analyses produced concordant results, we generated a
274 list of genes which were found to be significantly differently expressed by treatment across all three
275 analyses **(Supplemental 2).** There were six genes that were consistently highly-expressed in the WET
276 group and three genes that were highly-expressed in the DRY group. The six highly-expressed WET genes
277 are Insulin-like 3 (Insl3), Free-fatty acid receptor 4 (Ffar4), Solute carrier family 45 member 3 (Slc45a3),
278 Solute carrier family 38 member 5 (Slc38a5), Integrin alpha L (Itgal), and Transferrin (Trf). The three
279 highly-expressed DRY genes are Ras and Rab Interactor 2 (Rin2), Insulin-like growth factory binding
280 protein 3 (Igfbp3), and Connective tissue growth factor (Ctgf). Because the patterns of expression of these
281 nine genes were corroborated by multiple methodologies, we are confident that they are differentially
282 expressed between our treatments. Estimates of expression for these genes generated using the gene-level
283 edgeR analysis are plotted in **Figure 5**.

284 The significantly differently expressed genes were evaluated for gene function and chromosomal
285 location (**Table 6**). These genes occur throughout the genome; namely, they are located on different
286 chromosomes. The diverse functions of each gene will be described below. In addition, we generated
287 STRING diagrams (string-db.org) to view the protein-protein interactions for each of these nine genes
288 (Snel et al., 2000; Szklarczyk et al., 2015).

289 Slc38a5 and Slc45a3 are among the highly expressed genes in the WET group (they have lower
290 expression in the DRY group); these two solute carriers are members of a large protein family that is
291 responsible for cross-membrane solute transport (reviewed in Hediger et al., 2004; Hediger et al., 2013;
292 Cesar-Razquin et al., 2015). Slc38a5 is involved sodium-dependent amino-acid transport, while Slc45a3
293 is purported to transport sugars (Vitavska and Wieczorek, 2013; Schiöth, et al., 2013;
294 http://slc.bioparadigms.org/), thereby playing an important potential role in maintaining water balance via
295 management of oncotic pressures. Slc38a5 (**Figure 6a**) has interactions with multiple additional solute
296 carriers, including Slc1a5, Slc36a2, Slc36a3, and Slc36a4. Slc38a5 also has an interaction with disintegrin

297 and metalloproteinase domain-containing 7 (Adam7), which is involved in sperm maturation and the
298 acrosome reaction (Oh et al., 2005). In contrast, Slc45a3 (**Figure 6b**) does not have known protein
299 interactions with other solute carriers; however, this protein does interact with steroidogenic acute
300 regulatory protein (StAR), which is critical in steroidogenesis (Christenson and Strauss III, 2001).

301 Insl3 was lower expressed in the DRY group, and this hormone regulates fertility in male and
302 female mammals, by preventing apoptosis of germ cells in reproductive organs of both sexes (Kawamura
303 et al., 2004: Bathgate et al., 2012; Bathgate et al., 2013). In male rodents, Insl3 is critical to development
304 by facilitating testicular descent, and it is also present in testes of adults, where it binds to relaxin family
305 peptide receptor 2 (RXFP2), also known as Lrg8 (Bathgate et al., 2012; Bathgate et al., 2013). Within
306 adult rodent testes, luteinizing hormone (LH) stimulates expression of Insl3 in Leydig cells, and Insl3
307 binds to Lrg8 in seminiferous tubules, which results in inhibited apoptosis of germ-line cells, thus
308 increasing their availability (Kawarmura et al., 2004). Therefore, our finding of lower Insl3 expression in
309 DRY mice indicates less attenuation of germ-line cell apoptosis, which could result in the reduction of
310 sperm cells and lower fertility. Protein interaction data for Insl3 (**Figure 6c**) indicate that this hormone
311 interacts with RXFP2 and RFXP1, as well as other proteins, including leptin (Lep), a pleiotropic hormone
312 involved in reproduction, immunity, and metabolism (reviewed in Friedman, 2014).

313 Ffar4 was also down-regulated in the DRY group. Omega-3 fatty acid receptor 1 (O3Far1) is an
314 alias of Ffar4, and it has roles in metabolism and inflammation (Moniri, 2016). This protein interacts with
315 multiple other free fatty acid receptors and G-protein coupled receptors as well as Stanniocalcin 1 (Stc1)
316 (**Figure 6d**). Stc1 is involved in phosphate and calcium transportation (Wagner and Dimattia, 2006);
317 however, this protein's functional role in mice remains enigmatic (Chang et al, 2005).

318 Another of the lower expressed DRY group genes is Itgal (also known as CD11a), which has
319 multifaceted roles in lymphocyte-mediated immune responses (Bose et al., 2014). Concordantly, the
320 protein interactions with Itgal (**Figure 6e**) include numerous proteins integral to immunity, such as
321 Intracellular adhesion molecules (specifically, ICAM1,2,4), which are expressed on the cell surface of
322 immune cells and endothelial cells. Itgal is a receptor for these ICAM glycoproteins, which bind during
323 immune system responses (reviewed in Albelda, Smith and Ward, 1994). However, an additional role of
324 intercellular adhesion molecules has been proposed in spermatogenesis, whereby ICAMs may be integral
325 to transporting non-mobile developing sperm cells through the seminiferous epithelium (Xiao, Mruk and
326 Cheng, 2013).

327 The final gene with lower expression levels in the DRY treatment is Trf, which modulates the
328 amount of free-iron in circulation and binds to transferrin receptors on the surface of erythrocyte
329 precursors to deliver iron (reviewed in Gkouvastos Papanikolaou and Pantopoulos, 2012). TRF interacts
330 with multiple proteins (**Figure 6f**) involved in iron transport and uptake, including STEAP family member
331 3 (Steap3), hephaestin (HEPH), cerulopslamin (Cp), Solute carrier protein 40 member 1 (Slc40a1), and
332 several H+ ATPases. Furthermore, TRF is linked to apolipoprotein A-1 (Apoa1), which interacts with
333 immunoglobulin in a complex named sperm activating protein (SPAP) to activate the motility of sperm
334 when it inhabits the female genital tract (Akerlof et al., 1991; Leijonhufvud, Akerlof and Pousette, 1997).

335 One of the highly expressed genes in the DRY group is Rin2, which is involved in endocytosis
336 (reviewed in Doherty and McMahon, 2009) and membrane trafficking through its actions as an effector
337 protein for the GTPases in the Rab family within the Ras superfamily (reviewed in Stenmark and
338 Olkkonen, 2001). Rin2 protein-protein interactions **(Figure 7a**) include Ras related protein Rab5a and
339 Rab5b, which are involved in vesicle transport as well as vasopressin-regulated water reabsorption. This
340 mechanism for water reabsorption via Aquaporin 2 (AQP2) in the kidney has been thoroughly reviewed
341 by Boone and Deen (2008) and Kwon and colleagues (2013).

342 The second gene highly expressed in the DRY group is Igfbp3, which modulates the effects of
343 insulin growth factors. Thus, the protein directly interacts **(Figure 7b**) with insulin growth factors 1 and
344 2 (Igf1, Igf2), which are responsible for increasing growth in most tissues (reviewed in le Roth 1997;
345 Jones and Clemmons, 2008). Ctgf was also highly expressed in the DRY group, and this protein is
346 responsible for increased fibrosis and extracellular matrix formation (Reviewed in Moussad and
347 Brigstock, 2000). The protein interactions for Ctgf (**Figure 7c**) include many transcription activators in
348 the Hippo signaling pathway, including multiple TEA domain transcription factors (Tead1, 2, 3 and 4),
349 WW domain containing transcription regulator 1 (Wwtr1), as well as Yes-associated protein 1 (Yap1),
350 which is responsible for both increasing apoptosis and preventing cell proliferation to mitigate tumor
351 growth and control organ size (Reviewed in Pan, 2010).

352

353 **Discussion**

354 This is the first study to evaluate the reproductive correlates of acute dehydration in a desert-
355 specialized rodent. We analyzed differential gene expression levels for testes in male *Peromyscus*

356 *eremicus* (cactus mouse) in acute dehydration (DRY) versus a control group that was fully hydrated
357 (WET). Our results provide evidence suggesting that reproductive function is attenuated in acutely
358 dehydrated mice, which is surprising, given that this is not consistent with our understanding of *P.*
359 *eremicus* as a desert specialist. While acute dehydration is less common than chronic dehydration for
360 desert mammals, it is a selective force they must overcome. Indeed, throughout much of the described
361 range of the cactus mouse, rainfall events may occur several times per year. Cactus mice, and many other
362 rodents, are known to rehydrate during these rainfall events (MacManes, *personal observation*). Following
363 rehydration, cactus mice experience acute dehydration, followed by a steady state of chronic dehydration.

364 In male *P. eremicus*, we found strong evidence for reproductive suppression via down-regulation
365 of the Insl3 gene in acutely dehydrated animals. Insl3, which is a hormonal regulator of fertility among
366 mammals of both sexes, is known to inhibit germ line apoptosis in the testes (Kawamura et al., 2004;
367 Bathgate et al., 2012; Bathgate et al., 2013). Specifically, the binding of Insl3 to Lrg8 in rodent testes
368 decreases the apoptosis of germ-line cells, which increases sperm availability (Kawamura et al., 2004).
369 Lower Insl3 expression in the testes of acutely dehydrated mice suggests that fertility may be attenuated
370 due to acute water deprivation. Although the current study suggests reproductive suppression in acute
371 dehydration, future work characterizing the functional consequences of Insl3 down-regulation is needed.
372 Specifically, does the number or quality of sperm decrease, and does this decrease reduce the probability
373 of successful fertilization? Moreover, what are the temporal dynamics of reproductive suppression?
374 Logically, desert species with core reproductive functions that are suppressed by dehydration seem likely
375 to be rapidly outcompeted. Given this assertion, research characterizing the reproductive correlates of
376 chronic dehydration is a logical extension of this work, although doing so here is beyond the scope of this
377 study.

378 Solute carrier proteins, specifically Slc45a3 and Slc38a5, are also downregulated in acute
379 dehydration. These genes are part of a large family essential for transferring solutes across membranes
380 (reviewed in Hediger et al., 2004; Hediger et al., 2013; Cesar-Razquin et al., 2015). Another member of
381 this family, Solute carrier family 2 member 9 (Slc2a9), has been found to be undergoing positive selection
382 in studies on kidney transcriptomes of cactus mouse (MacManes & Eisen, 2014) and of other desert
383 rodents (Marra, Romero & DeWoody, 2014). Our previous work with the male reproductive transcriptome
384 of cactus mouse found evidence for positive selection in two additional solute carrier proteins: Slc15a3
385 and Slc47a1 (Kordonowy and MacManes, 2016). Therefore, our current findings that two solute carrier

386 proteins are lower expressed in the DRY treatment group is consistent with previous research in the kidney
387 and male reproductive transcriptomes for this species. This leads us to further support the hypothesis
388 originally proposed by Marra, Romero & DeWoody (2014) that this protein family is intrinsic to
389 osmoregulation in desert rodents. Indeed, the findings of MacManes and Eisen (2014) and Kordonowy
390 and MacManes (2016) also lend support to the essential role of solute carrier proteins for maintaining
391 homeostasis in the desert specialized cactus mouse.

392 In addition to their well characterized role in the maintenance of water and electrolyte balance, the
393 differential expression of solute carrier proteins may have important reproductive consequences,
394 particularly as they relate to hormone secretion. Indeed, the interaction between Slc38a5 and Adam7 is
395 relevant, because Adam7 is involved in sperm maturation and the acrosome reaction (Oh et al., 2005).
396 Furthermore, the protein-protein interactions between Slc45a3 with STaR and between Insl3 and Lep are
397 of particular interest because both STaR and Lep are integral to reproduction, as well as to homeostasis
398 (reviewed in Christenson and Strauss III, 2001; Anuka et al., 2013; Friedman, 2014; Allison and Myers,
399 2014). Thus, the protein interactions with reproductive implications are not restricted to solute carrier
400 proteins. The protein relationships between Itgal and intercellular adhesion molecules are also noteworthy
401 with respect to research hypothesizing an integral role for ICAMs in spermatogenesis (Xiao, Mruk and
402 Cheng, 2013). Furthermore, Trf is linked to Apoa1, which is a critical component of sperm activating
403 protein (Akerlof et al., 1991; Leijonhufvud, Akerlof and Pousette, 1997). While the relationship between
404 these differentially expressed genes and the hormones involved in reproductive function are currently
405 poorly-characterized, our findings that genes integral to sperm development and activation interact with
406 genes differentially expressed in acute dehydration provide strong evidence that, contrary to our
407 expectations, acute dehydration is linked to reproductive suppression in the cactus mouse.

408 In contrast to genes that are down-regulated in dehydration, the genes that were upregulated in the
409 DRY group are known to be responsible for water homeostasis and cellular growth. The significance of
410 Rin2 is notable, because this protein is an effector for Rab5, which as a GTPase involved in vasopressin-
411 regulated water reabsorption, a critical homeostatic process mediated through the AQP2 water channel in
412 kidneys (Boone and Deen, 2008; Kwon et al., 2013). It is not surprising that genes in addition to solute
413 carrier proteins, which are implicated in alternative processes for water homeostasis, are differentially
414 expressed in response to water limitation. The other two genes that are up-regulated in the DRY treatment
415 are indicative of modulated growth due to water limitation. Specifically, Igfb3 interacts directly with

416 insulin growth factors responsible for tissue growth (le Roth 1997; Jones and Clemmons, 2008), and Ctgf
417 is linked with numerous transcription factors in the Hippo signaling pathway, which modulates apoptosis,
418 proliferation and organ size control (Pan, 2010).

419 Emerging from this work is a hypothesis related to the reproductive response to water stress in the
420 cactus mouse, and perhaps other desert animals. Specifically, we hypothesize that while acute dehydration
421 is related to reproductive suppression, chronic dehydration is not. Indeed, it is virtually oxymoronic to
422 suggest that chronic dehydration, which is the baseline condition in desert animals, has negative
423 consequences for reproductive success. Generating an integrative, systems-level understanding of the
424 response to dehydration is required for testing this hypothesis. While understanding the renal response to
425 dehydration is critical for making predictions about survival, understanding the reproductive correlates is
426 perhaps even more relevant to evolutionary fitness. Though decades of research have characterized the
427 former, this study, to the best of our knowledge, is the first to describe the reproductive correlates of water-
428 limitation. Developing this understanding, particularly in light of global climate change, where increasing
429 climate variability is predicted, is necessary if we are to understand how climate change may modify the
430 distribution of extant organisms.

431

432 **Conclusion**

433 The genetic mechanisms responsible for physiological adaptations for survival and reproduction
434 in deserts remain enigmatic. Previous research has focused exclusively on adaptations related to survival,
435 specifically on renal adaptations to combat extreme water-limitation. In contrast, no research related to
436 the reproductive effects of acute and chronic dehydration on desert-adapted rodents has been previously
437 conducted. To this end, we characterized the reproductive correlates of acute dehydration in a desert-
438 specialized rodent, the cactus mouse, using a highly replicated RNAseq experiment. In contrast to
439 expectations, we describe a signal of reproduction suppression in dehydrated male mice. Specifically,
440 dehydrated mice demonstrated significantly lower expression of Insl3, which is a regulator of fertility, as
441 well as Slc45a3 and Slc38a5, both of which have interactions with genes important to reproductive
442 function. While the low number of differentially expressed genes between acutely dehydrated and control
443 mice might otherwise have suggested that this species is relatively unaffected by acute water-limitation,
444 the diminished expression of these genes, particularly Insl3, in dehydrated mice indicates the potential for
445 compromised fertility. Although an experimental demonstration of a functional relationship between this

446 gene expression pattern and reduced fertility is still needed, our finding that acute-dehydration alters the
447 expression of genes critical to reproductive function is concerning, particularly with respect to global
448 climate change. Climate change driven increased variabilities in weather patterns may result in a greater
449 frequency of acute water-stress, which could result in reduced reproductive function for the cactus mouse.
450 In addition, because global climate change is predicted to shift habitats toward extremes in temperature,
451 salinity, and aridity, and to alter species ranges, an enhanced understanding of the reproductive
452 consequences of these changes, and of the potential for organisms to rapidly adapt, may enable us to
453 effectively conserve innumerable species facing dramatic habitat changes.

454

455 Table 1: Transcriptome assembly performance metrics: contig number (n), TransRate score (Score),
456 BUSCO indices: % single copy orthologs (% SCO), % duplicated copy orthologs (% DCO), %
457 fragmented (% frag), and % missing (% miss), as well as Salmon mapping rates (% map) for the
458 optimized testes assembly.

459

Assembly Description	**n**	**Score**	**% SCO**	**% DCO**	**% frag**	**% miss**	**% map**
BinPacker CD-hit-est TransRate Corrected	155134	0.335	77	27	5.9	16	92.14

460

461

462 Table 2: Dammit transcriptome assembly annotation statistics. Dammit annotation includes searches in
463 the program TransDecoder for open reading frames (ORFs) and searches for homologous sequences in
464 five databases: Rfam, Pfam-A, Uniref90, OrthoDB, and BUSCO. Percentages were calculated from the
465 count number of each parameter divided by the total number of contigs in the transcriptome (155,134).
466 The only exception to this calculation is for complete ORFs, which were calculated as a percentage of
467 the total ORFs (75,482). The BUSCO results for the annotated assembly are not shown here as they are
468 identical to those for the un-annotated assembly (Table 2).

469

Search Type	***TransDecoder***		***Rfam***	***Pfam-A***	***Uniref90***	***OrthoDB***	***Dammit***
Parameter	Total ORFs	Complete ORFs	ncRNAs	Protein Domains	Proteins	Orthologs	Total Annotated Contigs
Count	75,482	43,028	937	25,675	62,865	51,806	77,915
Percentage	48.7%	57.0 %	0.6 %	16.6 %	40.5 %	33.4 %	50.2 %

470

471

472 Table 3: EdgeR determined significantly differentially expressed genes by treatment group in *P.*
473 *eremicus* testes. Of the 15 DGE, seven were significantly more highly expressed in WET mice (High in
474 WET) and eight were more highly expressed in DRY mice (High in DRY).

475

Ensembl ID	**log_2FC**	**logCPM**	**FDR**	**Gene ID**	**HIGH**
ENSMUSG00000079019.2	-4.354	1.650	5.82E-09	Insl3	WET
ENSMUSG00000054200.6	-3.734	0.619	1.82E-06	Ffar4	WET
ENSMUSG00000026435.15	-2.448	2.447	1.13E-03	Slc45a3	WET
ENSMUSG00000025020.11	-2.231	1.770	1.13E-03	Slit1	WET
ENSMUSG00000031170.14	-2.421	2.578	1.13E-03	Slc38a5	WET
ENSMUSG00000030830.18	-2.180	1.666	3.37E-02	Itgal	WET
ENSMUSG00000032554.15	-2.066	3.287	4.85E-02	Trf	WET
ENSMUSG00000001768.15	3.086	1.006	1.46E-07	Rin2	DRY
ENSMUSG00000025479.9	2.971	3.001	7.97E-05	Cyp2e1	DRY
ENSMUSG00000020427.11	2.681	3.887	1.13E-03	Igfbp3	DRY
ENSMUSG00000019997.11	2.314	3.235	1.13E-03	Ctgf	DRY
ENSMUSG00000040170.13	1.951	0.753	1.72E-03	Fmo2	DRY
ENSMUSG00000023915.4	1.534	1.290	2.02E-02	Tnfrsf21	DRY
ENSMUSG00000052974.8	2.077	0.647	2.26E-02	Cyp2f2	DRY
ENSMUSG00000027901.12	2.492	-0.620	4.78E-02	Dennd2d	DRY

476

477 Table 4: EdgeR determined significantly differentially expressed transcripts by treatment group in *P.*
478 *eremicus* testes. Of the 66 total DTE, 45 were significantly more highly expressed in WET mice (High
479 in WET) and 21 were more highly expressed in DRY mice (High in DRY). BLASTn matches to
480 Ensembl IDs and corresponding Gene IDs.
481

HIGH: WET					
Transcript ID	**log_2FC**	**logCPM**	**FDR**	**Ensembl ID**	**Gene**
BINPACKER.15365.1	-3.703	0.047	5.31E-11	ENSMUSG00000054200.6	Ffar4
BINPACKER.2960.1	-4.268	1.147	2.06E-09	ENSMUSG00000079019.2	Insl3
BINPACKER.17981.2	-2.975	0.436	6.29E-08	ENSMUSG00000026435.15	Slc45a3
BINPACKER.9961.2	-2.426	1.998	7.50E-07	ENSMUSG00000031170.14	Slc38a5
BINPACKER.3452.1	-2.507	-0.140	3.56E-06	no match	-
BINPACKER.724.4	-2.162	2.667	8.32E-06	ENSMUSG00000032554.15	Trf
BINPACKER.9604.1	-2.582	0.547	7.87E-05	no match	-
BINPACKER.31087.1	-2.908	-0.858	9.74E-05	no match	-
BINPACKER.24398.1	-2.440	-0.689	9.74E-05	ENSMUSG00000036596.6	Cpz
BINPACKER.9726.1	-3.474	-0.107	2.38E-04	ENSMUSG00000026435.15	Slc45a3
BINPACKER.9218.3	-1.578	1.525	2.76E-04	ENSMUSG00000021253.6	Tgfb3
BINPACKER.18534.1	-2.332	1.346	4.85E-04	ENSMUSG00000025020.11	Slit1
BINPACKER.17022.3	-2.899	-0.561	1.00E-03	no match	-
BINPACKER.13806.1	-2.442	-0.381	1.13E-03	ENSMUSG00000025172.2	Ankrd2
BINPACKER.7740.1	-2.790	1.095	1.13E-03	ENSMUSG00000057074.6	Ces1g
BINPACKER.10034.2	-4.420	0.387	1.23E-03	ENSMUSG00000026516.8	Nvl
BINPACKER.11560.2	-1.465	2.050	1.66E-03	ENSMUSG00000021913.7	Ogdhl
BINPACKER.13701.1	-1.312	1.804	2.28E-03	ENSMUSG00000025648.17	Pfkfb4
BINPACKER.3510.3	-2.163	0.906	2.95E-03	ENSMUSG00000027822.16	Slc33a1
BINPACKER.15806.1	-1.700	1.062	3.39E-03	ENSMUSG00000015702.13	Anxa9
BINPACKER.17992.1	-2.542	0.653	3.39E-03	ENSMUSG00000030830.18	Itgal
BINPACKER.9726.2	-2.119	0.560	3.48E-03	ENSMUSG00000026435.15	Slc45a3
BINPACKER.6383.3	-2.093	1.270	4.16E-03	ENSMUSG00000002109.14	Ddb2

BINPACKER.20716.2	-4.204	-0.566	5.75E-03	ENSMUSG00000013846.9	St3gal1
BINPACKER.20114.1	-1.661	0.501	5.97E-03	ENSMUSG00000030972.6	Acsm5
BINPACKER.18622.1	-1.645	1.704	6.36E-03	no match	-
BINPACKER.24914.1	-2.211	-0.159	9.83E-03	ENSMUSG00000003555.7	Cyp17a1
BINPACKER.31815.1	-1.905	-0.770	9.83E-03	no match	-
BINPACKER.6740.3	-3.090	-0.434	1.04E-02	no match	-
BINPACKER.20530.1	-1.626	0.545	1.12E-02	ENSMUSG00000038463.8	Olfml2b
BINPACKER.20656.1	-1.910	-0.531	1.22E-02	ENSMUSG00000029373.7	Pf4
BINPACKER.4855.1	-1.340	4.025	1.23E-02	ENSMUSG00000059991.7	Nptx2
BINPACKER.1846.1	-3.280	-0.792	1.23E-02	no match	-
BINPACKER.6494.2	-3.363	0.029	1.26E-02	ENSMUSG00000052861.13	Dnah6
BINPACKER.1818.1	-1.713	3.289	2.03E-02	ENSMUSG00000024125.1	Sbpl
BINPACKER.10743.2	-1.915	-0.525	2.06E-02	ENSMUSG00000041607.16	Mbp
BINPACKER.13054.2	-1.147	2.697	2.06E-02	ENSMUSG00000022994.8	Adcy6
BINPACKER.6807.1	-1.330	2.106	2.13E-02	ENSMUSG00000046687.5	Gm5424
BINPACKER.14160.1	-2.051	0.603	2.86E-02	ENSMUSG00000041556.8	Fbxo2
BINPACKER.16191.1	-1.431	0.926	3.42E-02	ENSMUSG00000028654.13	Mycl
BINPACKER.10141.3	-3.283	-1.191	3.68E-02	ENSMUSG00000024132.5	Eci1
BINPACKER.23790.1	-1.756	-0.275	4.51E-02	ENSMUSG00000001119.7	Col6a1
BINPACKER.22521.1	-1.841	-0.056	4.52E-02	ENSMUSG00000054083.8	Capn12
BINPACKER.1061.6	-1.807	1.943	4.93E-02	no match	-
BINPACKER.17734.1	-1.660	2.109	4.94E-02	ENSMUSG00000049608.8	Gpr55
HIGH: DRY					
Transcript ID	**log_2FC**	**logCPM**	**FDR**	**Ensembl ID**	**Gene**
BINPACKER.21794.1	2.434	3.117	4.41E-08	ENSMUSG00000020427.11	Igfbp3
BINPACKER.28731.1	2.484	1.634	4.41E-08	no match	-
BINPACKER.5662.4	2.061	2.419	1.32E-07	ENSMUSG00000019997.11	Ctgf
BINPACKER.87639.1	2.682	0.345	1.96E-07	ENSMUSG00000001768.15	Rin2
BINPACKER.35470.1	2.367	1.786	1.89E-04	no match	-
BINPACKER.52106.1	2.096	-0.542	6.83E-04	no match	-

BINPACKER.3957.3	6.309	1.579	1.02E-03	ENSMUSG00000019988.6	Nedd1
BINPACKER.116235.1	2.212	0.301	3.94E-03	no match	-
BINPACKER.4449.4	3.428	-0.538	6.74E-03	ENSMUSG00000005150.16	Wdr83
BINPACKER.28.2	4.183	2.295	1.05E-02	ENSMUSG00000075706.10	Gpx4
BINPACKER.56553.1	1.472	0.172	1.46E-02	no match	-
BINPACKER.93518.1	1.711	-0.793	1.57E-02	no match	-
BINPACKER.11512.1	1.187	3.654	1.70E-02	ENSMUSG00000031591.14	Asah1
BINPACKER.66588.1	1.851	-0.347	1.71E-02	no match	-
BINPACKER.42718.1	1.542	0.507	2.06E-02	ENSMUSG00000030790.15	Adm
BINPACKER.49203.1	1.639	-0.035	2.44E-02	no match	-
BINPACKER.147548.1	1.744	-0.007	2.99E-02	ENSMUSG00000042757.15	Tmem108
BINPACKER.23756.2	1.265	3.468	3.01E-02	ENSMUSG00000022061.8	Nkx3-1
BINPACKER.12709.1	3.906	2.611	3.01E-02	ENSMUSG00000028639.14	Ybx1
BINPACKER.5280.2	3.874	0.257	3.76E-02	ENSMUSG00000074582.10	Arfgef2
BINPACKER.58702.1	1.780	-0.500	4.93E-02	no match	-

485 Table 5: DESeq2 determined significantly differentially expressed genes by treatment group in *P.*
486 *eremicus* testes. Of the 20 DGE with a $-1 < \log_2$ fold change > 1, 16 were significantly more highly
487 expressed in WET mice (High in WET) and four were more highly expressed in DRY mice (High in
488 DRY).

Ensembl ID	**baseMean**	**$\log_2$FC**	**p-adjusted**	**Gene ID**	**HIGH**
ENSMUSG00000054200.6	8.77721485	-2.2659204	1.24E-27	Ffar4	WET
ENSMUSG00000026435.15	38.7630267	-2.2184407	1.16E-42	Slc45a3	WET
ENSMUSG00000079019.2	24.7158409	-1.6454793	4.55E-13	Insl3	WET
ENSMUSG00000031170.14	42.2322119	-1.6434261	6.64E-15	Slc38a5	WET
ENSMUSG00000038463.8	16.2605998	-1.4619721	3.55E-12	Olfml2b	WET
ENSMUSG00000030830.18	22.0478661	-1.4358002	3.41E-10	Itgal	WET
ENSMUSG00000032554.15	67.5197473	-1.3762549	7.26E-10	Trf	WET
ENSMUSG00000021253.6	31.2493344	-1.3551661	7.02E-14	Tgfb3	WET
ENSMUSG00000030972.6	13.8934534	-1.1709964	2.37E-07	Acsm5	WET
ENSMUSG00000059991.7	173.025492	-1.1528314	5.12E-11	Nptx2	WET
ENSMUSG00000046687.5	44.9527785	-1.0989949	8.31E-09	Gm5424	WET
ENSMUSG00000024125.1	101.5876	-1.0962074	9.77E-06	Sbpl	WET
ENSMUSG00000021913.7	46.5401886	-1.0876018	8.70E-07	Ogdhl	WET
ENSMUSG00000015702.13	27.7002506	-1.0603879	1.95E-05	Anxa9	WET
ENSMUSG00000036596.6	6.6698922	-1.0243046	9.04E-05	Cpz	WET
ENSMUSG00000025172.2	13.2622565	-1.0138171	0.00013318	Ankrd2	WET
ENSMUSG00000042757.15	14.5676529	1.00643936	0.00019556	Tmem108	DRY
ENSMUSG00000019997.11	64.49614	1.03331405	7.67E-05	Ctgf	DRY
ENSMUSG00000020427.11	92.3763518	1.56656207	4.55E-13	Igfbp3	DRY
ENSMUSG00000001768.15	12.3794312	1.72433255	8.16E-16	Rin2	DRY

489

490

491 Table 6: Functional information and chromosome (CHR) locations (*Mus musculus*) for the nine genes
492 differentially expressed across all three analyses in *P. eremicus* testes by treatment group

Gene Name	**Gene ID**	**Gene Function**	**CHR**	**HIGH**
Insulin-like 3	Insl3	testicular function and testicular development	8	WET
Free-fatty acid receptor 4	Ffar4	metabolism and inflammation	19	WET
Solute carrier family 45 member 3	Slc45a3	sugar transport	1	WET
Solute carrier family 48 member 5	Slc38a5	sodium-dependent amino acid transport	X	WET
Integrin alpha L	Itgal	lymphocyte-mediated immune responses	7	WET
Transferrin	Trf	iron transport and delivery to erythrocytes	9	WET
Ras and Rab Interactor 2	Rin2	endocytosis and membrane trafficking	2	DRY
Insulin-like growth factor binding protein 3	Igfbp3	modulates effects of insulin growth factors	11	DRY
Connective tissue growth factor	Ctgf	fibrosis and extracellular matrix formation	10	DRY

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494

Figure 1: Plot of edgeR determined differentially expressed genes. The 15 significant genes are in red, with positive values indicating increased expression in the DRY group, and negative values depicting increased expression in the WET group.

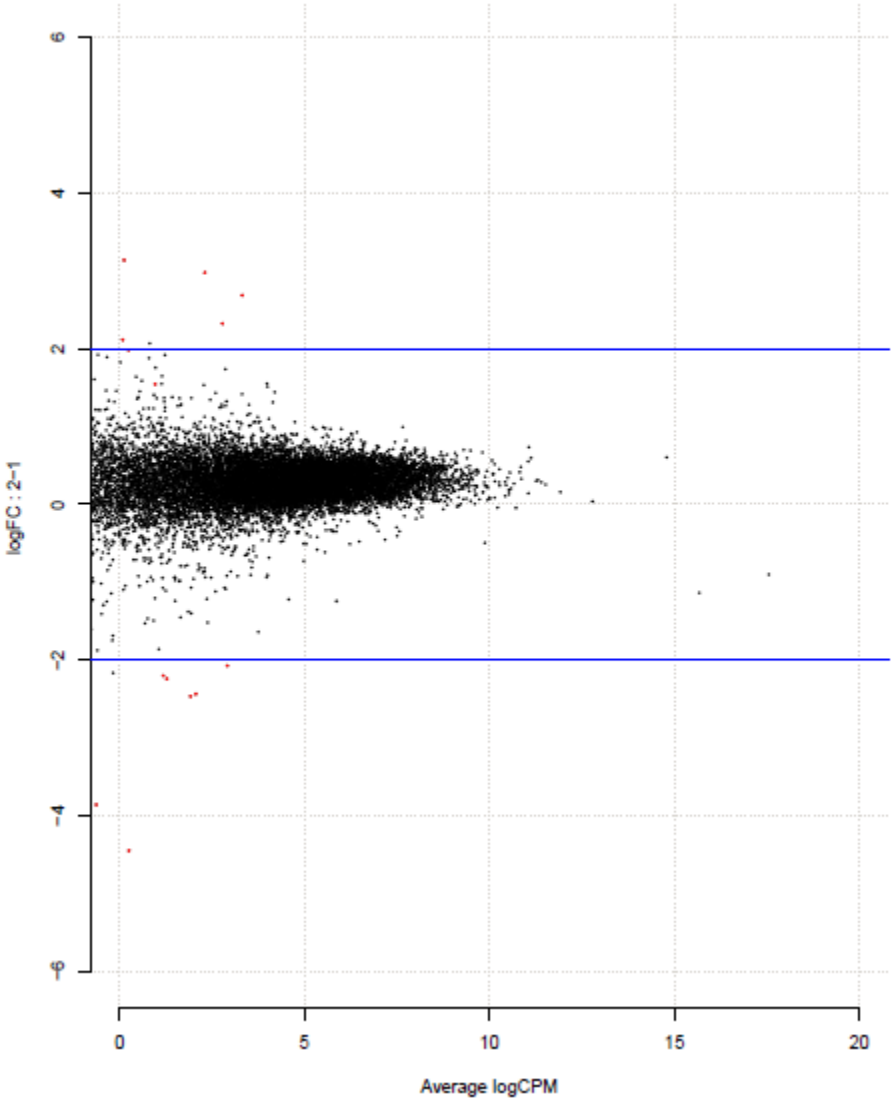
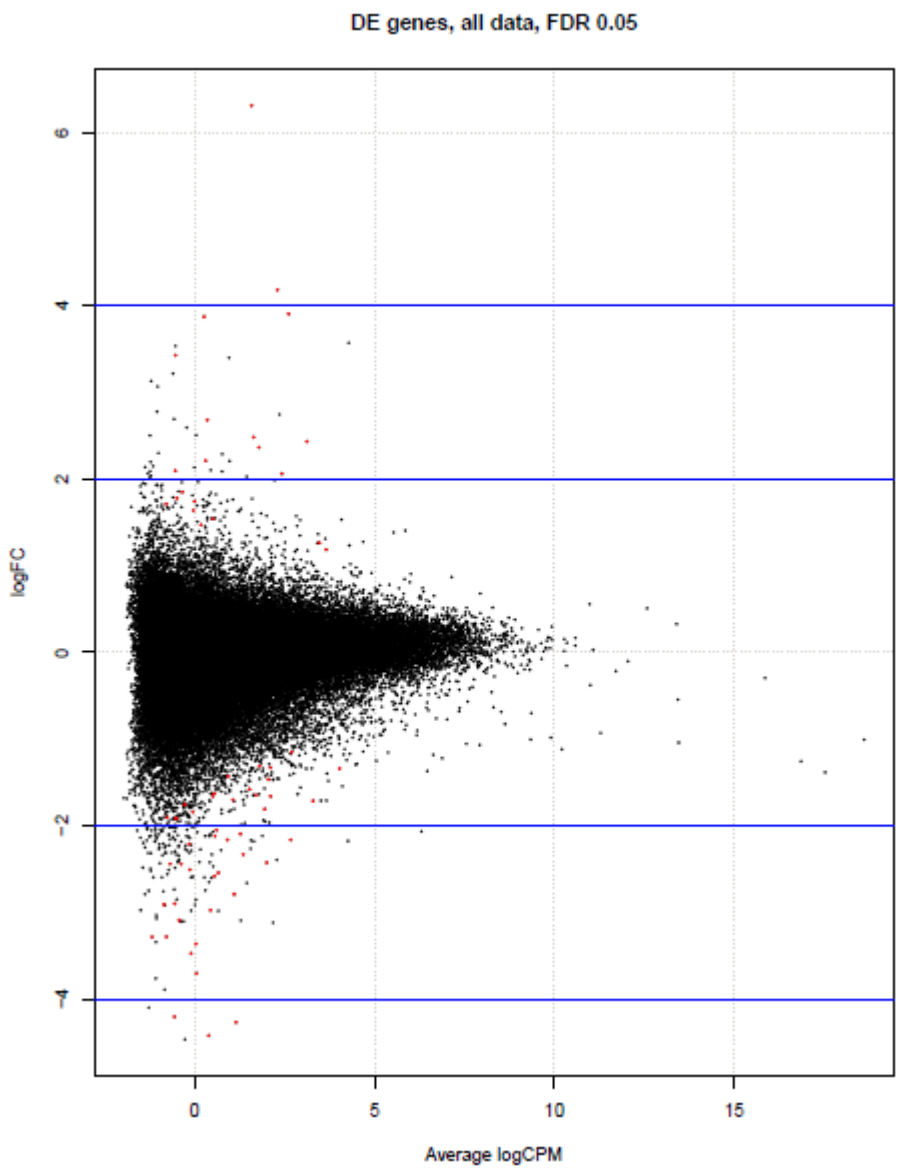
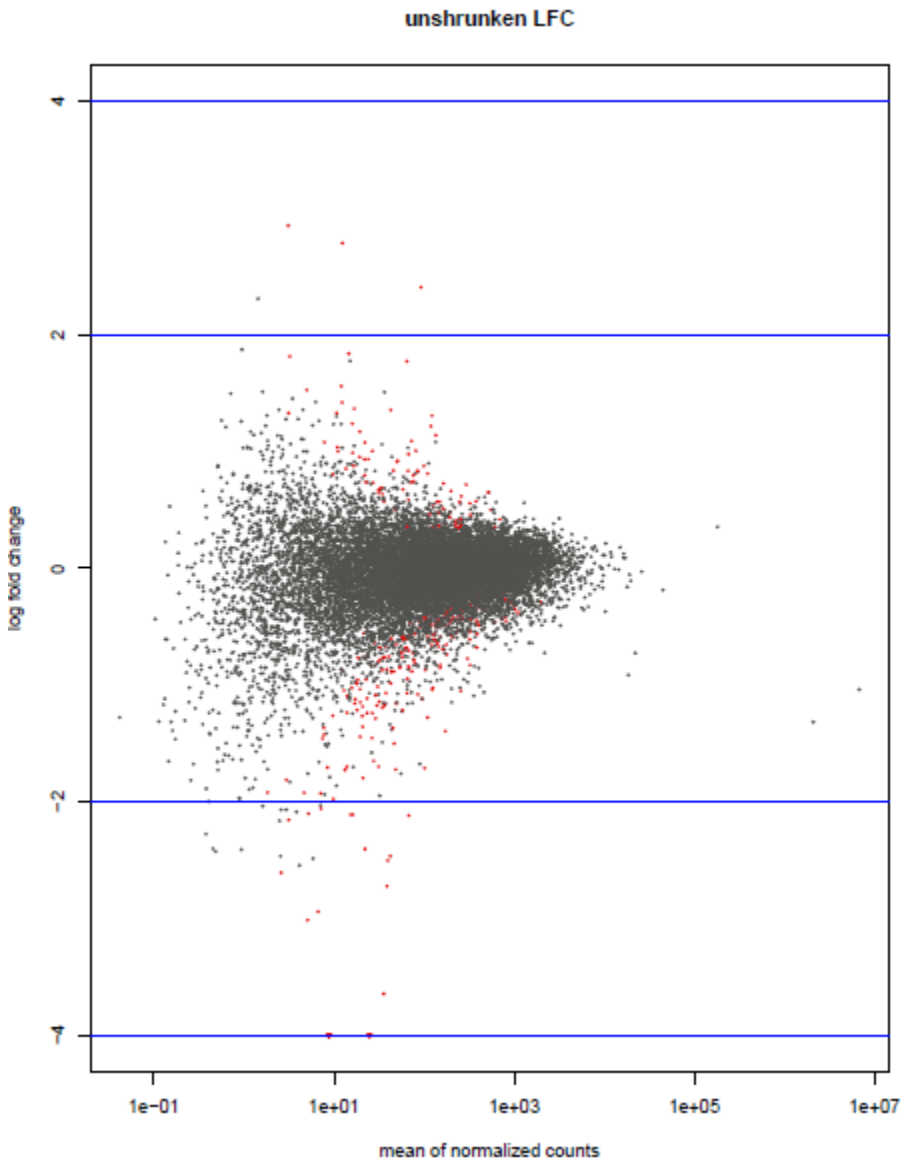


Figure 2: Plot of edgeR determined differentially expressed transcripts. The 66 significant transcripts are in red, with positive values indicating increased expression in the DRY group, and negative values depicting increased expression in the WET group.



512 Figure 3: Plot of DESeq2 determined differentially expressed transcripts. The 215 significant transcripts
513 are in red, with positive values indicating increased expression in the DRY group, and negative values
514 depicting increased expression in the WET group.
515



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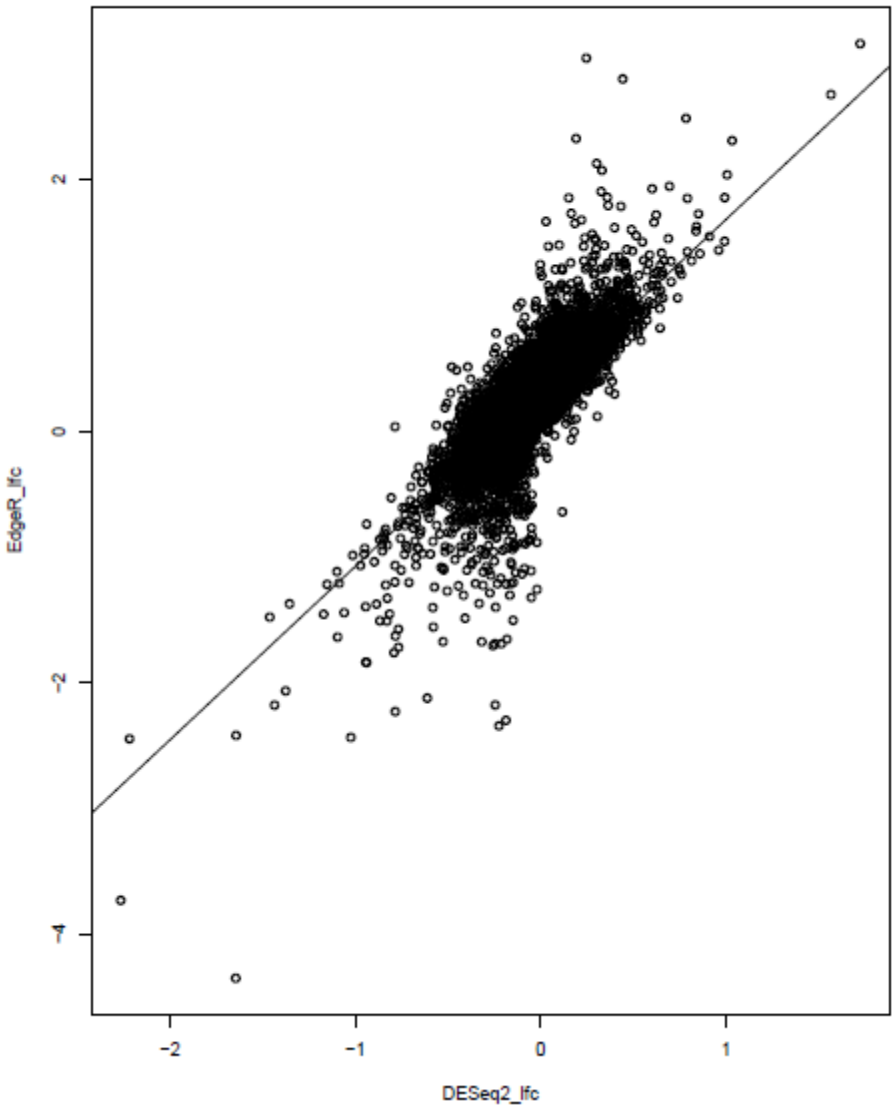
521 Figure 4: Correlation of $\log_2$ fold change results for all Ensembl ID gene matches from DESeq2 and
522 edgeR DGE analyses (Adj-R^2 = 0.6596; $F(1,14214) = 2.754 \times 10^4$; $p < 2.2 \times 10^{-16}$).
523



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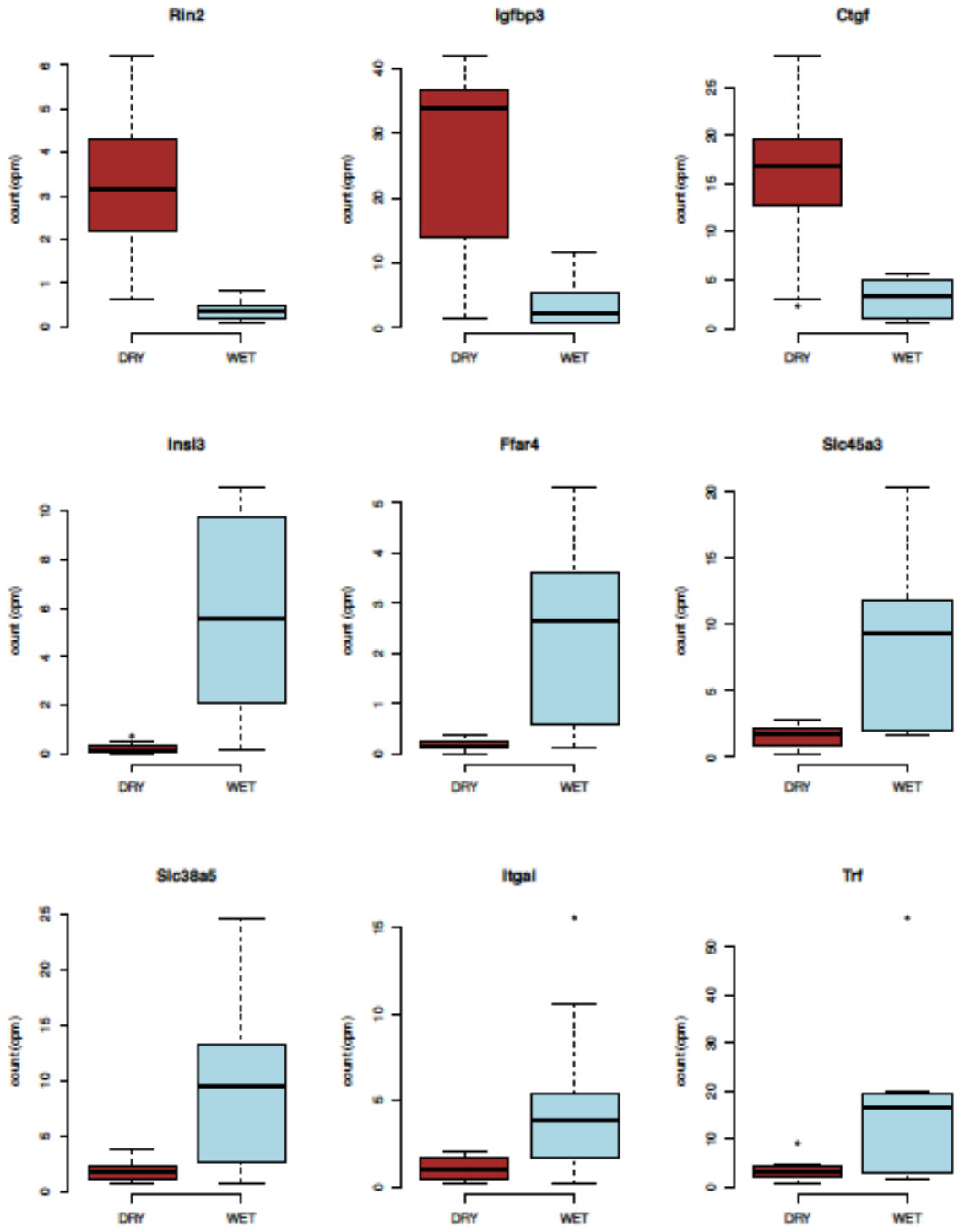
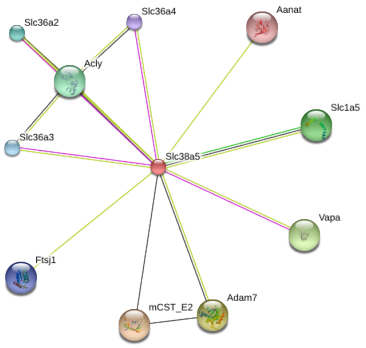
530 Figure 5: Box plots of edgeR analyzed differences in gene expression by treatment for the nine genes
531 significantly differentially expressed in all three analyses. Counts per million (cpms) for both treatments
532 (WET and DRY) are indicated.



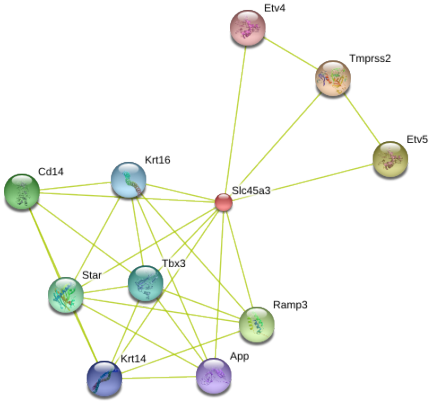
533

Figure 6: STRING diagrams of protein-protein interactions for genes significantly differentially expressed (highly expressed) in the WET treatment group. These six genes are (a) Slc38a5, (b) Slc45a3, (c) Insl3, (d) Ffar4 (also known as O3far1), (e) Itgal, and (f) Trf.

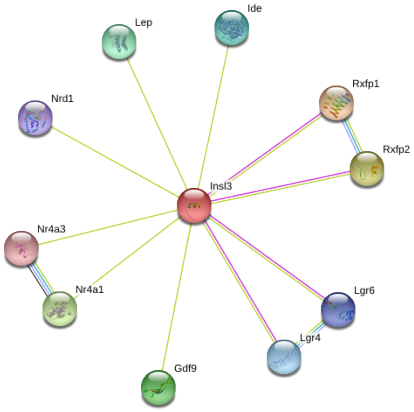
(a)



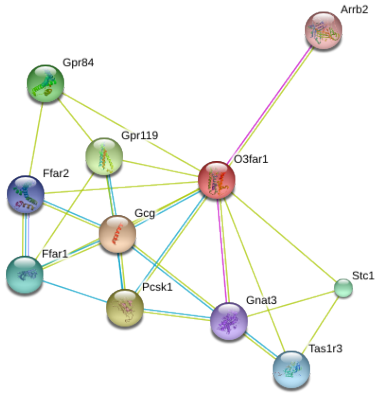
(b)



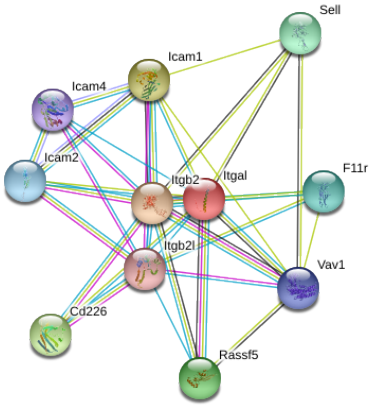
(c)



(d)


Arrb2
Gpr84
Gpr119
O3far1
Ffar2
Gcg
Ffar1
Pcsk1
Stc1
Gnat3
Tas1r3

(e)


Sell
Icam1
Icam4
Icam2
Itgb2
Itgal
F11r
Itgb2l
Vav1
Cd226
Rassf5

(f)

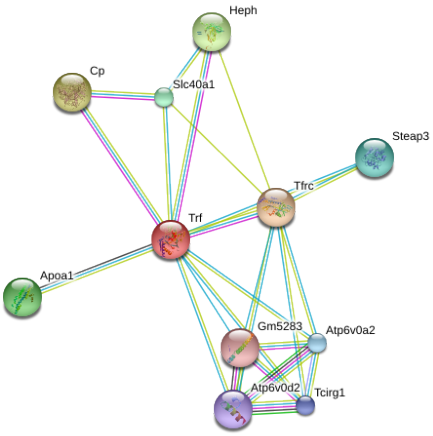
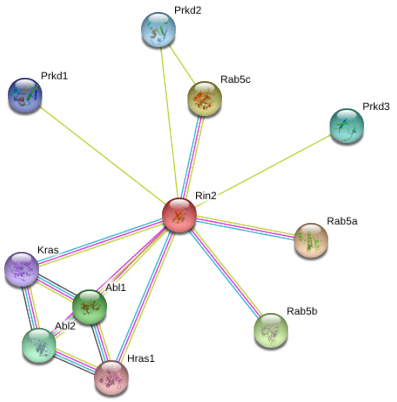
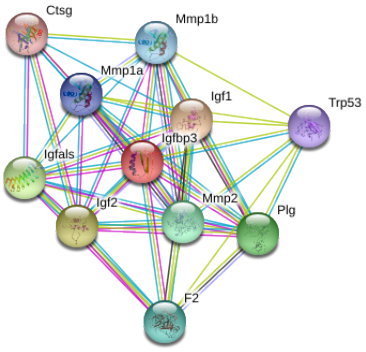

Heph
Cp
Slc40a1
Steap3
Tfrc
Trf
Apoa1
Gm5283
Atp6v0a2
Atp6v0d2
Tcirg1

Figure 7: STRING diagrams of protein-protein interactions for genes significantly differentially expressed (highly expressed) in the DRY treatment group. These three genes are (a) Rin2, (b) Igfbp3, and (c) Ctgf.

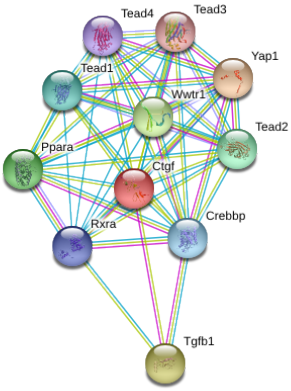
(a)



(b)



(c)



Supplemental 1: Testes read data statistics, including sample identification (Mouse ID), number of reads (# Reads), percent reads mapped to transcriptome (% Mapping), and treatment group (TRT). Mouse ID 335T* is the dataset which was used to assemble the testes transcriptome; therefore, these reads were not used for the differential expression analysis.

Mouse ID	# Reads	% Mapping	TRT
335T*	***45759114***	***85.46***	***wet***
3333T	15135923	82.56	Wet
2322T	12584407	82.37	Dry
382T	14305186	83.87	Dry
381T	14178847	83.23	Wet
376T	14588175	82.56	Dry
366T	13641731	82.95	Wet
349T	17289781	85.93	Wet
209T	11724617	84.02	Dry
265T	11536510	84.17	Dry
383T	13250034	81.46	Dry
384T	12152820	82.75	Dry
102T	11131941	84.84	Wet
400T	13259393	83.98	Wet
1357T	20603232	82.32	Wet
1358T	12240814	86.58	Wet
1359T	11144962	85.54	Wet
13T	11075885	83.55	Dry
343T	9423867	83.58	Dry
344T	17146134	85.36	Wet
355T	13948415	85.21	Wet
888T	18890387	86.52	Dry
999T	15213425	87.02	Dry

566 Supplemental 2: Significantly differentially expressed genes identified in the three analyses (DGE in
567 edgeR, DTE in edgeR, and DGE in DESeq2) by treatment group in *P. eremeicus* testes. Of the 34
568 different genes which were more highly expressed in WET mice, six were significant across all three
569 analyses (Gene IDs are italicized). Of the 17 genes which were more highly expressed in DRY mice,
570 three were significant across all three analyses (Gene IDs are italicized).

HIGH: WET			
Gene ID	**DGE edgeR**	**DTE edgeR**	**DGE DESeq2**
Insl3	x	X	x
Ffar4	x	X	x
Slc45a3	x	X	x
Slc38a5	x	X	x
Itgal	x	X	x
Trf	x	X	x
Slit1	x	X	
Cpz		X	x
Tgfb3		X	x
Ces1g		X	
Ankrd2		X	x
Nvl		X	
Ogdhl		X	x
Pfkfb4		X	
Slc33a1		X	
Anxa9		X	x
Ddb2		X	
St3gal1		X	
Acsm5		X	x
Cyp17a1		X	
Olfml2b		X	x
Pf4		X	
Nptx2		X	x

Dnah6		X	
Sbpl		X	x
Adcy6		X	
Gm5424		X	x
Mbp		X	
Fbxo2		X	
Mycl		X	
Eci1		X	
Capn12		X	
Col6a1		X	
Gpr55		X	
HIGH: DRY			
Gene ID	**DGE edgeR**	**DTE edgeR**	**DGE DESeq2**
Rin2	x	X	x
Igfbp3	x	X	x
Ctgf	x	X	x
Cyp2e1	x		
Fmo2	x		
Tnfrsf21	x		
Cyp2f2	x		
Dennd2d	x		
Nedd1		X	
Wdr83		X	
Gpx4		X	
Asah1		X	
Adm		X	
Tmem108		X	x
Nkx3-1		X	
Ybx1		X	
Arfgef2		X	

571 **DropBox Files (will be submitted to Dryad upon acceptance):**

572 Optimized final un-annotated transcriptome (good.BINPACKER.cdhit.fasta)

573 Annotated transcriptome (good.BINPACKER.cdhit.fasta.dammit.fasta)

574 Dammit gff3 file of annotation (good.BINPACKER.cdhit.fasta.dammit.gff3)

575 Salmon folder including salmon quant outputs for 22 individuals (salmon)

576 Salmon merged quant file (NEWmergedcounts.txt)

577 Gene ID by Transcript ID matrix (NEWESTfinalMUS.txt)

578 Transcripts without matches from edgeR DTE analysis (DTEno-matchBLASTnSequences.md)

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