Gene Regulatory Divergence Between Locally Adapted Ecotypes in Their Native Habitats

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Key words: *Mimulus*, local adaptation, allele-specific expression, salt tolerance, chromosome inversion

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Running title: Gene Expression Divergence in Nature

ABSTRACT

Local adaptation is a key driver of ecological specialization and the formation of new species. Despite its importance, the evolution of gene regulatory divergence among locally-adapted populations is poorly understood, especially how that divergence manifests in nature. Here, we evaluate gene expression divergence and allele-specific gene expression responses for locally-adapted coastal perennial and inland annual accessions of the yellow monkeyflower, *Mimulus guttatus*, in a field reciprocal transplant experiment. Overall, 6765 (73%) of surveyed genes were differentially expressed between coastal and inland habitats, while 7213 (77%) were differentially expressed between the coastal perennial and inland annual accessions. Further, 18% of transcripts had significant genotype x site (GxE) effects. Habitat-specific differential expression was found for 62% of the GxE transcripts (differential expression in one habitat, but not the other), while only 94 (~5%) GxE transcripts had crossing reaction norms. *Cis*-regulatory variation was pervasive, affecting 79% (5532) of differentially expressed genes. We detected *trans* effects for 52% (3611) of differentially expressed genes. Consistent with the supergene hypothesis of chromosome inversion evolution, a locally adaptive inversion was enriched for *cis*-regulatory divergence. These results provide multiple new insights into the evolution of transcriptome-wide gene regulatory divergence and plasticity among locally adapted populations.

46 **INTRODUCTION**

47 One of the most important forces driving the evolution of ecological specialization and the origin
48 of biodiversity is local adaptation (Clausen 1951; Colosimo et al. 2005; Sobel et al. 2010). Local
49 adaptation is characterized by reciprocal home-site advantage, whereby populations perform best
50 in their home habitat while performing poorly in foreign habitats (Kawecki & Ebert 2004;
51 Hoekstra et al. 2006; Hereford 2009; Wadgymar et al. 2017). Numerous reciprocal transplant
52 experiments have identified locally adapted populations (Leimu & Fisher 2008; Hereford 2009).
53 Recently, reciprocal transplant experiments have been combined with genetic studies to quantify
54 the role of individual loci adaptive divergence between local populations (Verhoeven et al. 2008;
55 Gardner & Latta 2006; Lowry et al. 2009; Lowry & Willis 2010; Anderson et al. 2011, 2013;
56 Leinonen et al. 2013; Ågren et al. 2013; Wadgymar et al. 2017). Despite this advance of
57 integrating transplant experiments with modern genetic techniques, we have a very limited
58 understanding of how gene expression divergence manifests between locally adapted populations
59 in nature.

60

61 One very promising route for understanding how gene expression regulation evolves between
62 locally adaptive populations is to conduct allele specific expression (ASE) analyses in natural
63 populations (Tung et al. 2009; Lovell et al. 2016; Wang et al. 2017). By simultaneously
64 evaluating different causes of gene expression, ASE analyses can determine the relative
65 contributions of variation in nearby *cis* elements versus distant *trans* factors on gene expression
66 divergence (Cowles et al. 2002; Wittkopp et al. 2004). Conducting ASE analyses in conjunction
67 with reciprocal transplant experiments offers the opportunity go beyond partitioning gene
68 expression into *cis* and *trans* effects by establishing how habitat context influences those sources

69 of variation in gene expression. ASE analysis is often conducted in F1 hybrids, where parental
70 alleles are evaluated for significant differences in expression (de Meaux et al. 2005; Guo et al.
71 2008; Zhang & Borevitz 2009; Cubillos et al. 2014; Coolon et al. 2014; Steige et al. 2015, 2017;
72 Lovell et al. 2016; Aguilar-Rangel et al. 2017). ASE relies on there being nucleotide
73 polymorphisms between parental alleles, so that those alleles can be individually counted in the
74 F1 transcript pool. *Cis*-regulatory allelic expression differences are expected to persist regardless
75 of whether alleles are expressed separately in parental genotypes or together in an F1 hybrid. In
76 this way, ASE allows for the identification of *cis* expression Quantitative Trait Loci (eQTL),
77 which can be evaluated for their responses to different environmental conditions (Lovell et al.
78 2016). Further, *trans* effects on gene expression are indicated by a change in allelic effects
79 between the parental generation and the F1 hybrids (Cowles et al. 2002; Wittkopp et al. 2004).
80 Despite its great potential, we are unaware of any field reciprocal transplant experiment that has
81 measured ASE to evaluate how gene expression divergence manifests across the habitats known
82 to be responsible for local adaptation.
83
84 In this study, we evaluated gene regulatory divergence in nature between locally adapted coastal
85 perennial and inland annual population accessions of the yellow monkeyflower, *Mimulus*
86 *guttatus* (*syn. Erythranthe guttata* (Fisch. ex DC.) G.L. Nesom). *M. guttatus* is a model system
87 for understanding the genetic underpinnings of evolutionary adaptations (reviewed in Wu et al.
88 2008; Twyford et al. 2015). Both annual and perennial populations are found throughout much of
89 the range of *M. guttatus* (van Kleunen 2007; Lowry & Willis 2010; Friedman & Willis 2013;
90 Oneal et al. 2014; Twyford et al. 2015). The large differences in morphology and life-history
91 among populations of *M. guttatus* has been shown to be genetically-based through common

92 garden experiments (Vickery 1952; Hall & Willis 2006; Lowry et al. 2008; Lowry & Willis
93 2010). In the inland Mediterranean climates of California's Coast Range and Sierra Foothills,
94 most populations of *M. guttatus* have an early flowering annual life-history, which they have
95 evolved to escape from the hot summer drought endemic to these regions. In contrast, coastal
96 populations of *M. guttatus* are sheltered from the summer drought by pervasive marine fog,
97 which supplies soil moisture and reduces transpiration rates (Corbin et al. 2005). As a result,
98 coastal populations uniformly have a late-flowering perennial life-history (Hall & Willis 2006;
99 Lowry et al. 2008). While coastal habitats are benign in terms of soil water availability, these
100 populations must contend with toxic oceanic salt spray, in response to which they have evolved
101 high salt tolerance (Lowry et al. 2008, 2009; Selby et al. 2014). Across previous reciprocal
102 transplant field experiments, inland plants survived to flower at 23.9 times the rate of coastal
103 plants in inland habitat, while coastal plants have survived at 2.5 times the rate of inland plants in
104 coastal habitat (Hall & Willis 2006; Lowry et al. 2008, 2009; Hall et al. 2010).
105
106 Population structure analysis has repeatedly demonstrated that coastal populations, all of which
107 are perennial, collectively constitute a single ecotype that is genetically distinct from inland
108 populations of the species (Lowry et al. 2008; Twyford & Friedman 2015). The individual genes
109 responsible for the divergence of coastal perennial and inland annual populations are unknown,
110 but it is clear that a large paracentric chromosomal inversion on chromosome 8 is responsible for
111 much of the divergence in traits and fitness between these populations (Lowry & Willis 2010;
112 Friedman 2014; Oneal et al. 2014; Gould et al. 2017). Smaller effect loci across the genome also
113 account for divergence between the coastal perennial and inland annual populations (Hall et al.
114 2006, 2010; Lowry et al. 2009; Friedman et al. 2015). The chromosome 8 inversion is also

115 involved in the divergence of inland annual populations from inland perennial populations
116 (Lowry & Willis 2010). Divergence of inland annual and perennial populations was not a focus
117 of this study.
118
119 The primary goal of our study was to characterize gene regulatory divergence between coastal
120 perennial and inland annual *M. guttatus* in the natural habitats that drove the evolution of local
121 adaptation among these ecotypes. In particular, our study aimed to 1) determine the prevalence
122 of genotype x environment (GxE) interactions affecting the expression of native and non-native
123 alleles across the divergent habitats, 2) characterize the relative contributions of *cis* and/or *trans*
124 regulatory variation in expression divergence, 3) establish the role of genome structure (e.g.
125 inversions) in the evolution of potentially locally adaptive regulatory variation, and 4) identify
126 candidate genes underlying local adaptation in this system. The results of our study provide new
127 insights into the gene regulatory evolution and suggest that coupling allele-specific expression
128 with reciprocal transplant experiments is a promising approach for modern local adaptation
129 research.
130
131 **MATERIALS AND METHODS**
132 ***Germplasm***
133 Original field collections of the seeds used in this experiment were made in the Spring of 2005
134 from the SWB (N39.0359', W123.6904') and LMC (N38.8640', W123.0839') populations
135 located in Mendocino County, California. The SWB and LMC populations have been the subject
136 of extensive research since then (Lowry et al. 2008; Lowry & Willis 2010; Wu et al. 2010;
137 Friedman & Willis 2013; Friedman 2014; Gould et al. 2017). Seeds from these populations were

138 inbred for multiple generations in the Duke University Greenhouses. The plants used for the field
139 experiment included the following: 1) An inbred (7 generations self-fertilized) inland line, LMC-
140 L1-2, henceforth referred to as LL; 2) An inbred (13 generation self-fertilized) coastal line,
141 SWB-S1-2, henceforth referred to as SS; and 3) An F1 hybrid between the two inbred lines,
142 henceforth referred to as F1 or SL. The F1s used in this experiment were made in the Michigan
143 State University (MSU) Growth Chamber Facilities through a cross between the two parental
144 inbred lines.
145
146 ***Field Reciprocal Transplant Experiment***
147 We conducted a field reciprocal transplant experiment in tandem with allele-specific expression
148 analyses to understand the causes (*cis*, *trans*) of gene expression divergence between locally
149 adapted coastal perennial and inland annual populations of *M. guttatus* (Fig. 1). The reciprocal
150 transplant experiment was conducted in 2016 using one coastal (Bodega Marine Reserve;
151 N38.3157', W123.0687') and one inland (Pepperwood Preserve; N38.5755', W122.7008') field
152 site located in Sonoma County, CA. SS, LL, and F1 seeds were originally sown in Sun Gro
153 Metromix 838 soil on February 3. The seeds were then stratified in the dark at 4°C until
154 February 9, when they were removed from the cold and germinated in the UC Berkeley Oxford
155 Track Greenhouses. Seedlings were moved to the Bodega Marine Reserve Greenhouses on
156 February 21 for acclimation. On February 22, the seedlings were transferred to native soil
157 collected from the field sites. Seedlings were planted in the field at Bodega on March 7 and at
158 Pepperwood on March 8. ~90 plants per type (SS, LL, F1) were planted at each field site. Plants
159 were evenly distributed among three blocks per field site and randomized within block. Tissue
160 for RNA sequencing was collected from Bodega on April 23 and Pepperwood on April 24. To

161 avoid effects of circadian rhythm on gene expression, all tissue was collected between 12:00pm
162 and 1:00pm. Both collection days had mostly clear skies, with mean local temperatures during
163 collection periods of 14° C at Bodega and 17° C at Pepperwood. Plants had progressed to
164 different developmental stages at the time of collection, with some plants approaching flowering
165 and others not. To best standardize tissue collection across genotypes, only the two youngest
166 pairs of fully expanded leaves were collected from each plant. Tissue was immediately flash
167 frozen on liquid nitrogen in the field. Samples were then shipped on dry ice to Michigan State
168 University for RNA extractions.
169
170 ***RNA-sequencing***
171 Total RNA was extracted from 80 leaf tissue samples with the Spectrum Plant Total RNA Kit
172 (Sigma-Aldrich, St. Louis, MO, USA). RNA was extracted from 12 replicates of both SS and LL
173 parental lines from the coastal field site and 13 replicates of both parental lines from the inland
174 field site. RNA from 15 F1 hybrids from each site was also extracted. Total RNA was submitted
175 for library preparation and sequencing at the MSU Genome Core
176 (https://rtsf.natsci.msu.edu/genomics/). RNA-Seq libraries were prepared using the Illumina
177 TruSeq Stranded mRNA Library Preparation Kit with a Perkin Elmer (Waltham, MA) Sciclone
178 NGS robot. Completed libraries were quality controlled and quantified using a combination of
179 Qubit dsDNA HS (Thermo Fisher Scientific, Waltham, MA), Caliper LabChipGX HS DNA
180 (Perkin Elmer, Waltham, MA), and Kapa Biosystems Illumina Library Quantification qPCR
181 (Wilmington, MA) assay kits. All libraries were barcoded and library concentrations were
182 normalized and pooled in equimolar amounts: 8 pools each consisting of 10 randomly chosen
183 libraries were made. Each of these pools was loaded on one lane of an Illumina HiSeq 2500 High

184 Output flow cell (v4) and sequenced in the 1x100bp (SE100) format using HiSeq SBS reagents
185 (also v4). Base calling was done by Illumina Real Time Analysis (RTA) v1.18.64 and output of
186 RTA was demultiplexed and converted to Fastq format with Illumina Bcl2fastq v1.8.4.
187
188 ***Reference Transcriptome Assembly***
189 Alignment of RNA-seq to a single reference genome can lead to major biases in ASE studies
190 (Stevenson et al. 2013). To overcome reference bias, we assembled reference transcriptomes for
191 the coastal perennial (SS) and inland annual (LL) parents. We chose this method over
192 construction of a pseudo-transcriptome reference (Shen et al. 2013) to avoid generating bias
193 caused by the reference genome and transcriptome for *M. guttatus*, which was assembled from
194 an inland annual line (IM62; Hellsten et al. 2013). Reference bias is of particular concern in *M.*
195 *guttatus* because pairwise polymorphism is generally high (π_S = 0.033-0.065; Brandvain et al.
196 2014; Gould et al. 2017; Puzey et al. 2017). To generate a reference transcriptome assembly that
197 contained allele-specific sequences for each parental genotype, we sequenced and conducted *de*
198 *novo* assembly using RNA-sequencing. Plants of each of the coastal (SS) and inland (LL) lines
199 were grown in the MSU greenhouses until both genotypes were flowering. Floral bud, leaf, and
200 root tissue were collected from each of the SS and LL lines, flash frozen with liquid nitrogen,
201 and stored at -80° C. Total RNA was extracted from each sample using the Spectrum Plant Total
202 RNA Kit and submitted to the MSU Genome Core for library construction and sequencing.
203 Strand-specific Tru-Seq Illumina RNA-seq libraries were prepared and sequenced along with
204 four libraries constructed from field collected leaf tissue from the reciprocal transplant
205 experiment (one sample per parental line per field site). These libraries were included in the
206 transcriptome assembly pipeline to capture sequences from genes that may only be expressed

207 under field conditions. Completed libraries were quality controlled and quantified using a
208 combination of Qubit dsDNA HS, Caliper LabChipGX HS DNA, and Kapa Biosystems Illumina
209 Library Quantification qPCR assay kits. A total of 10 libraries were barcoded with dual index
210 barcodes and pooled together. The entire pool was sequenced using 125 bp paired-end reads on a
211 single lane of the Illumina GA 2500 platform.
212
213 Raw output reads were quality checked using FastQC v.0.11.3 (Andrews 2015). Residual adapter
214 sequences were removed and reads were quality trimmed using Trimmomatic v.0.33 (Bolger et
215 al. 2014), discarding sequences less than 50 bp in length. Paired and un-paired reads from each
216 ecotype were combined and assembled into two separate *de novo* (non-reference guided)
217 assemblies using Trinity v20140413p1 (Grabherr et al. 2011) with *in silico* read normalization.
218 To check the quality of our two transcriptomes, we compared our *de novo* assemblies to the set
219 of primary transcripts from the IM62 reference transcriptome using Trinity utility scripts. We
220 measured how much of each *Mimulus* primary transcript was reconstructed in the two
221 assemblies. We found that 74.3% of *Mimulus* primary transcripts were reconstructed (to at least
222 50% of their full length) in the inland parent (L) transcriptome. In the coastal parent
223 transcriptome, 74.1% of *Mimulus* primary transcripts were reconstructed (to at least 50% of their
224 full length). Thus, the two assembled transcriptomes were of similar quality and completeness.
225 We aligned the filtered input reads back to the finished assemblies using Bowtie2 (Langmead et
226 al. 2012). A total of 98.98% and 99.26% of input reads aligned back to the inland (L) and coastal
227 (S) assemblies, respectively. We identified possible non-plant contaminant sequences in each
228 assembly through comparison to the NCBI nt database using blastn. We eliminated
229 transcriptome sequences with top blast sequence matches (e-value cutoff of 1e-20) to non-plant

230 organisms (*N* = 16,243 and *N* = 1,222 eliminated sequences for the inland and coastal
231 transcriptomes, respectively).
232
233 To construct a combined reference transcriptome that contained pairs of allelic sequences from
234 both lines, we used a reciprocal best blast strategy. Each transcriptome was compared against the
235 other (blastn) at an e-value cutoff of 1e-5 and reciprocal best hits were returned using a custom
236 Python (v. 2.7.2) script (https://github.com/lowrylab/Mimulus_Gene_Expression). We tested a
237 range of e-value cutoffs in this analysis and found that the choice of e-value between 1e-5 and
238 1e-50 had little effect on the total output number of best blast pairs (see Fig. S1). We considered
239 two sequences best blast hits if total high-scoring pair (HSP) coverage was 75% or greater
240 between sequences. We included only aligned sequence regions of the longest HSP in the
241 combined transcriptome.
242
243 To match transcripts in the combined *de novo* reference transcriptome with their corresponding
244 *Mimulus* genes, we compared *de novo* assembled alleles against the reference (IM62) set of
245 primary transcripts (blastn) at an e-value cut-off of 1e-5. In rare cases, where two alleles from a
246 *de novo* assembled transcript matched different *Mimulus* genes, the gene with the lowest e-value
247 alignment was chosen as a match for the allele pair. Note that with this annotation method, each
248 *de novo* assembled transcript has only one matching gene and thus, can be unambiguously
249 assigned a specific location in the genome. However, multiple transcripts can be assigned to the
250 same genomic location, which is typical of isoforms stemming from the same gene.
251
252 ***Differential Expression Analyses***

253 Raw reads from the 80 field RNA-seq libraries were de-multiplexed, quality filtered, and
254 trimmed using FastQC and Trimmomatic, as described above for the transcriptome assembly.
255 Filtered reads were aligned to the *de novo* combined reference transcriptome (described above)
256 using the un-gapped aligner Bowtie v1.0.0 (Langmead et al. 2009). We outputted only unique
257 alignments with zero mismatches. In this way, we analyzed only read count data for reads that
258 aligned uniquely and perfectly to only one parental allele in the combined reference
259 transcriptome. Only reads that overlap one or more polymorphisms between the parental alleles
260 provide useful information on differential expression between alleles. Approximately 52% of
261 reads per individual had one or more than one alignment to the transcriptome. Unaligned reads
262 fall mainly in areas that were not reconstructed in the reference transcriptomes. On average
263 28.6% of library reads per individual aligned uniquely to only one allele and were thus,
264 informative for allele-specific expression (range 23.7 – 30.7%). For confirmation, some
265 alignments were visually checked using IGV v.2.3.65 (Thorvaldsdóttir et al. 2013).
266
267 Read alignments to each transcript were quantified using a custom Python (v.2.7.2) script. Using
268 the raw count data, we identified allele pairs in the transcriptome for which there was a high
269 probability of residual heterozygosity in one of the parental inbred lines. Potential regions of
270 residual heterozygosity were indicated when a significant number of reads from a homozygous
271 parent aligned to the opposite parental allele in the transcriptome. We eliminated from further
272 analysis transcripts where the average number of reads aligning to the correct parental allele was
273 less than 5 times the average number of reads aligning to the alternate allele (N = 2255
274 eliminated transcripts). For example, we eliminated transcripts where the SS parental line did not
275 have at least 5 times more reads aligning to the S allele than the L allele in the reference

276 transcriptome. We also used the initial count data to test for mapping bias (Fig. S2). For both
277 field sites, mapping bias was present but well controlled. On average there were 1.9% more reads
278 aligned to the S allele than the L allele for F1 plants (Fig. S3). There was a small but statistically
279 significant relationship between transcript nucleotide divergence and differential expression
280 (equivalent to 0.001 increase in the absolute value of LFC between genotypes, per SNP). Count
281 data was normalized for library size using Trimmed Mean of M-values (TMM) normalization to
282 counts per million, which reduces biases generated by the presence of very highly and lowly
283 expressed transcripts (Robinson et al. 2010). We eliminated any transcript without at least one
284 library containing >= 10 counts per million reads. The remaining sequences constitute the
285 'expressed' set of transcripts (N = 10,122). The mean expressed library size for samples from the
286 coast and inland sites was not significantly different (t-tests: all libraries, P = 0.60; parental
287 libraries only, P = 0.22, Fig. S4, and is thus, unlikely to strongly affect statistical comparisons
288 between the two sites. Only 2 transcripts were expressed at only one site and only 7 transcripts
289 were expressed by only one parental line.
290
291 We analyzed the expressed transcript data using the limma (Ritchie et al. 2015) and edgeR
292 (Robinson et al. 2010) packages in R v.3.2.1 (R. Core Team 2012). Variance stabilizing
293 normalization of the data was performed using the voom (Law et al. 2014) function in limma,
294 with a design matrix corresponding to the particular linear model being used for analysis. Model
295 design matrices were coded using the default treatment-contrast parameterization scheme (see
296 Smyth et al. (2015), section 9.5.4) and the significance of model effects were calculated using
297 voom precision weights and the eBayes function. Model effects were judged significant if they

298 fell below a Benjamini-Hoechberg false-discovery rate corrected *P*-value of 0.05 using the
299 decideTests function.
300
301 We used several different linear models to analyze the count data for expressed transcripts,
302 modified from the method of Lovell et al. (2016). For this approach, it is important that the size
303 of the L and S sub-transcriptome in each F1 is approximately equal to produce unbiased results,
304 which we verified independently (Fig. S3). Each linear model had one or more fixed factors:
305 allele (L/S), environment (coastal field site vs. inland field site), generation (F0/F1), and
306 interaction terms. We analyzed the parental plants (F0 generation) separately, the F1 plants
307 separately, and both generations together in different models. We avoided analyzing models with
308 more than one categorical factor, except for the purposes of measuring interaction effects
309 because effect significance testing for crossing categorical factors only captures differences
310 present in the first level of each factor.
311
312 For data from the F0 plants, we ran models examining the allele (homozygous genotype) effect
313 within each environment separately. We also tested for environmental effects separately within
314 each parental genotype. We used a model with both genotype and environment to test the
315 significance of the genotype x environment interactions. Because the inland site had one extra
316 plant per genotype compared with the coastal site, we analyzed data from 12 randomly chosen
317 parental lines of each genotype to equalize the power to detect effects between sites. A *post-hoc*
318 power analysis was conducted using the R package *RnaSeqSampleSize* (Bi & Liu 2016).
319

320 We used data from all plants together (both F0 and F1) to test for *cis* and *trans* regulation. *Cis*-
321 regulatory allelic expression differences are expected to persist regardless of whether alleles are
322 expressed separately in parental genotypes or together in an F1 hybrid. However, in the F1
323 genotype, *trans* acting factors from both parental genotypes will influence the expression of an
324 allele, so *trans* regulation is indicated if allelic patterns of differential expression are different in
325 the parents vs. the F1 plants. In our model, a significant effect of allele is indicative of a *cis*-
326 regulatory difference in expression. Within each environment, we tested a model with the
327 following factors: allele (genotype), generation (F0/F1), and the interaction term. Any transcript
328 with any *cis* regulation had a significant allele effect (measured in the F1 generation only). Any
329 transcript with only *trans* regulation had a significant difference between generations, but a non-
330 significant allele effect in the F1 generation. Any transcript with both *cis* and *trans* regulation
331 had a significant generational effect and a significant allele effect in the F1.
332
333 To gauge the significance of *cis* x environment interactions, we tested the interaction term of a
334 model using only F1 data and the factors allele, environment, and interaction. To gauge the
335 significance of trans x environment interactions, we modeled all the data (F0 and F1) and
336 measured the significance of a three-way interaction term: allele x generation x environment.
337
338 ***Gene Ontology Enrichment and Expression Networks***
339 We tested for enrichment of gene-ontology (GO) terms among subsets of genes with particular
340 expression patterns (significant model effects). To annotate GO terms, all IM62 reference
341 *Mimulus* proteins were paired with their best *Arabidopsis* protein matches (blastp) at an e-value
342 cutoff of 10^-3. The set of expressed transcripts in our study was matched to the IM62 reference

343 genome genes, as described above, and corresponding GO terms were matched to the
344 *Arabidopsis* BLAST hits. Subsequent GO enrichment tests were done using the R
345 BIOCONDUCTOR package topGO (Alexa & Rahnenführer 2015). We used the Bioconductor
346 database 'org.At.tair.db' for annotation and the algorithm 'classic' (statistic = 'fisher') for
347 statistical tests. Because *P*-value distributions of GO enrichment tests do not typically conform to
348 a distribution amenable to Benjamini-Hoechberg FDR control, and because such control methods
349 can be overly conservative causing loss of valuable functional information (Alexa &
350 Rahnenführer 2015), we report GO terms with uncorrected enrichment *P*-values <= 0.01 (Table
351 S1). Enrichment tests were conducted at the level of genes rather than transcripts. For each gene
352 in the IM62 reference transcriptome, we determined the model effects for all matching expressed
353 transcripts in our data set. A model effect for a gene was considered significant if at least one
354 matching transcript had a significant effect. GO enrichment was then tested among all expressed
355 genes ($N = 9326$) with a significant effect vs. all expressed genes without a significant effect.
356 Enrichments for GxE effects were measured against all genes where either environmental or
357 allelic DE could be detected. Reaction norm plots were generated using the LSMean expression
358 level of all transcripts for each gene in each environment.
359
360 We conducted weighted gene co-expression network analysis within each parental genotype
361 using the R package WGCNA (Langfelder et al. 2008). The analysis identifies genes with
362 correlated patterns of expression across individuals (eigengenes), finding gene sets that
363 putatively form molecular networks (or modules). We conducted WGCNA separately for each
364 parental genotype and then identified which networks in each parent had expression that was
365 significantly different between field sites. Gene expression was quantified as the expression level

366 of the longest matching transcript for each gene. For all analyses, we calculated dissimilarity of
367 expression using a soft thresholding power of 6 and used the dynamicTreeCut function to define
368 modules with a minimum number of 30 genes. We calculated the correlation of each cluster
369 (eigengene) with habitat (Fig. S5). Gene ontology term enrichment was calculated for all
370 modules with a correlation value of $r > 0.75$ (Table S2).
371
372 ***Chromosomal Inversion Analyses***
373 Two inverted regions of the genome, one on chromosome 5 and one on chromosome 8, have
374 been linked to adaptive differences between inland and coastal ecotypes in previous studies
375 (Lowry & Willis 2010; Oneal et al. 2014; Twyford & Friedman 2015; Gould et al. 2017). When
376 examining genes in putatively inverted regions of the genome, we included reference genome
377 v1.1 scaffolds that are inferred to be in the inverted regions from a previous linkage mapping
378 study (Holeski et al. 2014). For the inversion on chromosome 5, we included v1.1 scaffolds 36,
379 149, 158, 170, 197, 266, 281, 288, 327, and 368. For the inversion on chromosome 8, we
380 included v1.1 scaffolds 11, 59, 76, 155 233, 604, and 1093. We tested whether genes in these
381 regions of the genome are enriched for any model effects. Enrichment tests of model effects
382 among annotated inversion and non-inversion genes were carried out using Fisher's Exact tests.
383 The magnitude of model effects among genes with significant effects were compared using
384 Wilcox rank sum tests in R. It should be noted that the presence of alternative orientations of
385 chromosome 8 inversion in the SS and LL parental lines has been confirmed by previous crosses
386 (Lowry & Willis 2010) while the chromosome 5 inversion was originally identified in a cross
387 between different inland and coastal lines (Holeski et al. 2014). High differentiation between the

388 SS and LL parental lines in both inversion regions has been demonstrated by an allele frequency
389 outlier analysis (Gould et al. 2017).
390
391 ***Patterns of Candidate Gene Expression***
392 In a study of species-wide genomic variation, Gould et al. (2017) identified a set of 667
393 candidate adaptation genes. These genes had unusually high differentiation in allele frequencies
394 between a set of 47 coastal and 50 inland *Mimulus* populations. High differentiation was detected
395 in either the genic or 1 kb upstream promoter regions of the genes, or both. We tested for
396 enrichment of differential expression patterns among the expressed candidate genes in the
397 current study. Enrichment was tested against all expressed non-candidate genes using Fisher's
398 Exact tests. The magnitude of model effects among genes with significant effects were compared
399 using Wilcox rank sum tests in R.
400
401 **RESULTS**
402
403 ***Reference Transcriptome Assembly***
404 From the tissues of the two *M. guttatus* parental lines we were able to reconstruct both parental
405 alleles for 25,893 transcripts, of which 708 pairs were removed because they contained no
406 polymorphism between lines. Eighty-two percent of transcripts could be matched back to
407 previously annotated *M. guttatus* genes (www.phytozome.org). Our transcriptome reconstructed
408 54.0% of *M. guttatus* primary transcripts at 50% coverage or greater (Fig S5). On average, there
409 were 2.8 polymorphisms (SNPs or indels) per 100 bp for each pair of alleles in the reference
410 transcriptome (Fig. S6C).

411

412 ***Differential Expression Analyses***

413 RNA-sequencing of plants from the field experiments produced approximately 25.9 million raw
414 reads per library (range 9.2-35.9 million reads). We were able to evaluate gene expression for
415 10,122 transcripts corresponding to 9,326 genes, after transcriptome reconstruction and filtering.
416 Significant expression differences (FDR = 0.05) between the inland and coastal field sites were
417 common (6765 genes; 73% of genes). Expression differences between parental genotypes within
418 field sites were also common (7213 genes; 77%; Table 1 and 2). Most transcripts that were
419 differentially expressed between parental lines were also sensitive to environment: only 7.2% of
420 transcripts were consistently differentially expressed between lines regardless of field site. We
421 found that 54% more genes were differentially expressed between parental lines at the inland site
422 than at the coastal site (Table 2). We can attribute only part of this discrepancy to differences in
423 power at the two sites. Average power to detect DE genes was only slightly higher at the inland
424 site than the coastal site (75% vs. 68% for a minimum fold-change of 2X).

425

426 Genotype x site (GxE) interactions were relatively common. In total, 1837 transcripts
427 (corresponding to 19% of expressed genes, Table 1, Table S3) had significant GxE interactions,
428 where the magnitude of expression differences between parental ecotypes depended on the field
429 site in which they were grown (Fig. 2A). Only 94 transcripts had crossing reaction norms (green
430 points, Fig. 2B). More often, expression differences between genotypes were significant at one
431 site and non-significant at the other (1137 transcripts, 11%, blue and red points, Fig. 2).

432

433 Most of the expression differences between the coastal and inland lines could be attributed to *cis*-
434 regulatory variation: 79% (5532 genes) of differentially expressed genes had *cis* regulatory
435 differences, while about half (3611 genes) of the differentially expressed genes had detectable
436 *trans* effects (Fig. 3 & 4, Table 1). At both coastal and inland field sites, the magnitudes of *cis*
437 and *trans* regulatory differences were similar (Table S4). However, we detected far more
438 significant *trans* effects in the inland field site than the coastal field site (Fig. 3 & 4, Table S4).
439 Overall, there was only moderate overlap between the sets of *cis*-regulated transcripts from the
440 two environments (Fig. S7. The same was true for the sets of *trans*-regulated transcripts from the
441 two environments.
442
443 ***Gene Ontology Enrichment and Expression Networks***
444 We explored the functional significance of genes with differential expression between
445 environments and between parental ecotypes. Differentially expressed genes in the coastal (SS)
446 parent between sites were enriched for glycosinolate/glucosinolate/S-glycoside biosynthesis and
447 metabolism, response to starvation, and others (Table S1). Differentially expressed genes in the
448 inland parent (LL) between sites were enriched for GO categories related to cuticle development,
449 meristem and floral development, and pigment production. Differentially expressed genes
450 between the parental lines (across sites) were enriched for genes involved with cell wall
451 formation, meristem initiation, and responses to viral pathogens. Genes with genotype x site
452 (GxE) interactions had the most significantly enriched GO terms ($N = 69$), including
453 brassinosteroid biosynthesis/metabolism, cell wall formation, regulation of development, and
454 pigment accumulation.
455

456 We used network clustering analysis to examine correlated expression of gene clusters (modules)
457 within habitats for the coastal and inland lines (Pearson's correlation, $r > 0.75$, Fig. S5. The
458 coastal perennial line (SS) preferentially expressed modules related to root and epidermal
459 development at its home site, while oxidative stress response, chlorophyll metabolism, and
460 circadian rhythm modules were highly expressed in inland habitat (Table S2). The inland annual
461 line preferentially expressed gene clusters related to nucleoside catabolism, sugar and starch
462 metabolism, pigment accumulation, and photoperiodism at the inland field site, but had no
463 modules strongly associated with the coastal field site.
464
465 ***Chromosomal Inversion Analyses***
466 Chromosomal inversions are thought to act as adaptation supergenes that hold together long
467 haplotypes containing multiple adaptive polymorphisms through suppressed recombination
468 (Kirkpatrick 2010; Joron et al. 2011; Thompson & Jiggins 2014). We were thus interested in
469 evaluating the hypothesis of whether the known inversions in this system hold together
470 haplotypes of differentially expressed genes. There were significantly more differentially
471 expressed genes inside the chromosome 5 inversion than expected by chance and there were
472 significantly more GxE interactions for genes in this inversion (Table 1). The adaptive (Lowry &
473 Willis 2010) chromosome 8 inversion also had significantly more differentially expressed genes,
474 but only at the coastal site (Table 2). Interestingly, the chromosome 8 inversion had a significant
475 enrichment of *cis*-eQTLs (Table 1), suggesting that it may maintain a higher level of regulatory
476 divergence caused by the evolution of *cis*-regulatory elements.
477
478 ***Patterns of Candidate Gene Expression***

479 To identify genes putatively linked to local adaptation, we cross-referenced our results with a
480 recent genomic outlier analysis, which identified 667 genes with a high level of allelic
481 differentiation between coastal and inland populations (Lowry et al. 2010). We found that the
482 magnitude of differential expression between environments was significantly elevated for
483 candidate genes with highly differentiated SNPs (from Gould et al. 2017) in their promoter
484 regions *(P* < 0.05, Table S5). The magnitude of differential expression between genotypes for
485 candidate genes with differentiated promoters was also higher than for non-candidate genes, but
486 not significantly so *(P* = 0.08). Candidate genes with differentiated gene regions tended to have
487 expression differences between lines or between environments that were lower in magnitude than
488 non-candidate genes.
489
490 We were able to examine gene expression in seven top candidate genes highlighted by the outlier
491 analysis and/or previous QTL mapping studies (Fig. 5). The candidate gene *ABA1* codes for the
492 first protein in the biosynthesis of the hormone abscisic acid, which controls responses to
493 osmotic stress, including drought (Xiong et al. 2002). An *ABA1* (abscisic acid deficient 1)
494 ortholog, Migut.H00431, is located within the chromosome 8 inversion and may contribute to
495 inland ecotype's adaptation to low soil water availability. *ABA1* was always more highly
496 expressed in the inland line and was upregulated in both lines at the inland field site (Fig. 5A).
497 Previously, we showed that coastal *M. guttatus* populations have evolved leaf tissue tolerance to
498 oceanic salt spray (Lowry et al. 2009). Leaf salt tolerance is generally mediated by a Na^+/H^+
499 antiporter (*SOS1*; Qiu et al. 2002), which is potentially activated by a calcium sensor (*CBL10*;
500 Kim et al. 2007). Both *SOS1* (Migut.E00570) and *CBL10* (Migut.A00138) were more highly

501 expressed in the coastal line than the inland line across habitats (Fig. 5B, C), a pattern consistent
502 with elevated salt tolerance of coastal populations.
503
504 **DISCUSSION**
505 Overall, our results identified multiple key patterns regarding the relationship between gene
506 expression and the evolution of local adaptation. Differential expression is substantial between
507 locally-adapted accessions in nature. Expression plasticity of alleles across habitats (genotype x
508 environment interactions) appear to be common and may minimize fitness trade-offs at
509 individual loci that contribute to the overall pattern of local adaptation. A large portion of that
510 differential expression is due to *cis*-regulatory divergence. Chromosomal inversions appear to act
511 as supergenes by holding together haplotypes of differentially expressed genes, but this pattern
512 depends on habitat context. We discuss each of these major findings as well as the limitations
513 and caveats associated with our study in detail below.
514
515 ***Sources of Variation in Gene Expression***
516 One of the major outstanding questions in local adaptation research is why the fitness effects of
517 individual loci on local adaptation are so often asymmetric across habitats. Theoretical models
518 (Levene 1953; Hedrick 1976, 1986; Dieckmann & Doebeli 1999) and studies in key model
519 systems (Abzhanov et al. 2004; Hoekstra et al. 2006; Barrett et al. 2009) have suggested trade-
520 offs at individual loci (e.g. antagonistic pleiotropy) to be common. However, field reciprocal
521 transplant experiments and experimental evolution studies have often found a pattern of
522 conditional neutrality, where loci have a strong effect on fitness in one environment but have an
523 undetectable effect on fitness in alternative environments (reviewed in Bono et al. 2017;

524 Wadgymar et al. 2017). This pattern has also been found in the coastal perennial/inland annual
525 *M. guttatus* system, where three salt tolerance QTLs have fitness effects in coastal habitat but no
526 detectable effects in inland habitat (Lowry et al. 2009). While a failure to statistically detect a
527 trade-off across habitats does not rule out the possibility of a small trade-off for an individual
528 locus, it does suggest that adaptive loci are often strongly asymmetric in their fitness effects
529 across habitats.
530
531 Gene regulatory divergence between locally adapted populations is one possible mechanism by
532 which trade-offs could be minimized across habitats for individual loci (Rutherford 2003;
533 Ghalambor et al. 2007; Des Marais et al. 2013, 2017; Dayan et al. 2015; Lohman et al. 2017).
534 While some gene regulatory differences between ecotypes may evolve due to genetic drift in
535 isolated populations, adaptive phenotypic plasticity can evolve due to natural selection.
536 Adaptations of organisms to maintain homeostasis when faced by environmental challenges can
537 be caused by genotype specific changes in gene expression (Gibson 2008; Des Marais et al.
538 2017). However, such environmentally dependent changes in gene expression would not be
539 expected to result in a cost if gene expression did not differ among genotypes in habitats that
540 lacked particular environmental stresses. In our study, we identified 1137 transcripts that were
541 differentially expressed between the coastal perennial and inland annual lines in one habitat
542 (coast or inland) but did not show the same expression difference in the alternative habitat (GxE
543 transcripts, Fig. 2). In contrast, fewer transcripts (733) had the same expression difference
544 between ecotypes regardless of environment (G-only transcripts). For the current experiment, we
545 cannot definitively determine whether any of the GxE or G-only transcripts cause asymmetries in
546 fitness effects across habitats. Further, some of the genotype specific changes in gene expression

547 could potentially be maladaptive (Ghalambor et al. 2007). Even so, our results do clearly show
548 that habitat-specific differential gene expression is common for locally adapted ecotypes. Future
549 studies should focus on making the critical linkages between patterns of gene expression and
550 fitness in the field to determine whether asymmetries of fitness effects across habitats are the
551 result of habitat-specific differential gene expression.
552
553 Overall, *cis*-regulatory evolution was responsible for at least 79% of the differentially expressed
554 genes. This is about twice the percentage of *cis*-regulated differentially expressed genes for
555 crosses both within and between *Drosophilia* species (Coolon et al. 2014). However, the
556 relatively high proportion of *cis*-regulatory divergence underlying differentially expressed genes
557 is not too surprising, given the high level of polymorphism among *M. guttatus* accessions
558 (Brandvain et al. 2014; Gould et al. 2017; Puzey et al. 2017). Further, in terms of the proportion
559 of *cis* regulated genes out of all genes surveyed (59% for our study), our results are not very
560 different from other study systems. Mack et al. (2016) found significant *cis* effects for 68% of
561 surveyed genes in crosses between house mice subspecies (*Mus musculus musculus* and *M. m.*
562 *domesticus*). Steige et al. (2015) found that 33-39% of genes surveyed had significant allele-
563 specific expression effects for hybrids between accessions of the plant *Capsella grandiflora*.
564
565 One striking pattern we observed in this study was a great deal more differential regulation
566 between ecotypes in the inland environment than the coastal environment (Table 2, Fig.3). We
567 can attribute only a small part of this difference to greater experimental power to detect
568 differences at the inland site (75% inland vs. 68% coast, for transcripts with a minimum fold-
569 change of 2X). Because mapping bias is well controlled in this study, we do not believe this

570 overall pattern is the result of an analytical artifact. We hypothesize that part of this difference is
571 due to more stressful conditions experienced by plants at the inland site; plants that survive to
572 maturity in coastal habitats are generally larger than plants growing at the inland habitat (Lowry
573 et al. 2008; Popovic & Lowry *unpublished data*). It remains to be seen whether the magnitude of
574 gene regulatory divergence between ecotypes varies across habitats in other systems as well.
575
576 ***Gene Regulatory Evolution and Chromosomal Inversions***
577 Chromosomal inversions have been thought to be involved in evolutionary adaptations since
578 classic studies in *Drosophila* found that inversion polymorphisms are frequently correlated with
579 environmental conditions over geographic space and through time (Dobzhansky 1951, 1970;
580 Kirpatrick & Barton 2006; Hoffman & Rieseberg 2008; Cheng et al. 2012; Ayala et al. 2013,
581 2014; Adrion et al. 2015). Inversions strongly suppress genetic recombination in heterokaryotic
582 (inversion heterozygotes) individuals because recombinant gametes are unbalanced (Dobzhansky
583 1970; Rieseberg 2001). Researchers have thus hypothesized that inversions could act as
584 supergenes by holding together long haplotypes containing multiple adaptive polymorphisms
585 through suppressed recombination (Dobzhansky 1970; Joron et al. 2011; Thompson & Jiggins
586 2014; Kirkpatrick & Barton 2006; Kirkpatrick & Barrett 2015).
587
588 The supergene hypothesis is a major reason why inversions are thought to frequently contribute
589 to the evolution of adaptations (Dobzhansky 1970; Kirkpatrick & Barton 2006; Hoffman &
590 Rieseberg 2008; Faria & Navarro 2009; Thompson & Jiggins 2014). Early researchers,
591 especially Dobzhansky (1970), argued that inversions could hold together co-adapted epistatic
592 complexes of genes through suppressed recombination. However, theoreticians have recently

593 shown that inversions can spread due to purely additive effects, when at least two locally
594 adaptive loci are captured within the inverted region (Kirkpatrick & Barton 2006; Kirkpatrick &
595 Barrett 2015). Regardless of the ultimate genetic mechanisms, these models all predict that
596 suppression of recombination by inversions maintains adaptive divergence at multiple linked
597 loci.
598
599 Global gene expression analyses offer one way to test whether inversions hold together
600 haplotypes of genetic variation potentially responsible for phenotypic divergence between locally
601 adapted populations. Research in a few systems has found evidence of increased levels of
602 expression divergence in inversions (Marquès-Bonet et al. 2004; Cassone et al. 2011; Fuller et al.
603 2016). For example, Marquès-Bonet et al. (2004) found 8.9% greater divergence (fold change) in
604 human versus chimp brain gene expression for chromosomal inversions when compared to the
605 rest of the genome. We did not observe a significant difference in fold change between inverted
606 and non-inverted regions in our study. However, genes within the region of the chromosome 5
607 inversion were 12.5% more likely to be differentially expressed. Genes within the adaptive
608 chromosome 8 inversion were 8.3% more likely to have a *cis*-eQTL.
609
610 The enrichment of gene expression differences in our study is consistent with patterns found in
611 inversions between chimpanzees and humans (Marquès-Bonet et al. 2004) and *Drosophila*
612 (Fuller et al. 2016; Said et al. 2018), but contrasts with results for the plant *Boechera stricta* (Lee
613 et al. 2017). While the finding of an enrichment of differential gene expression for inversions in
614 this study and others is consistent with the supergene hypothesis of inversion evolution, it is by
615 no means definitive proof. Demonstrating that an inversion has evolved as an adaptation

616 supergene requires confirmation that multiple genes within the inversion have effects on adaptive
617 phenotypes (Hoffman & Rieseberg 2008; Kirkpatrick 2010; Kirkpatrick & Kern 2012). There is
618 currently no study that we are aware of which has experimentally confirmed that two or more
619 genes within an inversion contribute additively or epistatically to adaptive phenotypic
620 differences between locally adapted populations, ecotypes, or species (but see Kunte et al. 2014).
621

622 ***Candidate Genes and Pathways***

623 Analysis of expression across both ecotypes and environments allowed us to examine the
624 detailed expression patterns of several candidate genes that may contribute to adaptive
625 divergence. We observed a diversity of expression patterns across candidate genes (Fig. 5). The
626 gene *ABA1* catalyzes the first step in the synthesis of the abiotic stress hormone ABA, and the
627 *Mimulus* ortholog of this gene is located in the adaptive chromosomal inversion on chromosome
628 8. In *Arabidopsis* and other plants, increased ABA production leads to drought tolerance by
629 triggering stomatal closure and resistance to osmotic stress (Jakab et al 2005). Previous work has
630 shown that the chromosome 8 inversion in *M. guttatus* contributes directly to fitness differences
631 between coastal and inland lines (Lowry & Willis 2010) and that the genic region of *ABA1*
632 within the inversion has an unusually high degree of differentiation (high F_{ST}) between coastal
633 and inland populations (Gould et al. 2017). In the present study, we found that both genotypic
634 and environmental differential regulation contribute to expression patterns at this locus. *ABA1*
635 was always more highly expressed in the inland ecotype and also upregulated in the inland
636 environment by both ecotypes (Fig. 5A). In contrast, the candidate gene P-glycoprotein19,
637 *PGP19* (Migut.H00909), also located in the adaptive chromosome 8 inversion (Gould et al.
638 2017), was differentially regulated between ecotypes only at the inland field site (Fig. 5E). The

639 *Arabidopsis* ortholog of *PGP19* is an ATP transporter involved in gravitropism, growth, and
640 development by mediating auxin signaling (Okamoto et al 2015).
641
642 Consistent with oceanic salt spray playing a key role in local adaptation (Lowry et al. 2009),
643 multiple salt tolerance candidate genes were differentially regulated between the coastal
644 perennial and inland annual ecotypes. Two major genes in the Salt Overly Sensitive (*SOS*)
645 pathway, *CBL10* and *SOS1,* were expressed at higher levels in the coastal ecotype than the inland
646 ecotype across habitats (Fig. 5B, C). *CBL10* is a calcineurin B-like calcium sensor that interacts
647 with the protein kinase *SOS2* in the shoots of plants to positively regulate Na^+ transporters in the
648 tonoplast of the vacuole as well as potentially in the plasma membrane (Quan et al. 2007; Kang
649 & Nam 2016; Egea et al. 2018). The function of *CBL10* in salt response has been shown to be
650 conserved across *Arabidopsis* (Quan et al. 2007)*, Populus* (Tang et al. 2014)*,* and tomatoes (Egea
651 et al. 2018). Interestingly, *CBL10* has been found to confer salt tolerance in plants, while at the
652 same time making them more susceptible to drought (Kang & Nam 2016), suggesting a potential
653 trade-off for local adaptation to coastal habitats that are inundated by salt spray. *SOS1* is a well
654 characterized Na^+/K^+ antiporter that pumps toxic Na^+ ions out of the cytoplasm of plant cells into
655 the surrounding apoplast (Shi et al. 2002). *SOS1* is potentially positively regulated by the
656 *CBL10/SOS2* complex in the shoots of plants (Quan et al. 2007). In addition to the *SOS* pathway,
657 there were G and E effects (but not GxE) for an ortholog of the High-Affinity K^+ Transporter1,
658 *HKT1*. *HKT1* has previously been linked in the evolution of salt tolerance adaptation in coastal
659 populations of *Arabidopsis thaliana* (Fig. 5D; Rus et al. 2006; Baxter et al. 2010).
660
661 ***Caveats, Limitations, and Future Directions***

662 There are important caveats and concerns to consider in the interpretation of the results of our
663 study. One of the foremost caveats of the study is its narrow scope. We only evaluated gene
664 expression differences between one inbred line per ecotype, at two field sites, and for gene
665 expression at one time point. Despite these drawbacks, this is the first study that we are aware of
666 to evaluate allele-specific expression in the field between known locally adapted populations
667 (Voelckel et al. 2017), which allowed us to detect novel *cis* x habitat and *trans* x habitat
668 interaction (Fig. 4; Table S6). The major outstanding questions include how variable gene
669 expression is across populations, field sites, and tissue types? In the future, it will be critical to
670 evaluate gene expression at different time points and across tissue types to gain a more
671 comprehensive understanding of how ecotypic differences in gene expression manifest
672 throughout development under natural field conditions.
673
674 Perhaps, the greatest challenge for interpreting gene expression results from reciprocal transplant
675 experiments is understanding how patterns of expression actually contribute to phenotypes and
676 fitness across habitats. Fitness trade-offs that are associated with local adaptation could
677 potentially be caused by any type of gene expression pattern. Constitutive expression differences
678 between ecotypes could cause stable phenotypic differences that are beneficial in one habitat but
679 deleterious in another. Gene expression characterized by genotype x environment interactions
680 could cause fitness trade-offs, but just as likely could mitigate trade-offs by helping ecotypes
681 maintain homeostasis across habitats.
682
683 Until recently, studies of gene regulation have for the most part been limited to the laboratory
684 setting. Coupling those studies with reciprocal transplant experiments to better understand local

685 adaptation is still rare (Kenkel & Matz 2016; Lohman et al. 2017; Voelckel et al. 2017). Allele-
686 specific expression is clearly an important tool for understanding the evolution of regulatory
687 differences between locally adapted populations, ecotypes, and species. However, we hope that
688 future reciprocal transplant experiments will go beyond RNA-sequencing by also examining
689 genotypic differences in genome-wide patterns of chromatin remodeling and protein translation
690 across divergent habitats (Rodgers-Melnick et al. 2016; Voelckel et al. 2017).
691

692 **DATA ACCSSIBILITY**

693 The assembled reference transcriptome and normalized count data for all transcripts in all
694 individuals has been archived at Data Dryad (see DOI: https://doi.org/10.5061/dryad.57d6118).
695

696 **ACKNOWLEDGMENTS**

697

698 We thank D. Des Marais, J. Tung, J. Puzey, J. Friedman, R. Guerrero, R. Rellán-Álvarez, J.
699 Lasky, and six anonymous reviewers for comments that helped to improve our manuscript. We
700 thank K. Childs and J. Lovell for guidance with expression analyses, B. Blackman and E.
701 Patterson for starting seeds for us at UC Berkeley, and D. Popovic for assistance in field
702 planting. The Bodega Marine Reserve and the Pepperwood Preserve kindly allowed us to
703 conduct this reciprocal transplant field experiment. Funding was provided by Michigan State
704 University through a start-up package to DBL. The authors declare no conflicts of interest.
705

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DATA ACCESSIBILITY

RNA-sequence data are deposited are available through the NCBI Sequence Read Archive under project identifier XX.

AUTHOR CONTRIBUTIONS

DBL designed and conducted the field experiment. YC conducted the laboratory work. BAG analyzed the data. DBL and BAG wrote the manuscript.

1130

1131 **TABLES**

1132

1133 Table 1: Summary of model effects on expressed genes.

	Inversion 5 (246)		Inversion 8 (311)		Non-Inversion (8805)		total (9326)
Differential Expression (model 1):	no.	LFC	no.	LFC	no.	LFC	no.
Genotypic DE	201	0.893	239	0.932	6773	0.86	7213
Environmental DE	185	0.667	230	0.808	6350	0.729	6765
Genotype by Environment	**60***	0.19	61	0.213	1659	0.196	1780
Regulation effects (model 2):							
total DE genes	**204*****		232		6490		6962
Cis regulation	158	1.023	**198***	1.04	5176	1.04	5532
Trans regulation	106	1.707	109	1.628	3396	1.229	3611

1134 *significantly greater percentage than non-inversion regions at p<0.05, **p<0.01.
1135 LFC: average magnitude (abs) log2 fold-change of genes with significant effects
1136

1137
1138
1139
1140 Table 2: Number and average effect size of differentially expressed genes within each habitat.

	Coast		Inland	
	no.	LFC	no.	LFC
Inversion 5 (246)	119	1.136	184	0.932
Inversion 8 (311)	**161***	1.092	210	0.993
Non-Inversion (8805)	3997	1.084	6195	0.897
Total (9326)	4277	1.085	**6589*****	**0.901*****

1141 *significantly greater than non-inversion regions at p<0.05
1142 *** significantly different than the coastal field site, p<0.001
1143 LFC: average magnitude (abs) log2 fold-change of genes with significant effects
1144

1145

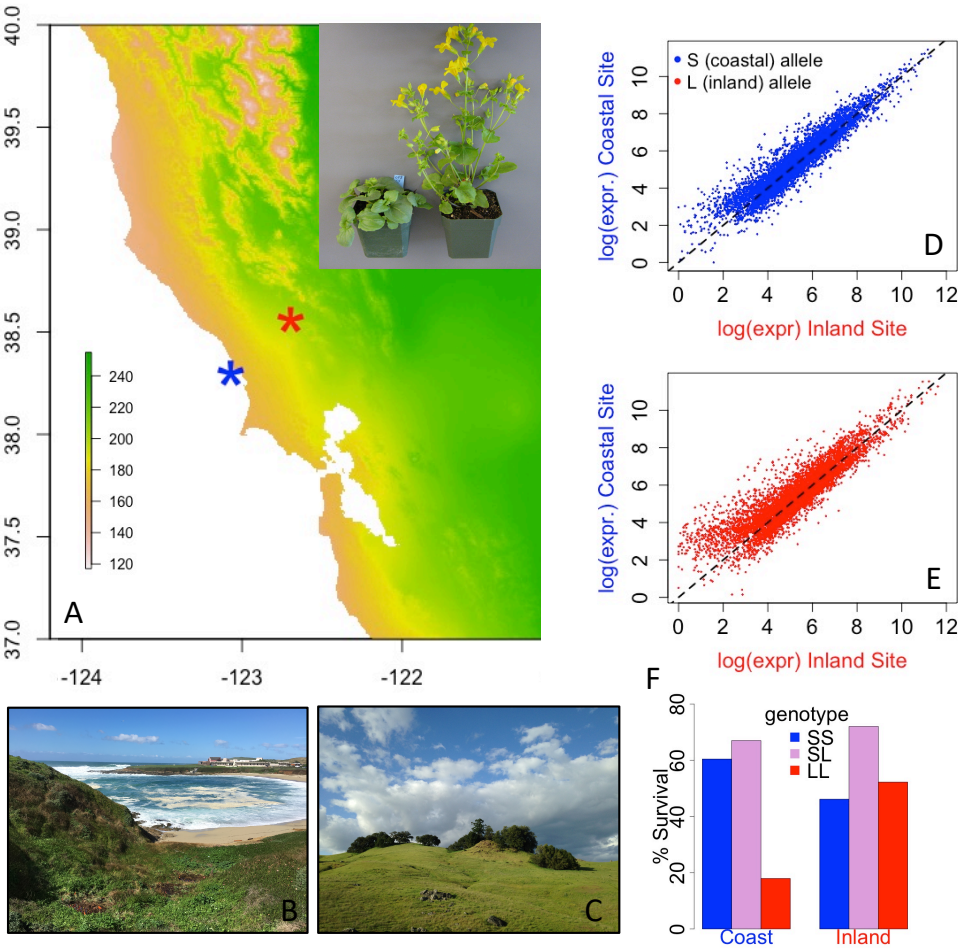

Fig. 1 A) Map of Northern California with the location of two field transplant sites. Colors represent mean temperature of the warmest quarter in degrees C x 10. Inset: left, coastal ecotype; right, inland ecotype. B) The coastal field site. C) The inland field site.
D) SS parental gene expression at two field sites. E) LL parental gene expression at two field sites. F) Survival of parental and F1 (SL) plants at the two field sites.

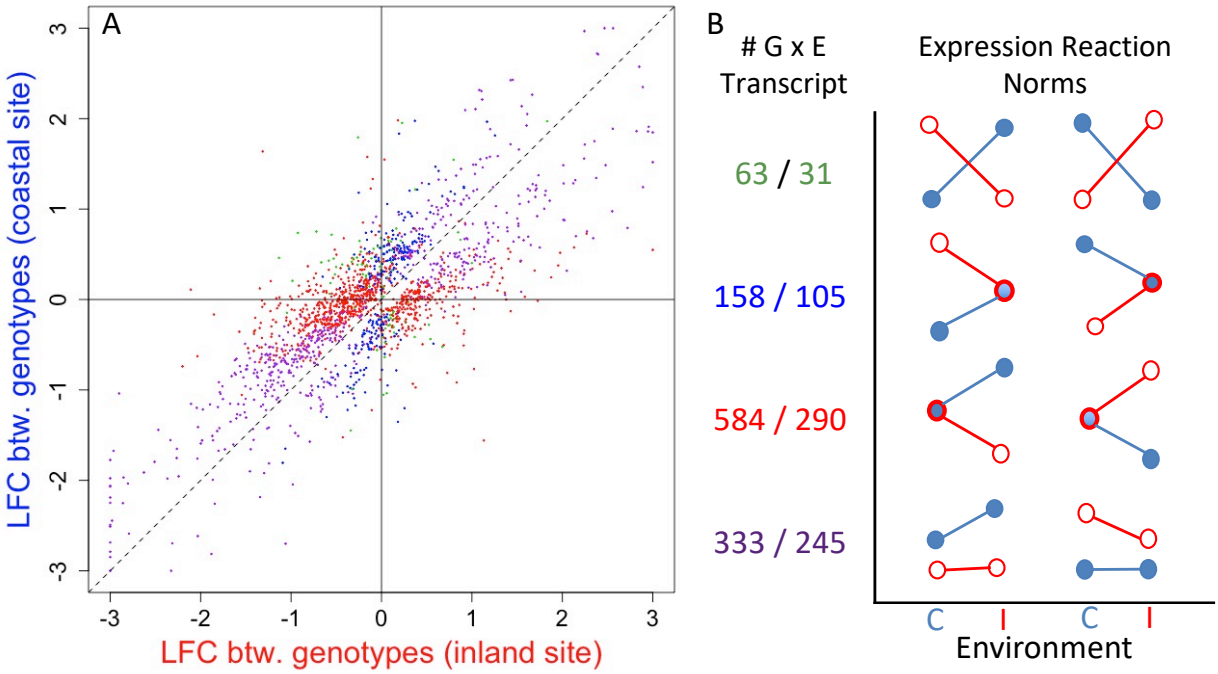

Fig. 2 A) Average log2 fold-change (LFC) differences between parental genotypes for expressed transcripts at the two field sites. B) Expression reaction norms for inland (red) and coastal (blue) lines. The numbers correspond to the colored points in part A of the figure. LFC has been restricted to <3 and <-3 for graphing purposes. Note, 28 transcripts with ambiguous expression patterns have been excluded.

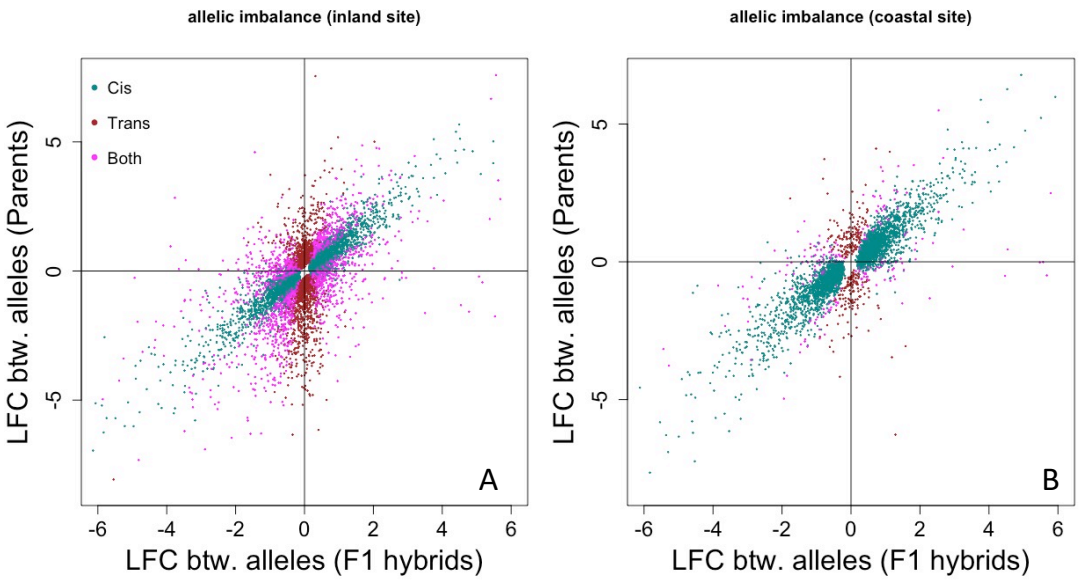

Fig.3. *Cis* and *trans* regulated transcripts detected at the coastal (A) and inland (B) field sites. Average log2-fold change (LFC) ratios are between inland and coastal alleles in the parental (y-axis) and F1 (x-axis) genetic backgrounds.

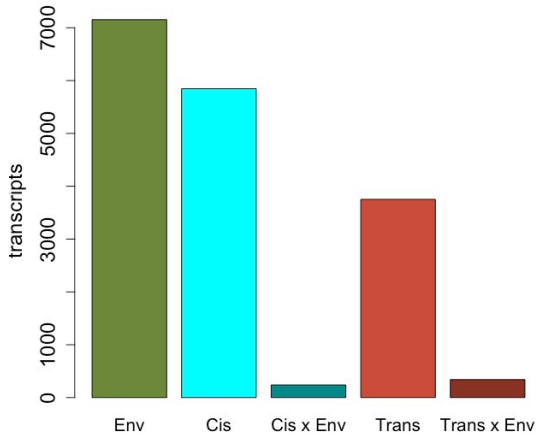

Fig.4. The frequency of significant model effects among all expressed transcripts.

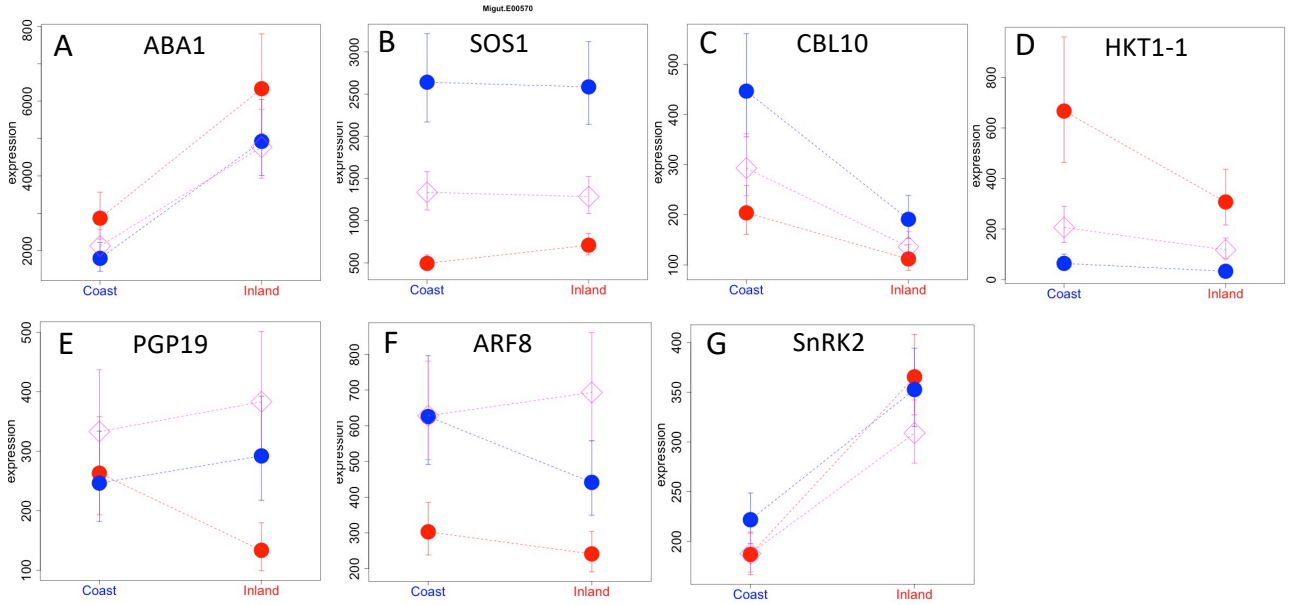

Fig.5 Reaction norms of expression at two field sites for seven candidate adaptive genes. A) ABA1 – abscisic acid deficient 1; B) SOS1 – salt overly-sensitive 1; C) CBL10 -calcineurin B-like protein 10; D) HKT1-1, high-affinity K+ transporter (functions in salt tolerance); E) PGP19, p-glycoprotein 19 (functions in auxin transport); F) ARF8 – auxin response factor 8; G) SnRK2, Snf-related protein kinase (functions in salt tolerance); Red, inland genotype. Blue, coastal genotype. Pink, F1 hybrid.