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11 Plasticity in gene expression facilitates invasion of the desert environment in house mice
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43 **Abstract**

44 Understanding how organisms adapt to new environments is a key problem in evolution,
45 yet it remains unclear whether phenotypic plasticity generally facilitates or hinders this
46 process. Here we studied the evolved and plastic responses to water stress in lab-born
47 descendants of wild house mice (*Mus musculus domesticus*) collected from desert and non-
48 desert environments. Using a full sib design, we measured organismal phenotypes and
49 gene expression under normal (hydrated) and water stressed (dehydrated) conditions.
50 After many generations in the lab, mice from the desert consumed significantly less water
51 than mice from other localities, indicating that this difference has a genetic basis. Under
52 water stress, desert mice lost less weight than non-desert mice, and desert mice exhibited
53 differences in blood chemistry related to osmoregulatory function. Gene expression in the
54 kidney revealed evolved differences between mice from different environments as well as
55 plastic responses between hydrated and dehydrated mice. Desert mice showed reduced
56 gene expression plasticity under water stress compared to non-desert mice. Importantly,
57 the non-desert mice generally showed shifts towards desert-like expression under water
58 stress, consistent with adaptive plasticity. Finally, patterns of gene expression identified
59 several candidate genes for adaptation to the desert, including *Aqp1* and *Apoe*. These
60 findings provide evidence for local adaptation in a recently introduced species and suggest
61 that adaptive plasticity may have facilitated the colonization of the desert environment.

62

63 Keywords: desert, plasticity, adaptation, *Mus*, gene expression

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64
Introduction

65 Understanding the origin and genetic architecture of complex traits associated with local
66 adaptation is a central goal of evolutionary biology. One ongoing debate concerns the
67 extent to which phenotypic plasticity may facilitate or constrain adaptation to new
68 environments (Baldwin, 1896; Price, Qvarnström, & Irwin, 2003; Ghalambor, McKay,
69 Carroll, & Reznick, 2007; Levis & Pfennig, 2016). For example, adaptive plasticity, defined
70 as an environmentally induced phenotypic change that brings individuals closer to the local
71 optimum, may enable organisms to invade new environments. Subsequent genetically
72 encoded changes in the same direction as the plastic changes may then accrue, bringing
73 individuals even closer to the optimum, as seen for coloration in lizards living on dark
74 substrates (Corl et al., 2018). Conversely, plastic changes may be non-adaptive, moving
75 individuals farther from the local optimum. In such cases, selection is expected to favor
76 genetic changes underlying phenotypes that go in the opposite direction of the plastic
77 change and thereby bring the individual closer to the optimum. This pattern of non-
78 adaptive plasticity is seen for gene expression changes in guppies reared in the absence of
79 predators (Ghalambor et al., 2015). Which of these two outcomes is most likely remains
80 unclear and may depend both on the phenotype in question and the environmental
81 heterogeneity to which populations have been exposed (e.g. Huang & Agrawal, 2016).
82
83 House mice (*Mus musculus domesticus*) provide an opportunity to study plastic and evolved
84 changes in the context of adaptation to novel environments. House mice are native to
85 western Europe but were recently introduced to the Americas with European colonization,
86 approximately 400-600 generations ago (Phifer-Rixey & Nachman, 2015). In this short

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87 time, they have colonized a wide variety of different environments. In eastern North
88 America, house mice show strong evidence of local adaptation for several complex
89 phenotypes such as body size, activity, and nest-building behavior (e.g. Lynch, 1992; Mack,
90 Ballinger, Phifer-Rixey, & Nachman, 2018; Phifer-Rixey et al., 2018).
91
92 In the Sonoran Desert of North America, house mice must contend with low to seasonally-
93 absent water as well as extreme heat. Although house mice are human commensals, they
94 frequently live in sheds, grain storage areas, barns, and other habitats where they are not
95 well shielded from the environment. They can also live in situations where they are not
96 associated with humans (Sage, 1981). House mouse urine concentration, a metric often
97 associated with specialization to xeric environments, is very high (Haines et al., 1973) and
98 falls within the range of many known desert specialists (Beuchat, 1990). Previous
99 experiments of wild mice brought into the lab have found that mice can survive beyond 14
100 days without access to free water on a diet of dried seeds (Koford, 1968). In other
101 experiments with varying relative humidity, mice survived up to 41 days without free
102 water (Haines & Schmidt-Nielsen, 1967).
103
104 Previous studies have examined the behavioral, morphological, and physiological
105 adaptations that allow desert mammals to persist under xeric conditions (reviewed in
106 Schmidt-Nielsen, 1964; Donald & Pannabecker, 2015) . Recent work has also begun to
107 identify some of the genes associated with these phenotypes (Giorello et al., 2018;
108 MacManes, 2017; Marra et al., 2014; Wu et al., 2014). Far less is known about the role of
109 phenotypic plasticity in the context of desert adaptation. Since all mice, including those

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110 living in more mesic environments, occasionally go through periods of water stress,
111 selection may have favored plastic responses that enable mice to survive periods of water
112 shortage (i.e. adaptive plasticity). Here we are interested in whether the evolved
113 differences between mice from more mesic environments and mice from more xeric
114 environments mirror the plastic responses seen within populations, both for organismal
115 level phenotypes and for gene expression in the kidney. Changes in gene expression in the
116 kidney may also help identify candidate genes for adaptation to xeric conditions.
117
118 To assess the contribution of plastic and evolved changes to a xeric environment, we
119 studied lab-born descendants of wild mice from two populations, one from Edmonton,
120 Canada and one from Tucson, Arizona. While neither population experiences high
121 precipitation, the annual precipitation in Edmonton is 57% more than in Tucson, and these
122 two locations differ dramatically in average temperature. We address four primary
123 questions. (1) Do progeny of wild mice from these two populations exhibit phenotypic
124 differences when reared in a common laboratory setting, indicating that the phenotypes
125 have a genetic basis? (2) Do these same phenotypes exhibit plastic (non-genetic) changes
126 when mice are exposed to water limitation? (3) Are plastic changes generally in the same
127 or opposite direction as the evolved changes? (4) Do gene co-expression networks identify
128 sets of genes and corresponding phenotypes that underlie adaptation to xeric conditions?
129 We find that in a common environment, Tucson mice drink less water than Edmonton mice
130 and differ in blood chemistry and gene expression in the kidney. These same traits exhibit
131 significant plasticity when mice are fully hydrated compared to mice under water stress,
132 and evolved differences are generally in the same direction as plastic differences, both for

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133 gene expression and for organism-level phenotypes. Finally, co-expression networks
134 identify groups of genes that likely underlie adaptation to xeric conditions. These findings
135 suggest an important role for adaptive plasticity in the colonization of the desert
136 environment.
137

138 **Materials and Methods**

139 *Mice*

140 To assess whether mice from the Sonoran Desert differ in water consumption compared to
141 mice from other habitats, we used wild-derived mouse lines developed in our lab from a
142 range of localities in the Americas. Wild house mice were caught from five populations in
143 different habitats and used to create inbred lines through sib-sib mating over multiple
144 generations. The five localities were Tucson, AZ, USA (TUC); Edmonton, Alberta, Canada
145 (EDM); Gainesville, FL, USA (GAI); Saratoga Springs, NY, USA (SAR); and Manaus,
146 Amazonas, Brazil (MAN). In nearly all cases, lines were established from unrelated
147 individuals. Lines were maintained in the laboratory for 6-19 generations on a diet of
148 standard mouse chow. All mice were handled in accordance with a UC Berkeley Animal
149 Care and Use protocol (protocol AUP-2016-03-8548-1).
150

151 *Measuring water consumption*

152 To quantify differences among populations, we measured water consumption over 72
153 hours in 163 adult males representing 45 different inbred lines (Table S1). In total, 40
154 individuals were measured from Tucson, 24 from Gainesville, 48 from Edmonton, 23 from
155 Saratoga Springs, and 28 from Manaus. Mice were between 90 and 200 days of age and

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156 were housed singly in cages at 23°C with a 10 hour dark and 14 hour light cycle on
157 standard Teklad Global rodent chow (18% protein, 6% fat). Body weight and amount of
158 water consumed after 72 hours were recorded, and relative water consumption (RWC) was
159 calculated (grams of water consumed per gram of mouse). Relative water consumption was
160 used as a metric due to the population level variation in body weight.
161

162 *Measuring phenotypic plasticity*

163 To study evolved and plastic responses to xeric conditions, we chose one wild-derived
164 inbred line each from Tucson and Edmonton [Tucson: TUSA4xA8 (TUCC/Nach), Edmonton:
165 TAS111x165 (EDMA/Nach)]. These lines were chosen because they showed large
166 differences in water consumption as well as little variance within lines. Male littermates
167 from these lines were assigned at random to either control or water restriction treatments
168 and housed individually post-weaning with water *ad libitum*. After 90 days of age, mice
169 were weighed and phenotyped for relative water consumption. Following phenotyping
170 (average age = 99 days), mice assigned to the water restriction treatment (Edmonton n=11,
171 Tucson n=7) were restricted from all water consumption for 72 hours. Mice assigned to the
172 control treatment (Edmonton n=10, Tucson n=6) were maintained with water *ad libitum*.
173 All mice were weighed every 24 hours and monitored for markers of drastically declining
174 health. All animal care was conducted in accordance with procedures approved by the UC
175 Berkeley Animal Care and Use Committee (protocol AUP-2017-05-9940). After sacrifice
176 with isofluorane, left kidney, liver, and caecum were immediately removed and stored in
177 RNAlater according to manufacturer's instructions. The right kidney was weighed and
178 stored in 10% neutral buffered formalin for morphological analysis at the UC Davis

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179 Comparative Pathology Laboratory. Five hydrated mice per population (ten mice total)
180 were phenotyped for renal cortex thickness and papilla thickness. Blood was extracted
181 from the heart and body cavity using a syringe and centrifuged in BD SST Microtainer tubes
182 to extract serum. Levels of blood urea nitrogen (BUN), total protein, creatinine, chloride,
183 potassium, and sodium levels were analyzed at the UC Davis Comparative Pathology
184 Laboratory for 20 mice (five individuals per population per treatment). These serum
185 solutes were measured to quantify kidney health and glomerular function in treated and
186 control samples.
187
188 *mRNA library preparation and sequencing*
189 RNA was extracted from half of a kidney preserved in RNAlater from twenty mice total,
190 (five mice per population per treatment), using the MoBio Laboratories Powerlyzer
191 Ultraclean Tissue & Cells RNA Isolation Kit. RNA libraries were prepared using the KAPA
192 Hyper Prep Kit and then pooled and sequenced across two lanes of 100bp PE Illumina
193 HiSeq4000 at the Vincent J. Coates Genomics Sequencing Center at UC Berkeley.
194
195 *mRNA read mapping and quantification of gene expression*
196 Reads were trimmed for quality and adaptor contamination with Trimmomatic v0.36
197 (Bolger et al., 2014) and mapped to the *Mus musculus* reference genome (GRCm38/mm10)
198 using STAR v2.6.0c (Dobin et al., 2013). Reads overlapping exons were counted using the
199 program HTSeq 0.6.1 (Anders et al., 2015) to estimate per-gene mRNA abundance. We
200 removed genes with a mean fewer than ten reads across samples from additional analyses.
201 The R package DESeq2 (Love et al., 2014) was used to test for differential expression

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202 between (1) treatments within each population, and (2) populations within each treatment.
203 Genes were retained as significant at a false-discovery rate of 5%.
204

205 *Gene co-expression analyses*

206 We used standard protocols (Langfelder & Horvath, 2008) to perform a weighted gene co-
207 expression network analysis on expression residuals for the 20 individuals to identify
208 expression modules. We tested for associations between eigengenes (the first principle
209 component of a module) and each of the nine phenotypes described (RWC, kidney weight,
210 proportion of weight maintained, serum BUN, serum creatinine, serum total protein, serum
211 chloride, serum potassium, and serum sodium) as well as population of origin and
212 treatment group. We were able to assign genes membership to expression modules as well
213 as position relative to the center of the module. Genes that are more central (i.e. those that
214 have more connections with other genes in a module) are good targets for putative
215 candidate genes related to phenotypes of interest, population of origin, or treatment group.
216 Additionally, to identify genes that show differential co-expression between Tucson and
217 Edmonton we used the program DGCA (Differential Gene Correlation Analysis) (McKenzie
218 et al., 2016), which calculates the average change in correlation between the two lines
219 across all gene pairs.
220

221 *Enrichment analyses*

222 GO category enrichment on gene sets of interests were performed with GOrilla (Eden et al.,
223 2009) by testing the foreground set against the background set of all genes expressed in
224 the kidney. Phenotype enrichment tests were performed with modPhea (Weng & Liao,

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225 2017) by comparing the foreground set against the background set of all genes expressed
226 in the kidney.
227
228 **Results**

229 *Relative water consumption is lowest in mice from the Sonoran desert*

230 To determine whether mice from Tucson, Arizona exhibit lower water consumption
231 compared to mice from other populations, we took advantage of a set of wild-derived
232 inbred lines of mice from five localities across the Americas (Figure 1a). We assayed
233 relative water consumption for 163 male mice representing 45 wild derived inbred lines
234 over a 72-hour period from five founder populations (Tucson (TUC), Edmonton (EDM),
235 Saratoga Springs (SAR), Gainesville (GAI), and Manaus (MAN). Mice from Tucson drank
236 significantly less water than mice from any other population except Saratoga Springs
237 (Median RWC: TUC: 0.34, SAR: 0.40, MAN 0.42, GAI: 43, EDM: 0.52)(Figure 1b). The
238 greatest difference was seen between mice from Tucson and mice from Edmonton (Mann-
239 Whitney U, $p < 0.00001$)(Figure 1b). For this reason, we chose to focus on comparisons
240 between lines from these two populations in all subsequent analyses.
241

242 *Evolved and plastic phenotypic differences associated with xeric conditions*

243 Water consumption is a complex trait with many factors contributing to the ultimate
244 phenotype. To further characterize this phenotypic variation, we compared the inbred line
245 from Tucson with the lowest average water consumption (TUCC/Nach) to the inbred line
246 from Edmonton with the greatest average water consumption (EDMA/Nach).
247

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248 First, we compared mice from these two lines under standard (hereafter, "hydrated")
249 conditions. In addition to the difference in water consumption seen between mice from
250 these lines (Figure S1), we found that hydrated mice from Tucson and Edmonton showed
251 several phenotypic differences related to fluid consumption and homeostasis in a standard
252 laboratory environment. Edmonton mice had heavier kidneys relative to their body weight
253 than Tucson mice (p = 0.00015)(Figure S2), but did not show significant differences in two
254 aspects of gross morphology: renal cortex thickness nor the ratio of papilla to cortex
255 thickness (Figure S3). The relative thickness of the papillae in the medulla is correlated
256 with urine concentrating ability; thicker medullas are often associated with animals
257 inhabiting more arid climates (Al-kahtani et al., 2004). Anecdotally, mice from Tucson
258 appeared to produce far less urine than mice from Edmonton, consistent with many desert
259 rodents, but this was not measured in this study due to difficulty in obtaining urine from
260 Tucson mice. In blood chemistry comparisons between hydrated Tucson and Edmonton
261 mice, Tucson mice had higher chloride (median mmol/L: Tucson: 114.9, Edmonton: 112.5,
262 p=0.03, Figure 2a) and creatinine (median mg/dL: Tucson: 0.17, Edmonton: 0.11, p=0.02,
263 Figure 2b) levels. Chloride, an electrolyte, is a marker of dehydration and thus expected to
264 be at higher concentrations in the blood of dehydrated animals (MacManes, 2017).
265 Creatinine is a waste product of normal muscle metabolism that is removed from the blood
266 to be excreted by the kidney mainly through glomerular filtration. Blood creatinine levels
267 increase as glomerular filtration, and thus kidney function, decreases and therefore is often
268 used as a measure of kidney health (Kassirer, 1971). The increased levels of serum chloride
269 and creatinine, commonly warnings for declining osmoregulatory function, in healthy

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270 Tucson mice suggests that their baseline kidney function differs from that of Edmonton
271 mice and they may be able to function normally despite higher blood osmolarity.
272
273 Next, we asked how mice from Tucson and mice from Edmonton differed in their response
274 to water stress. We took male full-siblings from the same litter as mice from our hydrated
275 comparison and withheld water from these mice for 72-hours. Hereafter, we refer to the
276 water-restricted group as "dehydrated." We found that Tucson mice lost significantly less
277 weight than Edmonton mice over the course of 72 hours without access to water (median
278 percent weight maintained: Tucson: 0.82, Edmonton: 0.78, Figure 2c) (Mann-Whitney U,
279 p=0.027) suggesting that Tucson mice are more buffered against water stress. Comparing
280 blood chemistry measures after mice were subjected to water stress, we found that Tucson
281 mice measured higher in BUN (median mg/dL: Tucson: 64.45, Edmonton: 43.80, p=0.03,
282 Figure 2d), chloride (median mmol/L, Tucson: 129.60, Edmonton: 111.95, p=0.03, Figure
283 2a), and potassium (median mmol/L: Tucson: 18.06, Edmonton: 7.78, p=0.03, Figure S4a)
284 than Edmonton mice. BUN is a waste product from the liver during the metabolism of
285 protein and increases as glomerular filtration rate and blood volume decreases (Baum et
286 al., 1975). Similarly, high levels of serum potassium often reflect a decrease in filtration of
287 the solute from the blood and thus decreased kidney function (Schwartz, 1955) although
288 this measure is particularly sensitive to lysed blood cells during collection and could reflect
289 the challenge of collecting blood from dehydrated animals. Regardless of treatment, Tucson
290 mice maintained higher serum chloride levels than Edmonton mice. In contrast, potassium
291 levels were higher in Tucson mice only in the dehydrated treatment. High levels of both of
292 these electrolytes are consistent with dehydration. Dehydrated mice from Tucson showed

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293 greater indicators for dehydration and kidney dysfunction than Edmonton mice but lost
294 less weight when water stressed. This may reflect a greater evolved capacity to respond to
295 the stress of dehydration. The fact that phenotypic differences in both hydrated and
296 dehydrated animals persist in a common environment indicates that they may have a
297 genetic basis.
298
299 While differences between the Tucson and Edmonton lines represent evolved differences,
300 differences between hydrated and dehydrated mice are evidence of plastic responses to
301 water stress. Many of the traits that differed between lines also exhibited phenotypic
302 plasticity in comparisons between hydrated and dehydrated mice within lines. Dehydrated
303 Tucson mice had higher levels of serum BUN compared with hydrated Tucson mice
304 (median mg/dL, Hydrated:25.20, Dehydrated: 64.45, p=0.008, Figure 2d). BUN differed
305 both between stressed and control mice from Tucson and was higher than in Edmonton
306 mice when water stressed. Dehydrated Edmonton mice had higher levels of serum
307 creatinine (median mg/dL: Hydrated: 0.11, Dehydrated:0.14, p=0.03, Figure 2b) and
308 sodium (mean mmol/L: Hydrated: 153, Dehydrated: 161, p=0.01, Figure S4b) compared
309 with hydrated Edmonton mice, reaching the levels for both solutes that were seen in
310 Tucson hydrated and dehydrated animals. The only solute that responded to water stress
311 in both lines was total protein (median g/dL: Edmonton: Hydrated: 5.29, Dehydrated: 6.89,
312 p=0.01, Tucson: Hydrated: 5.68, Dehydrated: 7.29, p=0.008). Total protein measures the
313 concentration of both albumin and gobulin in the blood which increases with dehydration
314 (Senay & Christensen, 1965). These results indicate that while both lines react

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315 physiologically to the stress of dehydration, they may do this through different
316 mechanisms.
317
318 *Evolved and plastic transcriptional responses to xeric conditions*
319 Changes in gene expression provide a flexible mechanism for rapidly responding to
320 changes in the local environment, and can also underlie evolutionary divergence. Kidneys
321 are the primary osmoregulatory organ and are essential for homeostasis and solute
322 excretion. To identify candidate genes underlying adaptation to low water environments
323 over short evolutionary timescales as well as plastic responses to water restriction, we
324 sequenced mRNA from kidneys of ten Tucson and ten Edmonton mice, five from the
325 dehydrated and five from the hydrated treatment. Differences in expression between
326 Tucson and Edmonton mice in a common environment represent evolved differences,
327 while differences between dehydrated and hydrated treatments represent a plastic
328 response to water restriction.
329
330 We sequenced a total of ~1.3 billion reads for an average of 26,406,068 uniquely mapped
331 reads per sample which were used to quantify mRNA expression levels and differential
332 expression between samples. Gene expression was measured in a total of 54,233 genes as
333 defined by Ensembl GRCm38 (mm10) with 19,105 genes expressed over a mean of ten
334 reads per sample. Sampling the 1000 genes with the greatest variance, we found that
335 Tucson and Edmonton individuals clustered separately, indicating that more of the gene
336 expression variation was partitioned between line-of-origin than between treatment group
337 (Figure 3a). Edmonton mice clustered into two distinct groups based on treatment

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338 (dehydrated vs. hydrated individuals), but dehydrated and hydrated Tucson mice did not
339 form distinct clusters (Figure 3a).
340
341 To identify differential gene expression, we used DESeq2 (Love et al. 2014) to perform
342 pairwise comparisons between: 1) Tucson hydrated *vs.* Edmonton hydrated, and 2) Tucson
343 dehydrated *vs.* Edmonton dehydrated, 3) Tucson dehydrated *vs*. Tucson hydrated, and 4)
344 Edmonton dehydrated *vs.* Edmonton hydrated individuals. Overall, we found more genes
345 were differentially expressed between the two lines than between the treatment groups
346 (FDR of 5%; see methods). A total of 3,935 genes were differentially expressed between
347 hydrated Edmonton and Tucson mice while a total of 3,419 genes were differentially
348 expressed between dehydrated Tucson and Edmonton individuals, a 51% overlap with
349 differences seen between hydrated Tucson and Edmonton mice.
350
351 Comparing the hydrated and dehydrated groups within each population (Tucson
352 dehydrated *vs*. Tucson hydrated, and Edmonton dehydrated *vs*. Edmonton hydrated), we
353 found that twice as many genes were differentially expressed in the Edmonton (1354) than
354 in the Tucson comparisons (677 genes) (Chi-square test with Yates Correction, $p < 0.0001$,
355 Figure 3b), with a 225 gene overlap. This 225 gene overlap represents the shared
356 transcriptional response to dehydration with respect to these two lines and is enriched for
357 phenotypes including dehydration (q=9x10^{-3}) and decreased vasodilation (q =3.8x10^{-2}) and
358 GO terms involved in regulation of blood pressure (q = 4.99x10^{-2}). Genes differentially
359 expressed between hydrated and dehydrated Edmonton mice were also enriched for GO
360 terms relevant to water stress, such as renal system processes (q = 5.08 x 10^{-2}) and

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361 regulation of body fluids (q = 1.30x10^{-2}). Within genes solely differentially expressed
362 between the Tucson groups, we saw enrichment for homeostasis related GO terms (see File
363 S1), but not for any kidney-specific categories. In addition to having a greater number of
364 differentially expressed genes in Edmonton comparisons, we also found that the average
365 magnitude of expression differences between hydrated and dehydrated treatments ($|\log_2$
366 fold change$|$) was significantly greater for Edmonton mice than for Tucson mice (Mann-
367 Whitney U, $p < 2.2 \times 10^{-16}$) (Figure 3c). Together, these results are consistent with
368 Edmonton mice being farther from their physiological optimum when water stressed than
369 are Tucson mice, consistent with the hypothesis that Tucson mice are locally adapted to a
370 water limited environment.
371
372 *Dehydrated Edmonton mice show shifts towards Tucson-like expression*
373 Next, we were interested in asking whether the plastic changes in response to water stress
374 in the non-xeric mice (i.e. Edmonton) go in the same or opposite direction as the evolved
375 differences between mice from xeric and non-xeric habitats. Specifically, we were
376 interested in whether Edmonton mice placed under water stress would show
377 transcriptional responses that make them more similar to the base-line condition of Tucson
378 mice. Therefore, we focused on differentially expressed genes between the Edmonton
379 hydrated and dehydrated groups and asked whether the Edmonton dehydrated group
380 showed shifts in expression in the direction of the Tucson hydrated condition (Figure 4a).
381 The majority of these genes (85%, 416 genes) showed changes in the same direction,
382 meaning that the putatively adaptive and plastic responses were in the same direction (+/+

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383 and -/-) (Binomial exact test, $P<0.0001$). Only 15% (74 genes) show changes in opposite
384 directions (+/- and -/+) (Figure 4b).
385
386 The group of 416 genes with evolved and plastic changes in the same direction was
387 enriched for GO terms involved in homeostasis and ion transport and for mutant
388 renal/urinary system phenotypes ($q = 0.035$). For example, one of these genes, *Aquaporin 1*
389 (*Aqp1*), showed differences in expression between hydrated and dehydrated Edmonton
390 mice ($q = 0.0016$) and between the hydrated conditions of both lines ($q = 0.00019$)(Figure
391 5a). In Tucson mice, there was no significant effect of treatment on expression level, but in
392 Edmonton mice, expression increased in response to water stress recapitulating the
393 constitutive expression level of the mice from Tucson. Aquaporins are a family of
394 membrane proteins which form channels used to transport water and small solutes across
395 cell membranes. *Aqp1* is expressed in the descending loop of Henle, and channels formed
396 from this protein are the main route through which water is reabsorbed in this region
397 (Chou et al., 1999). It is known to affect urine concentrating ability, response to
398 dehydration, and renal water transport in lab lines of house mice (Ma et al., 1998; Sohara et
399 al., 2005) and has been identified in a several studies related to desert adaptation in
400 rodents (reviewed in Pannabecker, 2015). In our analyses, expression of *Aqp1* was
401 associated with variation in six of the nine measured phenotypes (Creatinine, p=0.020;
402 total protein, p=0.012; potassium, p=0.0094; weight loss, p=0.016; kidney weight,
403 p=0.0077; RWC, p=0.022).
404

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405 Of the 416 genes where dehydrated Edmonton mice showed shifts towards Tucson-like
406 expression, the majority (87%) were not differentially expressed between hydrated and
407 dehydrated Tucson mice. For the 54 of these genes that were differentially expressed in the
408 Tucson comparison, in all cases the plastic response was observed to be changing in the
409 same direction as in Edmonton mice.
410
411 *Co-expressed sets of genes are associated with phenotypic variation in Tucson and Edmonton*
412 *mice*
413 To identify transcriptional networks associated with phenotypic variation in Tucson and
414 Edmonton mice, we performed a weighted gene co-expression network analysis (WGCNA).
415 This analysis uncovers genes with highly correlated expression profiles and groups them
416 into modules reflecting hypotheses about connectivity. We identified 54 co-expression
417 modules, with at least one module significantly associated with each of the nine
418 phenotypes (Figure S5). Of these, the "salmon" module (Figure S6a) was of particular
419 interest because it was significantly associated with all nine phenotypes (BUN, p=0.02;
420 Creatinine, p=0.006; total protein, $p = 1 \times 10^{-4}$; Chloride, $p = 0.002$; Potassium, $p = 0.001$;
421 Sodium level, $p = 0.01$; weight loss, $p = 0.007$; kidney weight, $p = 0.03$; RWC, p= 0.006) as
422 well as population of origin ($p = 0.006$) and treatment ($p = 0.001$). Genes in this module
423 were enriched for several metabolic processes, including glyceraldehyde-3-phosphate
424 metabolic process ($q = 6.85 \times 10^{-9}$), ribose phosphate metabolic process ($q = 1.45 \times 10^{-8}$),
425 and carbohydrate derivative metabolic process ($q = 1.3 \times 10^{-7}$). Visualizing the most
426 connected genes in the salmon module, we identified *Tmtc1*, *Apoe*, and *Sult1a1* as the most
427 centrally located hub genes (Figure S6a). All three of these genes were identified in our

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428 previous analysis as genes for which dehydrated Edmonton mice showed shifts towards
429 Tucson-like expression (Figure 4). *Apoe* is of particular interest because of its documented
430 role in kidney function. It is thought to play an important role in renal damage protection
431 (Bonomini et al., 2011; Wen et al., 2002), and laboratory mutants show a number
432 physiological and morphological changes similar to kidney disease phenotypes (Bonomini
433 et al., 2011). A*poe* expression responded in the same direction and in a similar magnitude
434 in both lines under water stress. Interestingly, under water stress, the expression level in
435 Edmonton recapitulated the constitutive expression level of hydrated Tucson mice (Figure
436 5b). *Apoe* expression was also correlated with eight of the nine measured phenotypes
437 (Creatinine, p=0.034; total protein, p=0.00077; chloride, p=0.0055; potassium, p=0.0041;
438 sodium, p=0.034; weight loss, p=0.014; kidney weight, p=0.025; RWC, p=0.0036).
439
440 In addition to shifts in the expression of entire co-expression modules, populations may
441 differ as a consequence of altered co-expression between pairs of genes, called differential
442 co-expression. In order to identify genes that show differential co-expression between
443 Tucson and Edmonton, we calculated the average change in correlation between the two
444 lines across all gene pairs using the program DGCA (Differential Gene Correlation Analysis)
445 (McKenzie et al., 2016). We identified 182 genes that showed significantly different co-
446 expression between Tucson and Edmonton individuals (q <0.10, see methods). These genes
447 were enriched for several GO categories including glycolysis: generation of precursor
448 metabolites and energy (q = 0.0013), cellular amino acid metabolic process (q = 0.00046),
449 and lipid metabolic process (q = 0.0014). This gene set was also enriched for mutant
450 phenotypes related to renal/urinary system (q = 0.00001), abnormal urine homeostasis

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451 (q = 0.005), and abnormal ion homeostasis (q = 0.01). One of the genes with significant
452 changes in co-expression was *Aqp1*, which is involved in renal water transport. We also
453 found that several solute carriers (*Slc22a19, Slc11a2, Slc36a1, Slc47a1, Slc12a1, Slco3a1*)
454 showed evidence of differential co-expression, including one (*Slc47a1*) that has been
455 shown to be under positive selection in the desert-adapted cactus mouse, *Peromyscus*
456 *eremicus* (Kordonowy et al., 2017). *Slc47a1* mouse mutants are also associated with
457 increased BUN, increased circulating creatinine level, and kidney degeneration (Tsuda et
458 al., 2009). Altogether, these results suggest that Tucson and Edmonton mice show shifts in
459 the expression of co-expression modules as well as changes in the co-expression
460 associations between sets of genes.

461 **Discussion**

462 Here we have described phenotypic and transcriptional divergence between descendants
463 of mice from a desert environment and descendants of mice from a more mesic
464 environment when reared under identical laboratory conditions. We also described plastic
465 responses in these mice under conditions of water stress. First, we showed that inbred
466 lines derived from the Sonoran desert consume less water than do mice from other
467 populations in the Americas. Next, comparisons between a single line from Tucson and a
468 single line from Edmonton revealed many phenotypic differences in a common
469 environment, both at the organismal level and at the gene expression level in the kidney.
470 The fact that these differences were present after multiple generations in the lab indicates
471 that they are genetically based. Nonetheless, these same traits reveal considerable
472 plasticity in comparisons between control mice and mice under conditions of water stress.
473 Notably, we found that Tucson mice showed attenuated responses to water stress. After a

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474 72 hour period without water, Tucson mice lost less weight and showed fewer expression
475 differences in the kidney. Surprisingly, the blood chemistry of Tucson mice was consistent
476 with higher levels of dehydration and reduced kidney function, both under standard and
477 water-restricted conditions. However, phenotypes that appear maladaptive in one genomic
478 or environmental context may be adaptive in another (e.g., Riddle et al., 2018). Altogether,
479 these results are consistent with genetic changes to Tucson mice following their invasion of
480 the desert environment.

481 Phenotypic plasticity may allow animals to persist in harsh new environments if the plastic
482 responses bring individuals closer to the local optimum (reviewed by Ghalambor et al.,
483 2007). Adaptive plasticity can be followed by genetic changes as populations become
484 established, in a process called "genetic assimilation" or the "Baldwin Effect" (Price et al.,
485 2003; Robinson & Dukas, 1999; Simpson, 1953; Waddington, 1942, 1952, 1953). We found
486 that the evolved differences in kidney gene expression between Tucson and Edmonton
487 mice were generally in the same direction as plastic changes in Edmonton individuals
488 under water stress. Consequently, under water stress, gene expression in Edmonton mice
489 becomes more similar to that of Tucson mice under hydrated conditions. This result is
490 consistent with the idea that plastic responses to short-term water stress are an example of
491 adaptive plasticity. Thus, plastic responses to water stress may have helped facilitate the
492 colonization and subsequent adaptation of house mice to the desert environment.

493 Our finding that plastic responses generally go in the same direction as evolved responses
494 stands in contrast to several recent studies. For example, an allele of the *Epas1* gene confers
495 adaptation to high altitude in Tibetans by attenuating the maladaptive plastic response of

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496 increased hemoglobin concentration (Beall et al., 2010; Jeong et al., 2018; Simonson et al.,
497 2010; Yi et al., 2010). Similarly, most gene expression changes in the brains of guppies
498 reared in the absence of predators go in the opposite direction of those seen in populations
499 that have evolved without predators (Ghalambor et al. 2015). In both cases, the selective
500 agent (hypoxia in humans or absence of predators in fish) may have not been present in the
501 recent history of the populations exhibiting non-adaptive plasticity. In contrast, we
502 speculate that occasional periods of water stress are probably common in many
503 populations of mice, including in places, like Edmonton, that are not in deserts. Under such
504 situations, selection is expected to favor an adaptive plastic response.

505 While adaptive plasticity may facilitate the colonization of new environments, it can also
506 slow or impede adaptive evolution if, by moving individuals closer to the optimum, genetic
507 variation is shielded from natural selection (Ghalambor et al., 2007; Price et al., 2003).
508 However, when plastic responses to a new environment are incomplete, directional
509 selection may favor a more extreme phenotype and lead to subsequent genetic changes
510 (Price et al. 2003). While all house mice, even those in mesic environments, likely undergo
511 short periods of water stress, water stress is likely to be more severe in desert
512 environments. Phenotypic comparisons between Edmonton and Tucson mice suggest that
513 plastic responses to water stress in Edmonton mice may be suboptimal. Tucson mice drink
514 less water on average and lose less weight in response to dehydration, indicating that these
515 animals are buffered against water stress in a way that Edmonton mice are not. The blood
516 chemistry comparisons reported here also suggest there are differences between Tucson
517 and Edmonton kidney function and homeostasis. Consequently, we suggest that while

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518 phenotypic plasticity likely helped house mice colonize the Sonoran desert, the xeric
519 environment still imposed sufficient selective pressure for subsequent genetic changes.

520 Finally, the comparison of gene expression changes both between treatments and between
521 lines has identified a few genes that may be important in the adaptive response. Expression
522 changes pinpoint a number of interesting candidates, including *Aqp1* and *Apoe*. Future
523 studies aimed at identifying *cis*-regulatory variation at these genes might help to pinpoint
524 causative mutations underlying adaptation to desert environments.

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706 **Data Accessibility Statement**

707 Illumina sequencing data from this study will be submitted to the NCBI BioProject

708 (https://www.ncbi.nlm.nih.gov/bioproject) under accession number xxxxxxx.

709

710 **Author Contributions**

711 This study was designed by all authors. NKJB conducted the experiments, and NKJB and

712 KLM analyzed the data. The paper was written by NKJB and edited by MWN and KLM.

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713 **Figure Legends**

714 **Figure 1.** Relative water consumption in lab-born descendants of wild mice from different
715 environments. (a) Sampling localities of wild-caught mice used to establish inbred lines in
716 this study (map obtained from Google satelites.pro): Edmonton, Canada (EDM), Tucson, AZ
717 (TUC), Gainesville, FL (GAI), Saratoga Springs, NY (SAR), and Manaus, Brazil (MAN). (b)
718 Relative water consumption (g water consumed/ g mouse) in descendants of mice from
719 different localities. Lines indicate comparisons that are significant ($p < 0.05$; Mann-Whitney
720 U tests). Vertical lines denote 1.5 * the interquartile range.
721
722 **Figure 2.** Evolved and plastic phenotypic variation among hydrated and dehydrated mice
723 from desert and non-desert environments. Asterisks denote significant comparisons ($p <$
724 0.05) either between lines (Tucson, Edmonton) or between treatments (hydrated,
725 dehydrated). (a) Tucson mice show higher levels of serum chloride than Edmonton mice,
726 both when hydrated and when dehydrated (p=0.03 for both). (b) Significant differences
727 between hydrated Edmonton and Tucson mice (p=0.02) and between hydrated and
728 dehydrated Edmonton mice (p=0.03) in creatinine. (c) Tucson mice show significantly less
729 weight loss after 72 hours of water restriction (p=0.027). (d) Significant differences in
730 serum BUN between hydrated and dehydrated Tucson mice (p = 0.008) and dehydrated
731 Tucson and Edmonton mice (p = 0.03). (e) Significant differences in total protein between
732 hydrated and dehydrated Tucson mice (p=0.008) and between hydrated and dehydrated
733 Edmonton mice (p=0.01). Vertical lines denote 1.5 * the interquartile range.
734
735 **Figure 3.** Evolved and plastic gene expression variation among hydrated and dehydrated
736 mice from desert and non-desert environments. (a) Heat map depicting relationships
737 among samples for the top 1000 genes with greatest variance in expression. Expression
738 patterns form two major groups, corresponding to line of origin (Tucson versus
739 Edmonton). Edmonton samples also form clusters based on treatment (hydrated versus
740 dehydrated) while Tucson samples do not. (b) Numbers of differentially expressed genes
741 between dehydrated and hydrated samples in Tucson and Edmonton. Edmonton mice
742 exhibit twice as many genes with differential expression between dehydrated and hydrated
743 conditions compared to Tucson mice. The 225 genes at the intersection represent the
744 shared transcriptional response to water stress. (c) Magnitude of fold changes in each
745 population between dehydrated and hydrated samples. Vertical lines denote 1.5 * the
746 interquartile range.
747
748 **Figure 4.** Plastic responses to dehydration in non-desert (Edmonton) mice are mostly in
749 the same direction as evolved differences between non-desert (Edmonton) and desert
750 (Tucson) hydrated mice. (a) Scatterplot comparing the evolved and plastic changes in gene
751 expression. Each point represents a gene and the log2 fold change between Edmonton
752 hydrated vs. dehydrated on the x-axis (plastic response) and Tucson hydrated vs.
753 Edmonton hydrated on the y-axis (evolved divergence). (b) Number of genes in which the
754 evolved and plastic transcriptional responses go in the same direction, and number of
755 genes in which the evolved and plastic transcriptional responses go in the opposite
756 direction.
757

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758 **Figure 5.** Expression variation at two genes that may underlie adaptation to a desert
759 environment. Normalized gene expression in hydrated and dehydrated mice from Tucson
760 (gold) and Edmonton (green). Asterisks denote significant comparisons ($p < 0.05$) either
761 between lines (Tucson, Edmonton) or between treatments (hydrated, dehydrated). (a)
762 *Aqp1*. (b) *Apoe*. For both genes, the dehydrated Edmonton mice recapitulate the baseline
763 expression level seen in hydrated Tucson mice. For *Aqp1*, the plastic response is attenuated
764 in Tucson mice (a), while for *Apoe*, it is not (b). Vertical lines denote 1.5 * the interquartile
765 range.
766

Figure 1 Relative water consumption in lab-born descendants of wild mice from different environments.

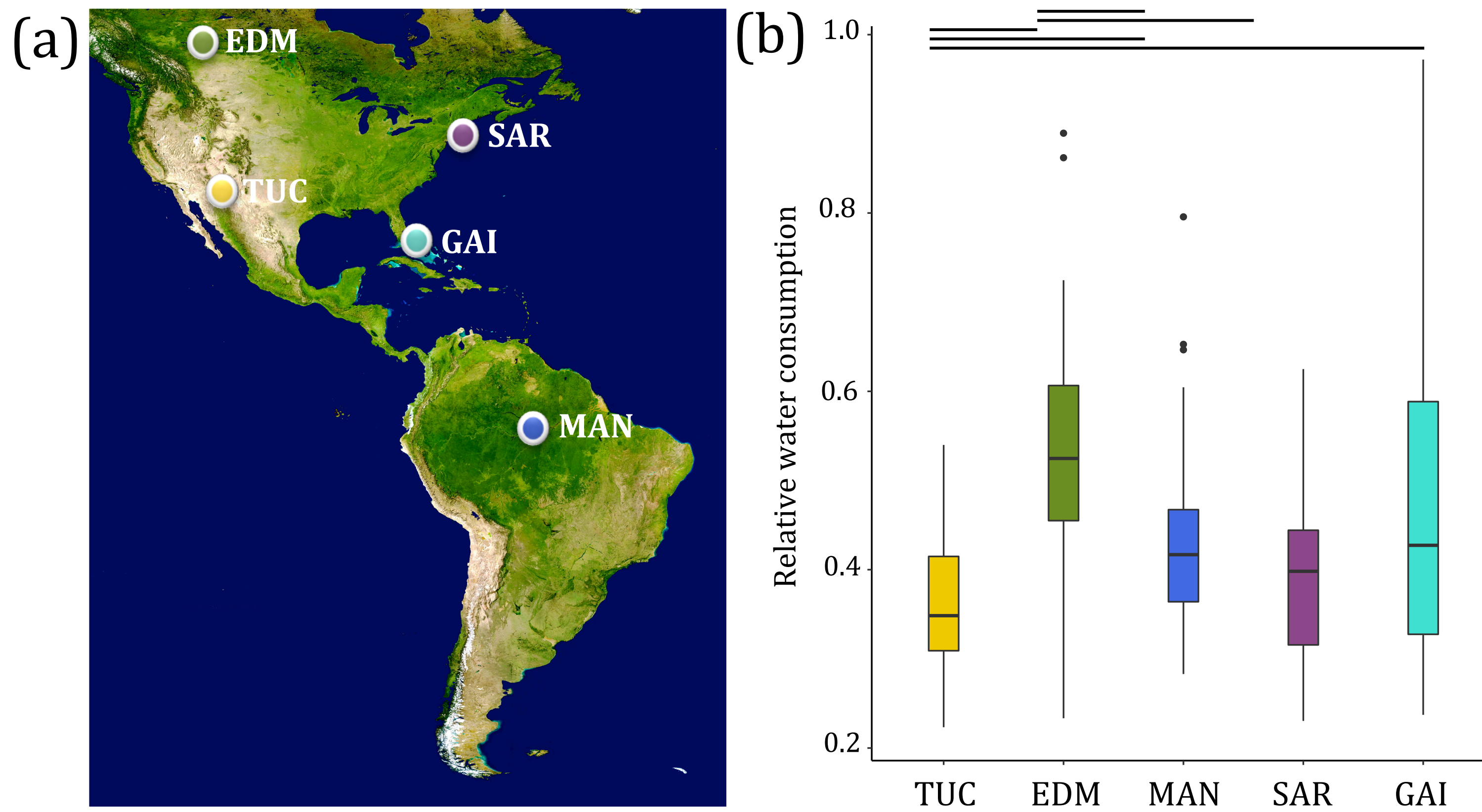

Figure 2 Evolved and plastic phenotypic variation among hydrated and dehydrated mice from desert and non-desert environments.

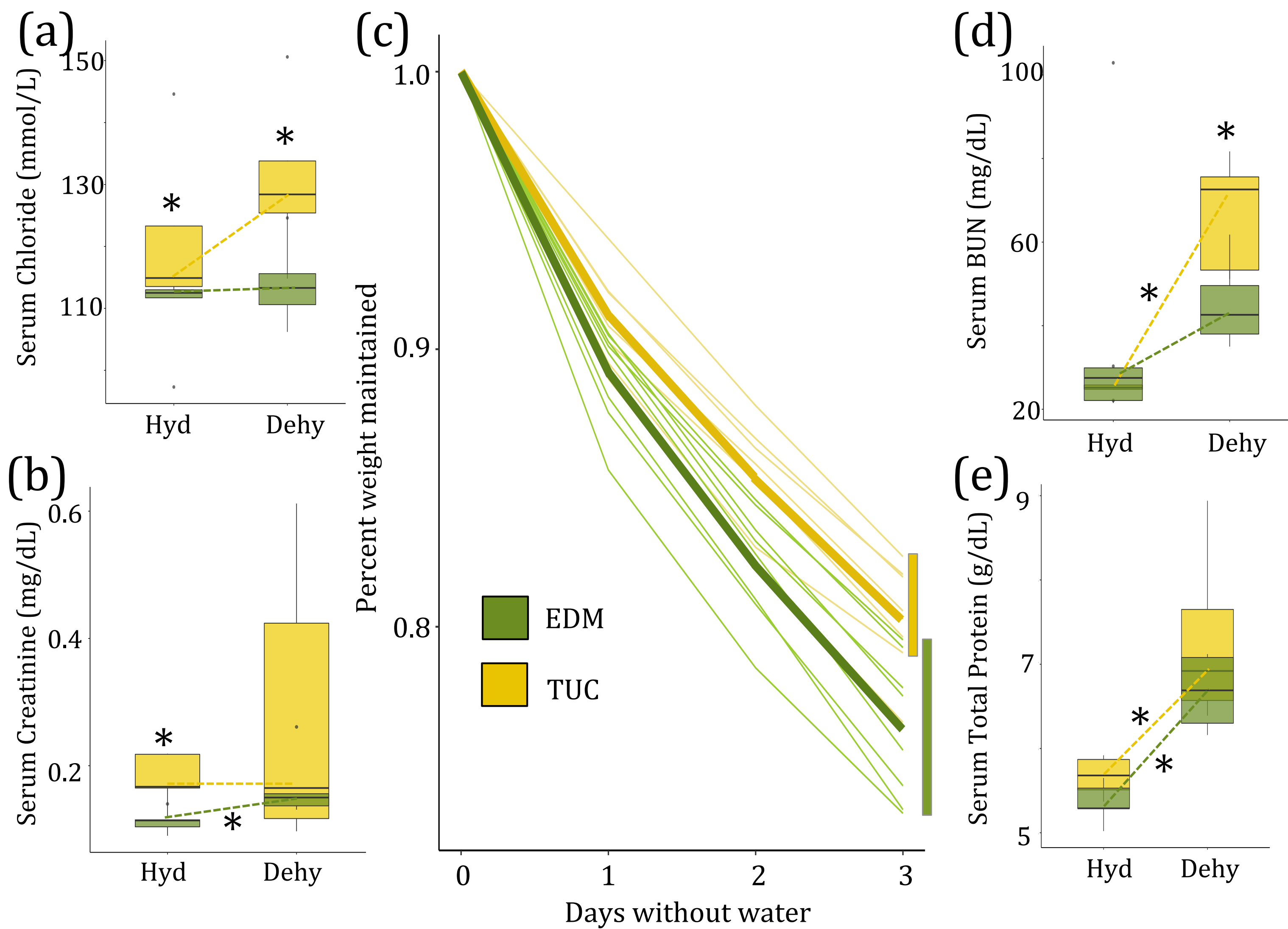

Figure 3 Evolved and plastic gene expression variation among hydrated and dehydrated mice from desert and non-desert environments.

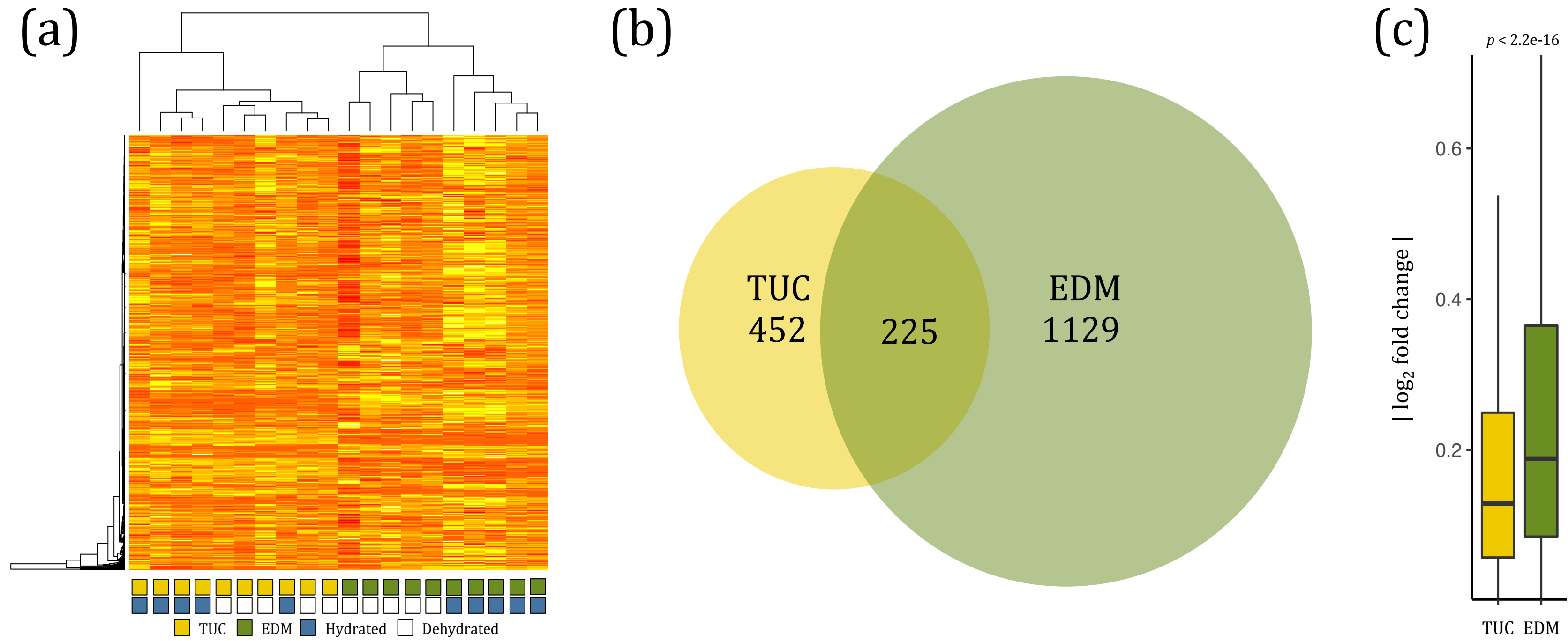

Figure 4 Plastic responses to dehydration in non-desert (Edmonton) mice are mostly in the same direction as evolved differences between non-desert (Edmonton) and desert (Tucson) hydrated mice.

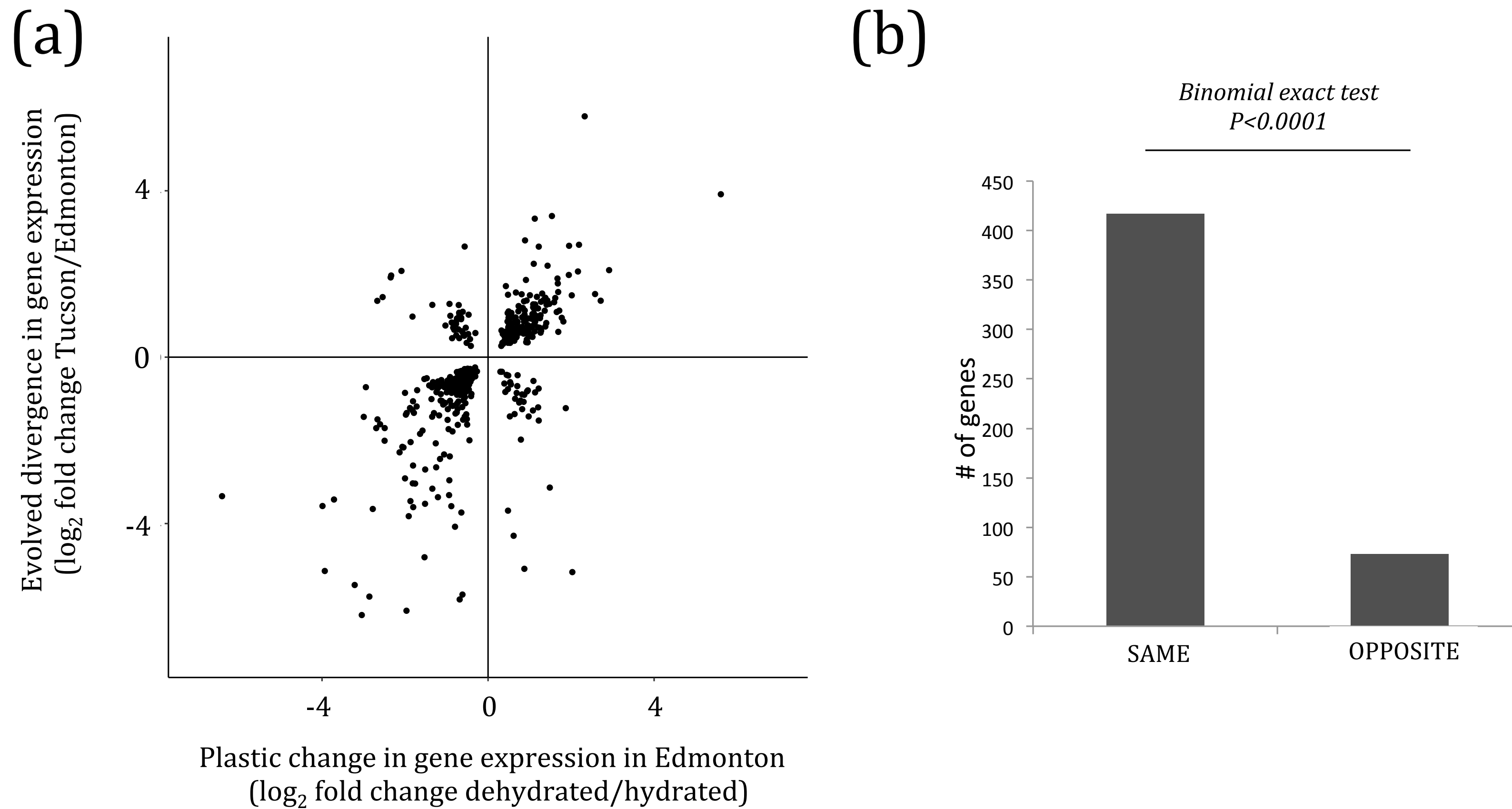

Figure 5 Expression variation at two genes that may underlie adaptation to a desert environment.