Gene Regulatory Divergence Between Locally Adapted Ecotypes in Their Native Habitats

Billie A. Gould[a], Yani Chen[a], David B. Lowry[a,b,c,*]

[a]Department of Plant Biology, Michigan State University, East Lansing, MI, USA

[b]Program in Ecology, Evolutionary Biology and Behavior, Michigan State University, East Lansing, MI 48824, USA

[c]Plant Resilience Institute, Michigan State University, East Lansing, MI 48824, USA

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[*]To whom correspondence should be addressed: David B. Lowry

Department of Plant Biology, Michigan State University, Plant Biology Laboratories, 612 Wilson Road, Room 166, East Lansing MI 48824, USA

Tel: (517) 432-4883

E-mail: dlowry@msu.edu

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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. Above and below mid-parent heterosis for gene expression was prevalent across habitats and heterotic genes were enriched for growth and development gene ontologies. 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 ecological specialization and the origin of
48 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). Case-studies, such as the evolution
52 of beak size in Darwin's Finches (Abzhanov et al. 2004) and coat color of Beach Mice (Hoekstra
53 et al. 2006), suggest that local adaptation should involve fitness trade-offs at the level of
54 individual genes. However, studies that have combined reciprocal transplant field experiments
55 with quantitative trait locus (QTL) mapping have observed that the majority of loci which confer
56 substantial fitness benefits in one habitat result in little or no cost in alternative habitats
57 (Verhoeven et al. 2008; Gardner & Latta 2006; Lowry et al. 2009; Lowry & Willis 2010;
58 Anderson et al. 2011, 2013; Leinonen et al. 2013; Agren et al. 2013; Wadgymar et al. 2017).
59 While multiple studies have now evaluated the effects of individual loci on fitness across
60 habitats, the patterns gene regulatory divergence that might explain habitat-dependent fitness
61 effects in those systems have not been evaluated under natural field conditions.
62
63 One very promising route for understanding how gene expression regulation evolves between
64 locally adaptive populations is to conduct allele specific expression (ASE) analyses in nature
65 (Tung et al. 2009; Lovell et al. 2016; Wang et al. 2017; Lohman et al. 2017). In contrast to
66 coarse QTL mapping, conducting ASE analyses in the field allows for the evaluation of the
67 patterns of gene expression responses to habitat context at the individual allele level. ASE
68 analysis is often conducted in F1 hybrids, where parental alleles are evaluated for significant

69 differences in expression (de Meaux et al. 2005; Guo et al. 2008; Zhang & Borevitz 2009;
70 Cubillos et al. 2014; Coolon et al. 2014; Steige et al. 2015, 2017; Lovell et al. 2016; Aguilar-
71 Rangel et al. 2017). ASE relies on there being nucleotide polymorphisms between parental
72 alleles, so that those alleles can be individually counted in the F1 transcript pool. *Cis*-regulatory
73 allelic expression differences are expected to persist regardless of whether alleles are expressed
74 separately in parental genotypes or together in an F1 hybrid. In this way, ASE allows for the
75 identification of *cis* expression Quantitative Trait Loci (eQTL), which can be evaluated for their
76 responses to different habitats. Further, *trans* effects on gene expression are indicated by a
77 change in allelic effects between the parental generation and the F1 hybrids (Cowles et al. 2002;
78 Wittkopp et al. 2004). Despite its great potential, we are unaware of any field reciprocal
79 transplant experiment that has measured ASE to evaluate how gene expression divergence
80 manifests across the habitats known to be responsible for local adaptation.
81
82 In this study, we evaluated gene regulatory divergence in nature between locally adapted coastal
83 and inland population accessions of the yellow monkeyflower, *Mimulus guttatus* (*syn.*
84 *Erythranthe guttata* (Fisch. ex DC.) G.L. Nesom). *M. guttatus* is a major study system for
85 understanding the genetic underpinnings of fundamental patterns in evolutionary ecology
86 (reviewed in Wu et al. 2008; Twyford et al. 2015). Inland populations of *M. guttatus* have
87 evolved an early-flowering annual life-history to escape from the seasonal summer drought that
88 occurs in the Mediterranean climate of the California Coastal Mountain Range. In contrast,
89 coastal populations of *M. guttatus* are sheltered from the summer drought by pervasive oceanic
90 fog, which supplies soil moisture and reduces transpiration rates (Corbin et al. 2005). As a result,
91 these populations have evolved a late-flowering perennial life-history (Hall & Willis 2006;

92 Lowry et al. 2008). While coastal habitats are benign in terms of soil water availability, these
93 populations must contend with toxic oceanic salt spray, in response to which they have evolved
94 high salt tolerance (Lowry et al. 2008, 2009; Selby et al. 2014). Across previous reciprocal
95 transplant field experiments, inland plants have survived to flower at 23.9 times the rate of
96 coastal plants in inland habitat, while coastal plants have survived at 2.5 times the rate of inland
97 plants in coastal habitat (Hall & Willis 2006; Lowry et al. 2008, 2009; Hall et al. 2010).
98
99 Population structure analysis has repeatedly demonstrated that coastal populations collectively
100 constitute a single ecotype, which is distinct genetically from inland annual populations (Lowry
101 et al. 2008; Twyford & Friedman 2015). The individual genes responsible for the divergence of
102 coastal and inland ecotypes are unknown, but it is clear that a large paracentric chromosomal
103 inversion on chromosome 8 is responsible for much of the divergence in traits and fitness
104 between coastal and inland ecotypes (Lowry & Willis 2010; Friedman 2014; Oneal et al. 2014;
105 Gould et al. 2017). Smaller effect loci across the genome also account for divergence between
106 the coastal and inland ecotypes (Hall et al. 2006, 2010; Lowry et al. 2009; Friedman et al. 2015).
107 In addition, significant heterosis occurs for multiple traits (fitness, flowering time, internode
108 length) in F1 hybrids between coastal and inland accessions (Lowry et al. 2008; Lowry & Willis
109 2010). The extent to which gene expression also shows patterns of heterosis in this system is
110 unknown.
111
112 The primary goal of our study was to characterize gene regulatory divergence between coastal
113 perennial and inland annual *M. guttatus* in the natural habitats that drove the evolution of these
114 distinct coastal and inland ecotypes. In particular, our study aimed to 1) determine the prevalence

115 of genotype x environment (GxE) interactions affecting the expression of native and non-native
116 alleles across the divergent habitats, 2) characterize the relative contributions of *cis* and/or *trans*
117 regulatory variation in expression divergence, 3) establish the role of genome structure (e.g.
118 inversions) in the evolution of locally adaptive regulatory variation, 4) quantify gene expression
119 heterosis across habitats, and 5) identify candidate genes underlying local adaptation in this
120 system. The results of our study provide new insights into the gene regulatory evolution and
121 suggest that coupling allele-specific expression with reciprocal transplant experiments is a
122 promising approach for modern local adaptation research.
123

124 **MATERIALS AND METHODS**

125 ***Germplasm***

126 Original field collections of the seeds used in this experiment were made in the Spring of 2005
127 from the SWB (N39.0359', W123.6904') and LMC (N38.8640', W123.0839') populations
128 located in Mendocino County, California. The SWB and LMC populations have been the subject
129 of extensive recent research (Lowry et al. 2008; Lowry & Willis 2010; Wu et al. 2010; Friedman
130 & Willis 2013; Friedman 2014; Gould et al. 2017). Seeds from these populations were inbred for
131 multiple generations in the Duke University Greenhouses. The plants used for the field
132 experiment included the following: 1) An inbred (7 generations self-fertilized) inland line, LMC-
133 L1-2, henceforth referred to as LL; 2) An inbred (13 generation self-fertilized) coastal line,
134 SWB-S1-2, henceforth referred to as SS; and 3) An F1 hybrid between the two inbred lines,
135 henceforth referred to as F1 or SL. The F1s used in this experiment were made in the Michigan
136 State University (MSU) Growth Chamber Facilities through a cross between the two parental
137 inbred lines.

138

139 ***Field Reciprocal Transplant Experiment***

140 We conducted a field reciprocal transplant experiment in tandem with allele-specific expression
141 analysis to understand the causes (*cis*, *trans*) of gene expression divergence between locally
142 adapted coastal and inland populations of *M. guttatus* (Fig. 1). The reciprocal transplant
143 experiment was conducted in 2016 using one coastal (Bodega Marine Reserve; N38.3157',
144 W123.0687') and one inland (Pepperwood Preserve; N38.5755', W122.7008') field site located
145 in Sonoma County, CA. SS, LL, and F1 seeds were originally sown in Sun Gro Metromix 838
146 soil on February 3. The seeds were then stratified in the dark at 4°C until February 9, when they
147 were removed from the cold and germinated in the UC Berkeley Oxford Track Greenhouses.
148 Seedlings were moved to the Bodega Marine Reserve Greenhouses on February 21 for
149 acclimation. On February 22, the seedlings were transferred to native soil collected from the field
150 sites. Seedlings were planted in the field at Bodega on March 7 and at Pepperwood on March 8.
151 ~90 plants per type (SS, LL, F1) were planted at each field site. Plants were evenly distributed
152 among three blocks per each field site and randomized within block. Tissue for RNA
153 sequencings was collected from Bodega on April 23 and Pepperwood on April 24. To avoid
154 effects of circadian rhythm on gene expression, all tissue was collected between 12:00pm and
155 1:00pm. Both collection days had mostly clear skies, with mean local temperatures during
156 collection periods of 14° C at Bodega and 17° C at Pepperwood. Plants had progressed to
157 different developmental stages at time of collection, with some plants approaching flowering and
158 others not. To best standardize tissue collection across genotypes, only the two youngest pairs of
159 fully expanded leaves were collected from each plant. Tissue was immediately flash frozen on

160 liquid nitrogen in the field. Samples were then shipped on dry ice to Michigan State University
161 for RNA extractions.
162
163 ***RNA-sequencing***
164 Total RNA was extracted from 80 leaf tissue samples with the Spectrum Plant Total RNA Kit
165 (Sigma-Aldrich, St. Louis, MO, USA). RNA was extracted from 12 replicates of both SS and LL
166 parental lines from the coastal field site and 13 replicates of both parental lines from the inland
167 field site. RNA from 15 F1 hybrids from each site was also extracted. Total RNA was submitted
168 for library preparation and sequencing at the MSU Genome Core
169 (https://rtsf.natsci.msu.edu/genomics/). RNA-Seq libraries were prepared using the Illumina
170 TruSeq Stranded mRNA Library Preparation Kit on a Perkin Elmer (Waltham, MA) Sciclone
171 NGS robot. Completed libraries were quality controlled and quantified using a combination of
172 Qubit dsDNA HS (Thermo Fisher Scientific, Waltham, MA), Caliper LabChipGX HS DNA
173 (Perkin Elmer, Waltham, MA), and Kapa Biosystems Illumina Library Quantification qPCR
174 (Wilmington, MA) assay kits. All libraries were barcoded and library concentrations were
175 normalized and pooled in equimolar amounts: 8 pools each consisting of 10 randomly chosen
176 libraries were made. Each of these pools was loaded on one lane of an Illumina HiSeq 2500 High
177 Output flow cell (v4) and sequenced in the 1x100bp (SE100) format using HiSeq SBS reagents
178 (also v4). Base calling was done by Illumina Real Time Analysis (RTA) v1.18.64 and output of
179 RTA was demultiplexed and converted to Fastq format with Illumina Bcl2fastq v1.8.4.
180
181 ***Reference Transcriptome Assembly***

182 Alignment of RNA-seq to a single reference genome can lead to major biases in ASE studies
183 (Stevenson et al. 2013). To overcome reference bias, we assembled reference transcriptomes for
184 the coastal perennial (SS) and inland annual (LL) parents. We chose this method over
185 construction of a pseudo-transcriptome reference (Shen et al. 2013) to avoid generating bias
186 caused by the reference genome and transcriptome for *M. guttatus* (IM62; Hellsten et al. 2013),
187 which was generated from an inland annual line. Reference bias is of particular concern in *M.*
188 *guttatus* because pairwise polymorphism is generally high (π_S = 0.033-0.065; Brandvain et al.
189 2014; Gould et al. 2017; Puzey et al. 2017). To generate a reference transcriptome assembly that
190 contained allele-specific sequences for each parental genotype, we sequenced and conducted *de*
191 *novo* assembly using RNA-sequencing. Plants of each of the coastal (SS) and inland (LL) lines
192 were grown in the MSU greenhouses until both genotypes were flowering. Floral bud, leaf, and
193 root tissue were collected from each of the SS and LL lines, flash frozen with liquid nitrogen,
194 and stored at -80° C. Total RNA was extracted from each sample using the Spectrum Plant Total
195 RNA Kit and submitted to the MSU Genome Core for library construction and sequencing.
196 Strand-specific Tru-Seq Illumina RNA-seq libraries were prepared and sequenced along with 4
197 libraries constructed from field collected leaf tissue from the reciprocal transplant experiment (1
198 sample per parental line per field site). These libraries were included in the transcriptome
199 assembly pipeline to capture sequences from genes that may only be expressed under field
200 conditions. Completed libraries were quality controlled and quantified using a combination of
201 Qubit dsDNA HS, Caliper LabChipGX HS DNA, and Kapa Biosystems Illumina Library
202 Quantification qPCR assay kits. A total of 10 libraries were barcoded with dual index barcodes
203 and pooled together. The entire pool was sequenced using 125 bp paired-end reads on a single
204 lane of the Illumina GA 2500 platform.

205

206 Raw output reads were quality checked using FastQC v.0.11.3 (Andrews 2015). Residual adapter
207 sequences were removed and reads were quality trimmed using Trimmomatic v.0.33 (Bolger et
208 al. 2014), discarding sequences less than 50 bp in length. Paired and un-paired reads from each
209 ecotype were combined and assembled into two separate *de novo* (non-reference guided)
210 assemblies using Trinity v20140413p1 (Grabherr et al. 2011) with *in silico* read normalization.
211 To check the quality of our two transcriptomes, we compared our *de novo* assemblies to the set
212 of primary transcripts from the IM62 reference transcriptome using Trinity utility scripts. We
213 found that 74.3% of primary transcripts represented at least 50% sequence coverage in the inland
214 (L) transcriptome and 74.1% in the coastal (S) transcriptome. We also aligned the filtered input
215 reads back to the finished assemblies using Bowtie2 (Langmead et al. 2012). A total of 98.98%
216 and 99.26% of input reads aligned back to the inland (L) and coastal (S) assemblies, respectively.
217 We identified possible non-plant contaminant sequences in each assembly through comparison to
218 the NCBI nt database using blastn. We eliminated transcriptome sequences with top blast
219 sequence matches (e-value cutoff of 1e-20) to non-plant organisms (N = 16243 and N = 1222
220 eliminated sequences for the inland and coastal transcriptomes, respectively).

221

222 To construct a combined reference transcriptome that contained pairs of allelic sequences from
223 both lines, we used a reciprocal best blast strategy. Each transcriptome was compared against the
224 other (blastn) at an e-value cutoff of 1e-5 and reciprocal best hits were returned using a custom
225 Python (v. 2.7.2) script (https://github.com/lowrylab/Mimulus_Gene_Expression). We tested a
226 range of e-value cutoffs in this analysis and found that the choice of e-value between 1e-5 and
227 1e-50 had little effect on the total output number of best blast pairs (see Fig. S1). We considered

228 two sequences best blast hits if total high-scoring pair (HSP) coverage was 75% or greater
229 between sequences. We included only aligned sequence regions of the longest HSP in the
230 combined transcriptome.
231
232 To match transcripts in the combined *de novo* reference transcriptome with their corresponding
233 *Mimulus* genes, we compared *de novo* assembled alleles against the reference (IM62) set of
234 primary transcripts (blastn) at an e-value cut-off of 1e-5. In rare cases, where two alleles from a
235 *de novo* assembled transcript matched different *Mimulus* genes, the gene with the lowest e-value
236 alignment was chosen as a match for the allele pair. Note that with this annotation method, each
237 *de novo* assembled transcript has only one matching gene and thus, can be unambiguously
238 assigned a specific location in the genome. However, multiple transcripts can be assigned to the
239 same genomic location, which is typical of isoforms stemming from the same gene.
240
241 ***Differential Expression Analyses***
242 Raw reads from the 80 field RNA-seq libraries were de-multiplexed, quality filtered, and
243 trimmed using FastQC and Trimmomatic, as described above for the transcriptome assembly.
244 Filtered reads were aligned to the *de novo* combined reference transcriptome (described above)
245 using the un-gapped aligner Bowtie v1.0.0 (Langmead et al. 2009). We outputted only unique
246 alignments with zero mismatches. In this way, we analyzed only read count data for reads that
247 aligned uniquely and perfectly to only one parental allele in the combined reference
248 transcriptome. Using these criteria, on average 28.6% of library reads per individual were
249 aligned (range 23.7 – 30.7%). For confirmation, some alignments were visually checked using
250 IGV v.2.3.65 (Thorvaldsdóttir et al. 2013).

251

252 Read alignments to each transcript were quantified using a custom Python (v.2.7.2) script. Using
253 the raw count data, we identified allele pairs in the transcriptome with a high degree of residual
254 heterozygosity in the parental inbred lines. This is potentially indicated where a significant
255 number of reads from a homozygous parent align to the opposite parental allele in the
256 transcriptome. We eliminated from further analysis any pair of alleles where the average number
257 of reads aligning to the correct parental allele was less than 5 times the average number of reads
258 aligning to the alternate allele (N = 2255 eliminated transcripts). We also used the initial count
259 data to test for mapping bias (Fig. S2). For both field sites mapping bias was present but well
260 controlled. There was a small but statistically significant relationship between transcript
261 nucleotide divergence and differential expression (equivalent to 0.001 increase in the absolute
262 value of LFC between genotypes, per SNP). Count data was normalized for library size using
263 Trimmed Mean of M-values (TMM) normalization to counts per million, which reduces biases
264 generated by the presence of very highly and lowly expressed transcripts (Robinson et al. 2010).
265 We eliminated any transcript without at least one library containing >= 10 counts per million
266 reads. The remaining sequences constitute the 'expressed' set of transcripts (N = 10,122). The
267 mean expressed library size for samples from the coast and inland sites was not significantly
268 different (t-tests: all libraries, P = 0.60; parental libraries only, P = 0.22, Fig. S3A), and is thus,
269 unlikely to strongly affect statistical comparisons between the two sites. Only 2 transcripts were
270 expressed at only one site and only 7 transcripts were expressed by only one parental line.
271

272 We analyzed the expressed transcript data using the limma (Ritchie et al. 2015) and edgeR
273 (Robinson et al. 2010) packages in R v.3.2.1 (R. Core Team 2012). Variance stabilizing

274 normalization of the data was performed using the voom (Law et al. 2014) function in limma,
275 with a design matrix corresponding to the particular linear model being used for analysis. Model
276 design matrices were coded using the default treatment-contrast parameterization scheme (see
277 Smyth et al. (2015), section 9.5.4) and the significance of model effects were calculated using
278 voom precision weights and the eBayes function. Model effects were judged significant if they
279 fell below a Benjamini-Hoechberg false-discovery rate corrected *P*-value of 0.05 using the
280 decideTests function.
281
282 We used several different linear models to analyze the count data for expressed transcripts,
283 modified from the method of Lovell et al. (2016). For this approach, it is important that the size
284 of the L and S sub-transcriptome in each F1 is approximately equal to produce unbiased results,
285 which we verified independently (Fig. S4). Each linear model had one or more fixed factors:
286 allele (L/S), environment (coastal field site vs. inland field site), generation (F0/F1), and
287 interaction terms. We analyzed the parental plants (F0 generation) separately, the F1 plants
288 separately, and both generations together in different models. We avoided analyzing models with
289 more than one categorical factor, except for the purposes of measuring interaction effects
290 because effect significance testing for crossing categorical factors only captures differences
291 present in the first level of each factor.
292
293 For data from the F0 plants, we ran models examining the allele (homozygous genotype) effect
294 within each environment separately. We also tested for environmental effects separately within
295 each parental genotype. We used a model with both genotype and environment to test the
296 significance of the genotype x environment interactions. Because the inland site had one extra

297 plant per genotype compared with the coastal site, we analyzed data from 12 randomly chosen
298 parental lines of each genotype to equalize the power to detect effects between sites. A *post-hoc*
299 power analysis was conducted using the R package *RnaSeqSampleSize* (Bi & Liu 2016).
300
301 We used data from all plants together (both F0 and F1) to test for *cis* and *trans* regulation. *Cis*-
302 regulatory allelic expression differences are expected to persist regardless of whether alleles are
303 expressed separately in parental genotypes or together in an F1 hybrid. However, in the F1
304 genotype, *trans* acting factors from both parental genotypes will influence the expression of an
305 allele, so *trans* regulation is indicated if allelic patterns of differential expression are different in
306 the parents vs. the F1 plants. In our model, a significant effect of allele is indicative of a *cis*-
307 regulatory difference in expression. If a transcript has a significant allele x generation interaction
308 effect, this indicates *trans* regulation. Note that use of linear models is limited in its ability to
309 detect *cis* and *trans* effects when those effects are antagonistic (Lovell et al. 2016). In such cases,
310 significant allelic differential expression can be detected between parental genotypes, but
311 becomes non-significant in the F1 generation. Within each environment, we tested a model with
312 the following factors: allele (genotype), generation (F0/F1), and the interaction term. Any
313 transcript with any *cis* regulation had a significant allele effect (measured in the F1 generation
314 only). Any transcript with only *trans* regulation had a significant difference between generations,
315 but a non-significant allele effect in the F1 generation. Any transcript with both *cis* and *trans*
316 regulation had a significant generational effect and a significant allele effect in the F1.
317
318 To gauge the significance of *cis* x environment interactions, we tested the interaction term of a
319 model using only F1 data and factors allele, environment, and interaction. To gauge the

320 significance of trans x environment interactions we modeled all the data (F0 and F1) and
321 measured the significance of a three-way interaction term: allele x generation x environment.
322
323 ***Gene Ontology Enrichment and Expression Networks***
324 We tested for enrichment of gene-ontology (GO) terms among subsets of genes with particular
325 expression patterns (significant model effects). To annotate GO terms, all IM62 reference
326 *Mimulus* proteins were paired with their best *Arabidopsis* protein matches (blastp) at an e-value
327 cutoff of 10^-3. The set of expressed transcripts in our study was matched to the IM62 reference
328 genome genes, as described above, and corresponding GO terms were matched to the
329 *Arabidopsis* BLAST hits. Subsequent GO enrichment tests were done using the R
330 BIOCONDUCTOR package topGO (Alexa & Rahnenführer 2015). We used the Bioconductor
331 database 'org.At.tair.db' for annotation and the algorithm 'classic' (statistic = 'fisher') for
332 statistical tests. Because *P*-value distributions of GO enrichment tests do not typically conform to
333 a distribution amenable to Benjamini-Hoechberg FDR control, and because such control methods
334 can be overly conservative causing loss of valuable functional information (Alexa &
335 Rahnenführer 2015), we report GO terms with uncorrected enrichment *P*-values <= 0.01 (Table
336 S1). Enrichment tests were conducted at the level of genes rather than transcripts. For each gene
337 in the IM62 reference transcriptome, we determined the model effects for all matching expressed
338 transcripts in our data set. A model effect for a gene was considered significant if at least one
339 matching transcript had a significant effect. GO enrichment was then tested among all expressed
340 genes ($N = 9326$) with a significant effect vs. all expressed genes without a significant effect.
341 Enrichments for GxE effects were measured against all genes where either environmental or

342 allelic DE could be detected. Reaction norm plots were generated using the LSMean expression
343 level of all transcripts for each gene in each environment.
344
345 We conducted clustering analysis of gene expression within each parental genotype using the R
346 package WGCNA (Langfelder et al. 2008). Gene expression was quantified as the expression
347 level of the longest matching transcript for each gene. For all analyses, we calculated
348 dissimilarity of expression using a soft thresholding power of 6 and used the dynamicTreeCut
349 function to define modules with a minimum number of 30 genes. We calculated the correlation
350 of each cluster (eigengene) with habitat (Fig. S5). Gene ontology term enrichment was calculated
351 for all modules with a correlation value of $r > 0.75$ (Table S2).
352
353 ***Chromosomal Inversion Analyses***
354 Two inverted regions of the genome, one on chromosome 5 and one on chromosome 8, have
355 been linked to adaptive differences between inland and coastal ecotypes in previous studies
356 (Lowry & Willis 2010; Oneal et al. 2014; Twyford & Friedman 2015; Gould et al. 2017). When
357 examining genes in putatively inverted regions of the genome, we included reference genome
358 v1.1 scaffolds that are inferred to be in the inverted regions from a previous linkage mapping
359 study (Holeski et al. 2014). For the inversion on chromosome 5, we included v1.1 scaffolds 36,
360 149, 158, 170, 197, 266, 281, 288, 327, and 368. For the inversion on chromosome 8, we
361 included v1.1 scaffolds 11, 59, 76, 155 233, 604, and 1093. We tested whether genes in these
362 regions of the genome are enriched for any model effects. Enrichment tests of model effects
363 among annotated inversion and non-inversion genes were carried out using Fisher's Exact tests.
364 The magnitude of model effects among genes with significant effects were compared using

365 Wilcox rank sum tests in R. It should be noted that the presence of alternative orientations of
366 chromosome 8 inversion in the SS and LL parental lines has been confirmed by previous crosses
367 (Lowry & Willis 2010) while the chromosome 5 inversion was originally identified in a QTL
368 cross between different inland and coastal lines (Holeski et al. 2014). High differentiation
369 between the SS and LL parental lines in both inversion regions has been demonstrated by an
370 allele frequency outlier analysis (Gould et al. 2017).
371
372 ***Expression Heterosis***
373 We analyzed heterosis of gene expression separately for each field site using a model with a
374 single factor for genotype (SS/LL/SL). Data from the inland site was down-sampled to 12 lines
375 for each parental genotype to equalize power to detect effects between sites. Specific contrasts of
376 the F1 genotype (SL) were made to each parental genotype (LL/SS) using makeContrasts in
377 limma. Contrasts were judged significant if they fell below a Benjamini-Hoechberg false-
378 discovery rate corrected *P*-value of 0.05. Heterosis of gene expression for each transcript was
379 classified into 7 categories depending on contrasts between the parental genotype with lower
380 expression (LP), parental genotype with higher expression (HP), and the F1:
381 i. additive expression (no heterosis): LP<F1<HP
382 ii. low parent heterosis: F1<LP<HP
383 iii. below mid-parent heterosis: F1=LP<HP
384 iv. above mid-parent heterosis: LP<HP=F1
385 v. high parent heterosis: LP<HP<F1
386 vi. ambiguous: LP<HP, but LP=F1=HP
387 vii. no differential expression: LP=HP

388

389 Transcripts with ambiguous expression patterns were excluded from enrichment tests (Table S1).

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 and heterosis among the expressed candidate genes

397 in the current study. Enrichment was tested against all expressed non-candidate genes using

398 Fisher's Exact tests. The magnitude of model effects among genes with significant effects were

399 compared 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. On average, there were 43

409 polymorphisms (SNPs or indels) between each pair of alleles in the reference (Fig. S3B).

410

411 ***Differential Expression Analyses***

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

422

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

429

430 Most of the expression differences between the coastal and inland lines could be attributed to *cis*-
431 regulatory variation: 79% (5532 genes) of differentially expressed genes had *cis* regulatory
432 differences, while about half (3611 genes) of the differentially expressed genes had detectable
433 *trans* effects (Fig. 3A-C, Table 1). At both coastal and inland field sites, the magnitudes of *cis*

434 and *trans* regulatory differences were similar (Table S4). However, we detected far more
435 significant *trans* effects in the inland field site than the coastal field site (Fig. 3A-C, Table S4).
436 Overall, there was only moderate overlap between the sets of *cis*-regulated transcripts from the
437 two environments (Fig. S6). The same was true for the sets of *trans*-regulated transcripts from
438 the two environments.
439
440 ***Gene Ontology Enrichment and Expression Networks***
441 We explored the functional significance of genes with differential expression between
442 environments and between parental ecotypes. Differentially expressed genes in the coastal (SS)
443 parent between sites were enriched for glycosinolate/glucosinolate/S-glycoside biosynthesis and
444 metabolism, response to starvation, and others (Table S1). Differentially expressed genes in the
445 inland parent (LL) between sites were enriched for GO categories related to cuticle development,
446 meristem and floral development, and pigment production. Differentially expressed genes
447 between the parental lines (across sites) were enriched for genes involved with cell wall
448 formation, meristem initiation, and responses to viral pathogens. Genes with genotype x site
449 (GxE) interactions had the most significantly enriched GO terms ($N = 69$), including
450 brassinosteroid biosynthesis/metabolism, cell wall formation, regulation of development, and
451 pigment accumulation.
452
453 We used network clustering analysis to examine correlated expression of gene clusters (modules)
454 within habitats for the coastal and inland lines (Pearson's correlation, $r > 0.75$, Fig. S5). The
455 coastal perennial line (SS) preferentially expressed modules related to root and epidermal
456 development at its home site, while oxidative stress response, chlorophyll metabolism, and

457 circadian rhythm modules were highly expressed in inland habitat (Table S2). The inland annual
458 line preferentially expressed gene clusters related to nucleoside catabolism, sugar and starch
459 metabolism, pigment accumulation, and photoperiodism at the inland field site, but had no
460 modules strongly associated with the coastal field site.
461
462 ***Chromosomal Inversion Analyses***
463 Chromosomal inversions are thought to act as adaptation supergenes that hold together long
464 haplotypes containing multiple adaptive polymorphisms through suppressed recombination
465 (Kirkpatrick 2010; Joron et al. 2011; Thompson & Jiggins 2014). We were thus interested in
466 evaluating the hypothesis of whether the known inversions in this system hold together
467 haplotypes of differentially expressed genes. There were significantly more differentially
468 expressed genes inside the chromosome 5 inversion than expected by chance and there were
469 significantly more GxE interactions for genes in this inversion (Table 1). The adaptive (Lowry &
470 Willis 2010) chromosome 8 inversion also had significantly more differentially expressed genes,
471 but only at the coastal site (Table 2). Interestingly, the chromosome 8 inversion had a significant
472 enrichment of *cis*-eQTLs (Table 1), suggesting that it may maintain a higher level regulatory
473 divergence caused by the evolution of *cis*-regulatory elements.
474
475 ***Expression Heterosis***
476 Heterosis is commonly found in crosses among locally-adapted populations and can potentially
477 facilitate gene flow between those populations (Lowry et al. 2008; Charlesworth & Willis 2009;
478 Baack et al. 2016). Heterosis is typically quantified at the level of an individual trait, including
479 expression traits by comparing the trait value of hybrids to the trait values of the parents

480 (Springer & Stupar 2007a,b; Niemi et al. 2015; Lovell et al. 2016). Consistent with previous
481 studies (Lowry et al. 2008; Lowry & Willis 2010), we have found robust heterosis for multiple
482 phenotypic traits in F1 hybrids between coastal and inland lines (Fig. 1F). In the current study,
483 we also found nearly 73% of differentially expressed transcripts had heterotic (non-additive)
484 expression in the F1 (Fig. 3D, Table S5). The percentage of transcripts with heterotic expression
485 did not vary strongly by site (Fig 3D), although only 54% of heterotic genes were shared
486 between sites. Heterotic genes were enriched for functional terms associated with multiple
487 growth and development terms at both field sites (Table S6).
488
489 ***Patterns of Candidate Gene Expression***
490 To identify genes putatively linked to local adaptation, we cross-referenced our results with a
491 recent genomic outlier analysis, which identified 667 genes with a high level of allelic
492 differentiation between coastal and inland populations (Lowry et al. 2010). We found that the
493 magnitude of differential expression between lines and between field sites was significantly
494 elevated for candidate genes with highly differentiated SNPs (from Gould et al. 2017) in their
495 promoter regions (Table S7).
496
497 We were able to examine gene expression in seven top candidate genes highlighted by the outlier
498 analysis and/or previous QTL mapping studies (Fig. 3E-G, Fig. S7). The candidate gene *ABA1*
499 mediates production of abscisic acid, which controls responses to osmotic stress, including
500 drought (Xiong et al. 2002). An *ABA1* homolog, Migut.H00431, is located within the
501 chromosome 8 inversion and may contribute to inland ecotype's adaptation to low soil water
502 availability. *ABA1* was always more highly expressed in the inland line and was upregulated in

503 both lines at the inland field site (Fig. 3E). Previously, we showed that coastal *M. guttatus*
504 populations have evolved leaf tissue tolerance to oceanic salt spray (Lowry et al. 2009). Leaf salt
505 tolerance is generally mediated by a Na^+/H^+ antiporter (*SOS1*; Qiu et al. 2002), which is
506 activated by a calcium sensor (*CBL10*; Kim et al. 2007). Both *SOS1* (Migut.E00570) and *CBL10*
507 (Migut.A00138) were more highly expressed in the coastal line than the inland line across
508 habitats (Fig. 3G, F), a pattern consistent with elevated salt tolerance of coastal populations.
509

510 **DISCUSSION**

511 Overall, our results identified multiple key patterns regarding the relationship between gene
512 expression and the evolution of local adaptation. Differential expression is substantial between
513 locally-adapted accessions in nature. Expression plasticity of alleles across habitats (genotype x
514 environment interactions) appear to be common and may minimize fitness trade-offs at
515 individual loci that contribute to the overall pattern of local adaptation. A large portion of that
516 differential expression is due to *cis*-regulatory divergence. Chromosomal inversions appear to act
517 as supergenes by holding together haplotypes of differentially expressed genes, but this pattern
518 depends on habitat context. Heterosis of gene expression is pervasive and heterotic genes are
519 enriched for growth and developmental functions. We discuss each of these major findings as
520 well as the limitations and caveats associated with our study in detail below.
521

522 ***Sources of Variation in Gene Expression***

523 One of the major outstanding questions in local adaptation research is why the fitness effects of
524 individual loci on local adaptation are so often asymmetric across habitats. Theoretical models
525 (Levene 1953; Hedrick 1976, 1986; Dieckmann & Doebeli 1999) and studies in key model

526 systems (Abzhanov et al. 2004; Hoekstra et al. 2006; Barrett et al. 2009) have suggested trade-
527 offs at individual loci (e.g. antagonistic pleiotropy) to be common. However, field reciprocal
528 transplant experiments and experimental evolution studies have often found a pattern of
529 conditional neutrality, where loci have a strong effect on fitness in one environment, but have an
530 undetectable effect on fitness in alternative environments (reviewed in Bono et al. 2017;
531 Wadgymar et al. 2017). While a failure to statistically detect a trade-off across habitats does not
532 rule out the possibility of a small trade-off for an individual locus, it does suggest that adaptive
533 loci are often strongly asymmetric in their fitness effects across habitats.
534
535 Adaptive phenotypic plasticity mediated through gene regulatory divergence between locally
536 adapted populations is one possible mechanism by which trade-offs could be minimized across
537 habitats for individual loci (Rutherford 2003; Ghalambor et al. 2007; Des Marais et al. 2013,
538 2017; Dayan et al. 2015; Lohman et al. 2017). Adaptations of organisms to maintain homeostasis
539 when faced by environmental challenges can be caused by genotypic specific changes in gene
540 expression (Gibson 2008; Des Marais et al. 2017). However, such environmentally dependent
541 changes in gene expression would not be expected to result in a cost if gene expression did not
542 differ among genotypes in habitats that lacked particular environmental stresses. In our study, we
543 identified 1137 transcripts that were differentially expressed between the coastal perennial and
544 inland annual lines in one habitat (coast or inland), but were not differentially expressed in the
545 alternative habitat. We cannot definitively determine whether any of these genes are responsible
546 for asymmetries in fitness effects across habitats. Further, some of the genotype specific changes
547 in gene expression could potentially be maladaptive (Ghalambor et al. 2007). However, our
548 results do clearly show that habitat-specific differential gene expression is common for locally

549 adapted ecotypes. Future studies should focus on making the critical linkages between patterns of
550 gene expression and fitness in the field to determine whether asymmetries of fitness effects
551 across habitats are the 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). Indeed, the degree of allele
559 frequency differentiation in promoter regions between the coastal and inland ecotypes (Gould et
560 al. 2017) was predictive of differential expression in this study. Further, in terms of the
561 proportion of *cis* regulated genes out of all genes surveyed (59% for our study), our results are
562 not very different from other study systems. Mack et al. (2016) found significant *cis* effects for
563 68% of surveyed genes in crosses between house mice subspecies (*Mus musculus musculus* and
564 *M. m. domesticus*). Steige et al. (2015) found that 33-39% of genes surveyed had significant
565 allele-specific expression effects for hybrids between accessions of the plant *Capsella*
566 *grandiflora*.
567
568 ***Gene Regulatory Evolution and Chromosomal Inversions***
569 Chromosomal inversions have been thought to be involved in evolutionary adaptations since
570 classic studies in *Drosophila* found that inversion polymorphisms are frequently correlated with
571 environmental conditions over geographic space and through time (Dobzhansky 1951, 1970;

572 Kirpatrick & Barton 2006; Hoffman & Rieseberg 2008; Cheng et al. 2012; Ayala et al. 2013,
573 2014; Adrion et al. 2015). Inversions strongly suppress genetic recombination in heterokaryotic
574 (inversion heterozygotes) individuals because recombinant gametes are unbalanced (Dobzhansky
575 1970; Rieseberg 2001). Researchers have thus hypothesized that inversions could act as
576 supergenes by holding together long haplotypes containing multiple adaptive polymorphisms
577 through suppressed recombination (Dobzhansky 1970; Joron et al. 2011; Thompson & Jiggins
578 2014; Kirkpatrick & Barton 2006; Kirkpatrick & Barrett 2015).
579
580 The supergene hypothesis is a major reason why inversions are thought to frequently contribute
581 to the evolution of adaptations (Dobzhansky 1970; Kirkpatrick & Barton 2006; Hoffman &
582 Rieseberg 2008; Faria & Navarro 2009; Thompson & Jiggins 2014). Early researchers,
583 especially Dobzhansky (1970), argued that inversions could hold together co-adapted epistatic
584 complexes of genes through suppressed recombination. However, theoreticians have recently
585 shown that inversions can spread due to purely additive effects, when at least two locally
586 adaptive loci are captured within the inverted region (Kirkpatrick & Barton 2006; Kirkpatrick &
587 Barrett 2015). Regardless of the ultimate genetic mechanisms, these models all predict that
588 suppression of recombination by inversions maintains adaptive divergence at multiple linked
589 loci.
590
591 Global gene expression analyses offer one way to test whether inversions hold together
592 haplotypes of genetic variation potentially responsible for phenotypic divergence between locally
593 adapted populations. Research in a few systems has found evidence of increased levels of
594 expression divergence in inversions (Marquès-Bonet et al. 2004; Cassone et al. 2011; Fuller et al.

595 2016). For example, Marquès-Bonet et al. (2004) found 8.9% greater divergence (fold change) in
596 human versus chimp brain gene expression for chromosomal inversions when compared to the
597 rest of the genome. We did not observe a significant difference in fold change between inverted
598 and non-inverted regions in our study. However, genes within the region of the chromosome 5
599 inversion were 12.5% more likely to be differentially expressed. Genes within the adaptive
600 chromosome 8 inversion were 8.3% more likely to have a *cis*-eQTL.
601
602 The enrichment of gene expression differences in our study is consistent with patterns found in
603 inversions between chimpanzees and humans (Marquès-Bonet et al. 2004) and *Drosophila*
604 (Fuller et al. 2016), but contrasts with results for the plant *Boechera stricta* (Lee et al. 2017).
605 While the finding of an enrichment of differential gene expression for inversions in this study
606 and others is consistent with the supergene hypothesis of inversion evolution, it is by no means
607 definitive proof. Demonstrating that an inversion has evolved as an adaptation supergene
608 requires confirmation that multiple genes within the inversion have effects on adaptive
609 phenotypes (Hoffman & Rieseberg 2008; Kirkpatrick 2010; Kirkpatrick & Kern 2012). There is
610 currently no study that we are aware of which has experimentally confirmed that two or more
611 genes within an inversion contribute additively or epistatically to adaptive phenotypic
612 differences between locally adapted populations, ecotypes, or species (but see Kunte et al. 2014).
613
614 ***Heterosis of Gene Expression***
615 We have previously found that heterosis is common in F1 hybrids between coastal perennial and
616 inland annual accessions (Lowry et al. 2008; Lowry & Willis 2010). In this study, we observed a
617 high level of above mid-parent heterosis for gene expression. However, only a small percentage

618 (<1%) of genes had above high-parent or below low-parent heterosis. Heterosis often causes
619 increased growth and vigor (reviewed in Hochholdinger & Hoecker 2007; Charlesworth & Willis
620 2009). Consistent with this pattern, heterotic genes were enriched for functional terms associated
621 with growth and development at both field sites (Table S1). The enrichment of heterotic genes
622 involved with growth and development likely reflects the downstream responses of the ultimate
623 genetic causes of heterosis.
624
625 Even though heterosis is a well-documented phenomenon occurring in hybrids, the ultimate
626 causes of heterosis are still not well characterized (Hochholdinger & Hoecker 2007;
627 Charlesworth & Willis 2009). The dominance hypothesis postulates that heterosis is the result of
628 masking of deleterious recessive loci through complementation genome-wide in hybrids.
629 Alternatively, the overdominance hypothesis proposes that heterosis is the result of heterozygote
630 advantage at multiple loci across the genome. A third major alternative hypothesis is that
631 heterosis is conferred by epistatic interactions.
632
633 Some readers may question our use of the term heterosis to describe the observation of non-
634 additive gene expression in F1 hybrid individuals. Many in the field have used other terms to
635 describe non-additive gene expression in allele-specific expression studies, often labeling mid-
636 parent heterosis as "overdominance" and below mid-parent heterosis as "underdominance"
637 (Gibson et al. 2004; Swanson-Wagner et al. 2006; Rockman & Kruglyak 2006; Bell et al. 2013).
638 However, the use of overdominance in this way conflates the phenomena of heterosis with one of
639 the hypotheses for its ultimate cause (i.e. heterosis is conflated with the overdomiance
640 hypothesis; Hochholdinger & Hoecker 2007; Niemi et al. 2015). Further, it is important to

641 recognize that the quantification of heterosis is not limited to fitness and yield traits. In the plant
642 biology and crop literature, heterosis is routinely quantified for any trait measured in F1 hybrids,
643 including leaf shape, root morphology, and flowering time (Blum et al. 1977; Barth et al. 2003).
644 Gene expression is also a trait. Thus, heterosis can be quantified as transcript levels for
645 individual genes, as has been done in previous studies (Huang et al. 2006; Stupar & Springer
646 2006; Ji et al. 2014; Niemi et al. 2015; Lovell et al. 2016).
647
648 ***Caveats, Limitations, and Future Directions***
649 There are important caveats and concerns to consider in the interpretation of the results of our
650 study. One of the foremost caveats of the study is its narrow scope. We only evaluated gene
651 expression differences between one inbred line per ecotype, at two field sites, and for gene
652 expression at one time point. Despite these drawbacks, this is the first study that we are aware of
653 to evaluate allele-specific expression in the field between known locally adapted populations
654 (Voelckel et al. 2017), which allowed us to detect novel *cis* x habitat and *trans* x habitat
655 interaction (Fig. 3; Table S8). The major outstanding questions include how variable gene
656 expression is across populations, field sites, and tissue types? In the future, it will be critical to
657 evaluate gene expression at different time points and across tissue types to gain a more
658 comprehensive understanding of how ecotypic differences in gene expression manifest
659 throughout development under natural field conditions.
660
661 Perhaps, the greatest challenge for interpreting gene expression results from reciprocal transplant
662 experiments is understanding how patterns of expression actually contribute to phenotypes and
663 fitness across habitats. Fitness trade-offs that are associated with local adaptation could

664 potentially be caused by any type of gene expression pattern. Constitutive expression differences
665 between ecotypes could cause stable phenotypic differences that are beneficial in one habitat but
666 deleterious in another. For example, in our study, expression of *SOS1* does not appear to be
667 plastic in either ecotype and it may be a major contributor to the fitness trade-offs observed
668 between habitats (Fig. 3F). Gene expression characterized by genotype x environment
669 interactions could cause fitness trade-offs, but just as likely could mitigate trade-offs by helping
670 ecotypes maintain homeostasis across habitats.
671
672 Until recently, studies of gene regulation have for the most part been limited to the laboratory
673 setting, and coupling those studies with reciprocal transplant experiments to better understand
674 local adaptation is still rare (Kenkel & Matz 2016; Lohman et al. 2017; Voelckel et al. 2017).
675 Allele-specific expression is clearly an important tool for understanding the evolution of
676 regulatory differences between locally adapted populations, ecotypes, and species. However we
677 hope that future reciprocal transplant experiments will go beyond RNA-sequencing by also
678 examining genotypic differences in genome-wide patterns of chromatin remodeling and protein
679 translation across divergent habitats (Rodgers-Melnick et al. 2016; Voelckel et al. 2017).
680
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682
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689

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1077 **DATA ACCESSIBILITY**
1078 RNA-sequence data are deposited are available through the NCBI Sequence Read Archive under
1079 project identifier XX.
1080
1081 **AUTHOR CONTRIBUTIONS**
1082 DBL designed and conducted the field experiment. YC conducted the laboratory work. BAG
1083 analyzed the data. DBL and BAG wrote the manuscript.
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1090 **TABLES**

1091

1092 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

1093 *significantly greater percentage than non-inversion regions at p<0.05, **p<0.01.
1094 LFC: average magnitude (abs) log2 fold-change of genes with significant effects
1095

1096

1097

1098 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*** **

1099 *significantly greater than non-inversion regions at p<0.05

1100 *** significantly greater than the coastal field site, p<0.001

1101 LFC: average magnitude (abs) log2 fold-change of genes with significant effects

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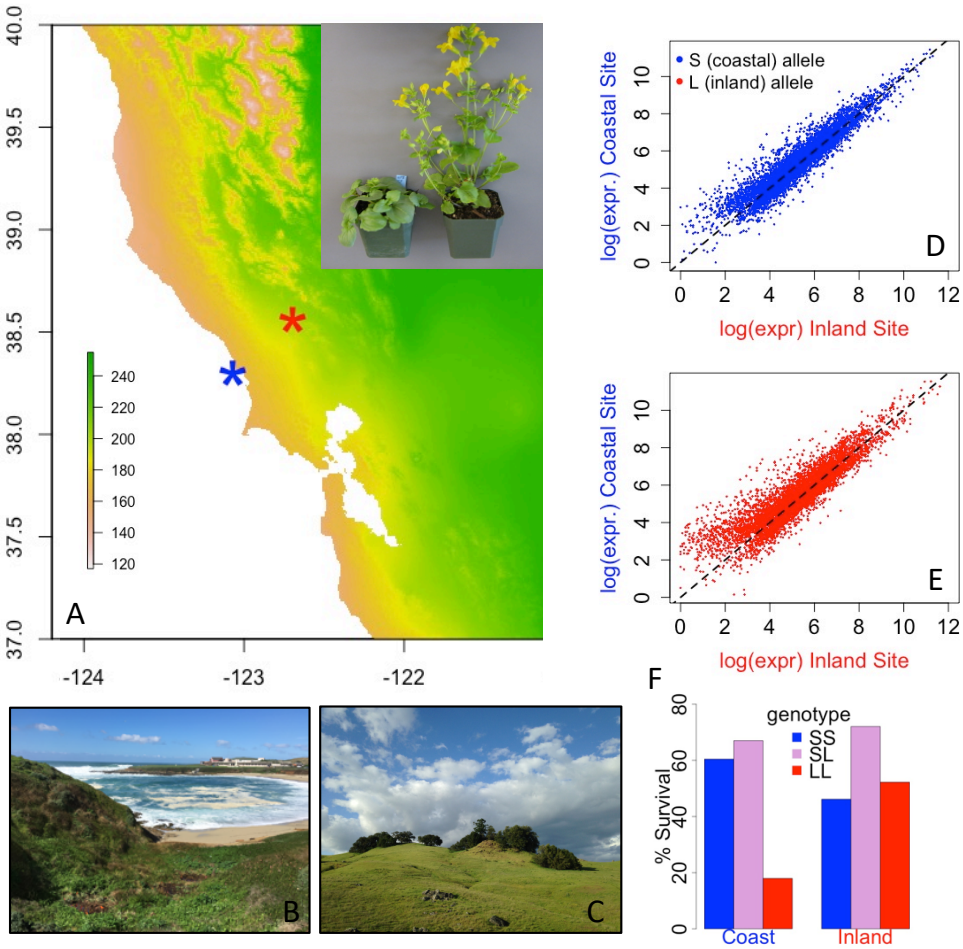

Figure 1. A) Map of Northern California with the location of two field reciprocal transplant sites. Colors represent mean temperature of the warmest quarter in °C x 10. Inset: left, coastal ecotype; right, inland ecotype. B) The coastal field site. C) The inland field site. D) SS parental gene expression 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.

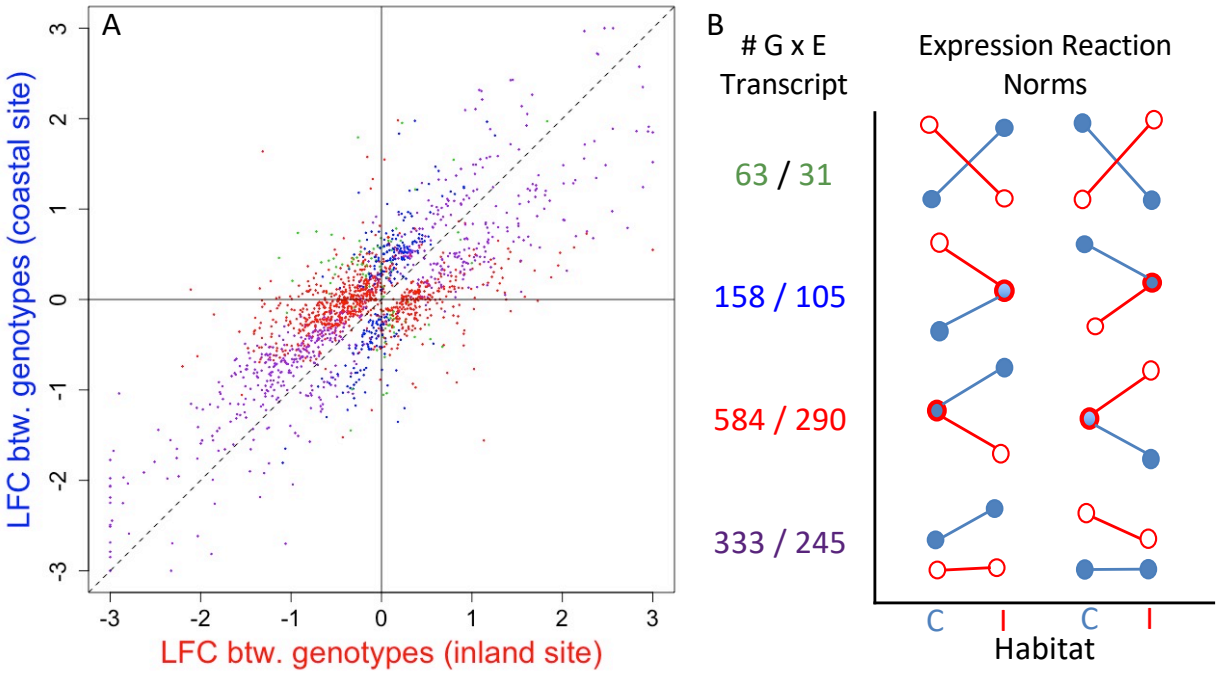

Figure 2. A) Average log2 fold-change (LFC) differences between parental genotypes for expressed transcripts at two field sites. B) Expression reaction norms corresponding to colored points in A and B. LFC has been restricted to <3 and <-3 for graphing purposes. Note, 28 transcripts with ambiguous expression patterns are not included.

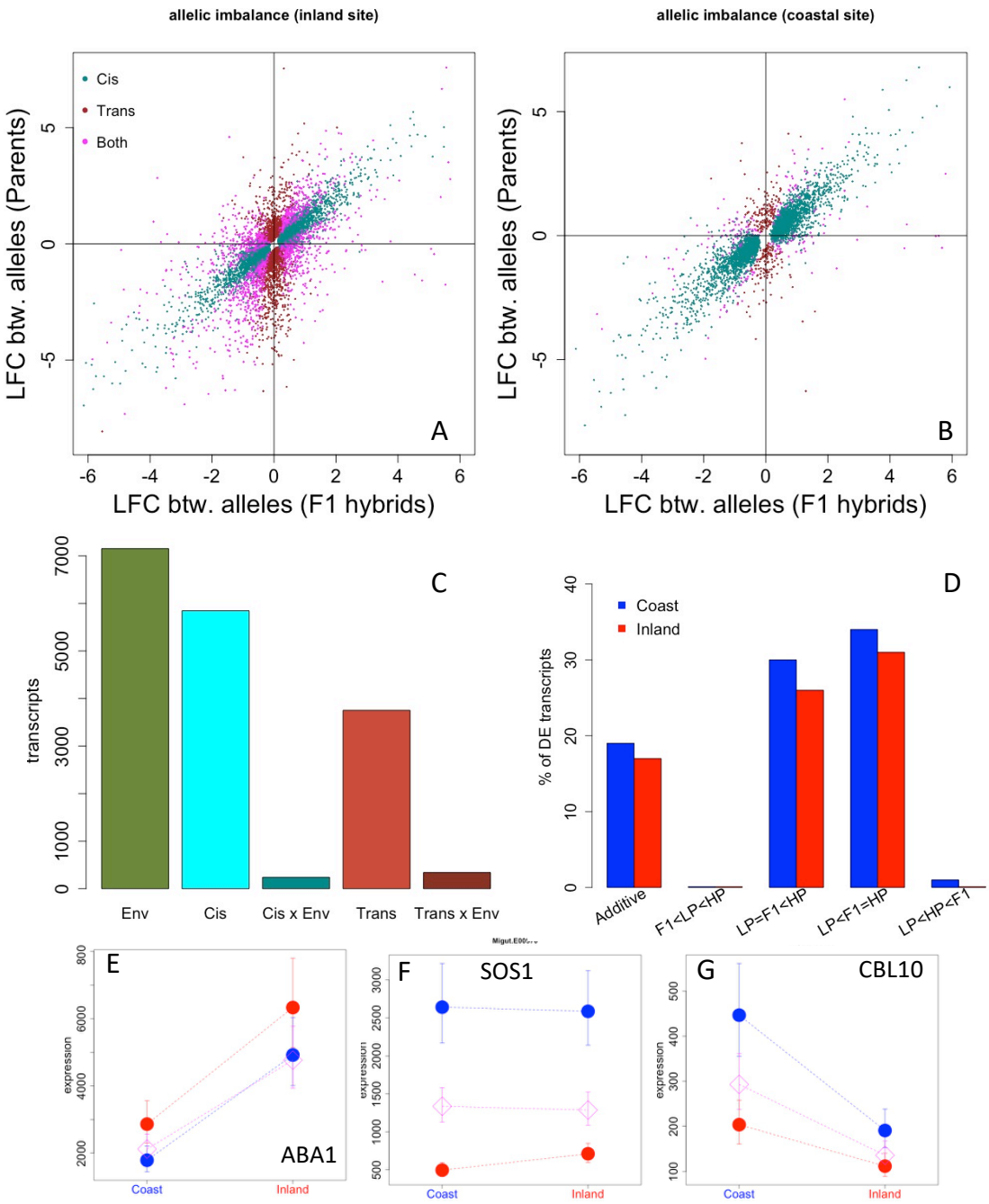

Figure 3. *Cis* and *trans* regulated transcripts detected at the coastal (A) and inland (B) field sites. Average log2-fold change (LFC) ratios are between inland and coastal alleles in the parental (y-axis) and F1 (x-axis) genetic backgrounds. C) The frequency of significant model effects among expressed genes. D) The frequency of expression heterosis in F1 plants at the inland and coastal field sites. LP, low parent; HP, high parent. E-G) Reaction norms of expression at the two field sites for three candidate adaptation genes. Red, inland parent; Blue, coastal parent; Pink, F1 .