1   **Gene Regulatory Divergence Between Locally Adapted Ecotypes in Their Native Habitats**

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

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6   [a]Department of Plant Biology, Michigan State University, East Lansing, MI 48824, USA

7   [b]Counsyl Inc., South San Francisco, CA 94108, USA

8   [c]Program in Ecology, Evolutionary Biology and Behavior, Michigan State University, East

9   Lansing, MI 48824, USA

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

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

17  Department of Plant Biology, Michigan State University, Plant Biology Laboratories, 612

18  Wilson Road, Room 166, East Lansing MI 48824, USA

19  Tel: (517) 432-4883

20  E-mail: dlowry@msu.edu

21

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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. 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

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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

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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

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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

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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 ($\pi_S$ = 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

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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***

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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

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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

14

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

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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

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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

17

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](http://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).

18

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

20

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***

21

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;

23

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

24

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

25

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

26

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

27

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

28

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***

29

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

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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**

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**DATA ACCESSIBILITY**

1123   RNA-sequence data are deposited are available through the NCBI Sequence Read Archive under

1124   project identifier XX.

1125

**AUTHOR CONTRIBUTIONS**

1127   DBL designed and conducted the field experiment. YC conducted the laboratory work. BAG

1128   analyzed the data. DBL and BAG wrote the manuscript.

1129

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
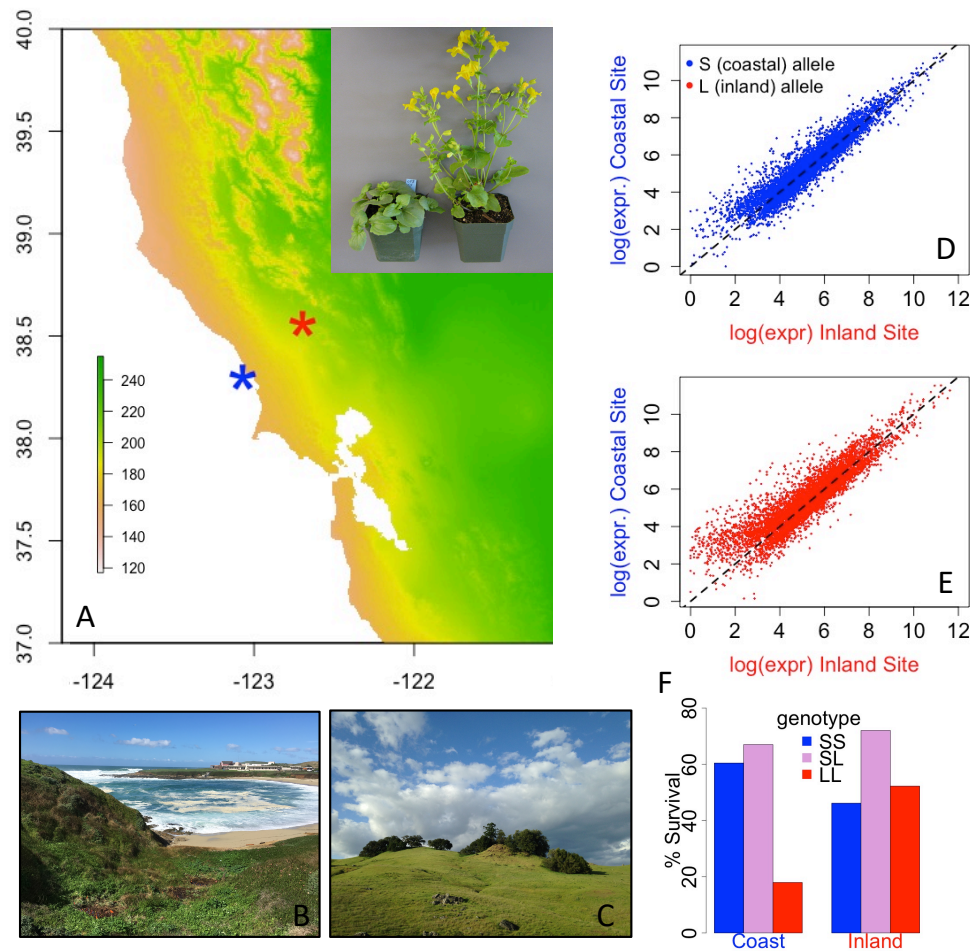1144

1145

51

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.
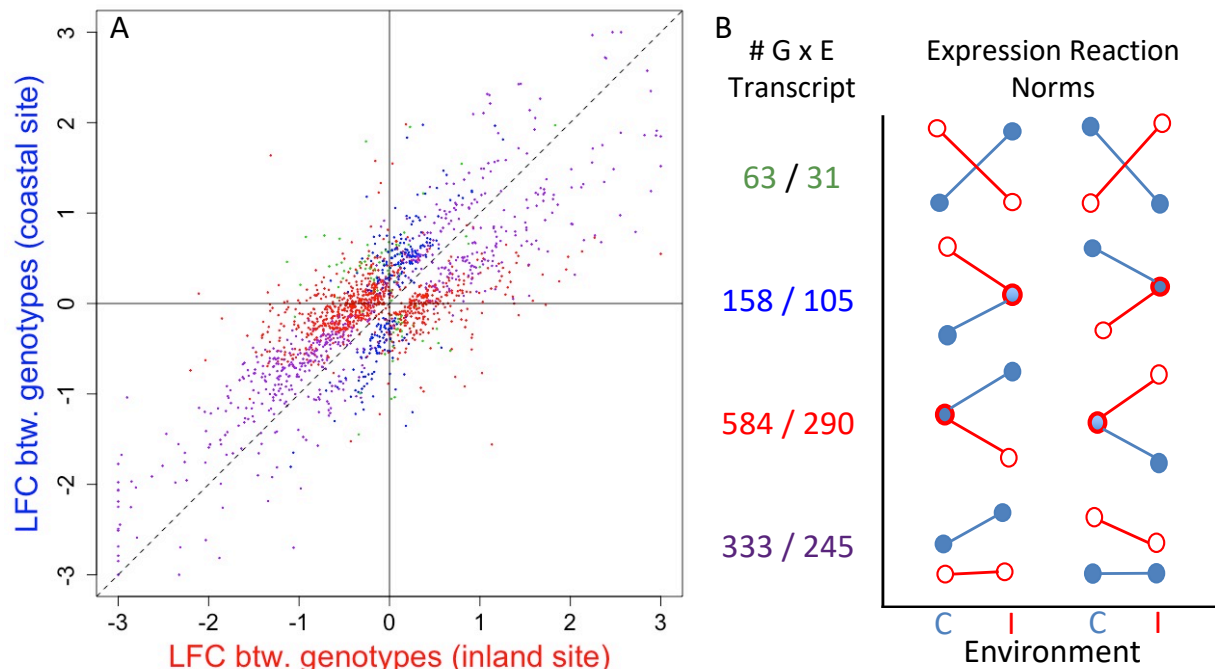
Fig. 2 A) Average log2 fold-change (LFC) differences between parental genotypes for expressed transcripts at the two field sites. B) Expression reaction norms for inland (red) and coastal (blue) lines. The numbers correspond to the colored points in part A of the figure. LFC has been restricted to <3 and <-3 for graphing purposes. Note, 28 transcripts with ambiguous expression patterns have been excluded.
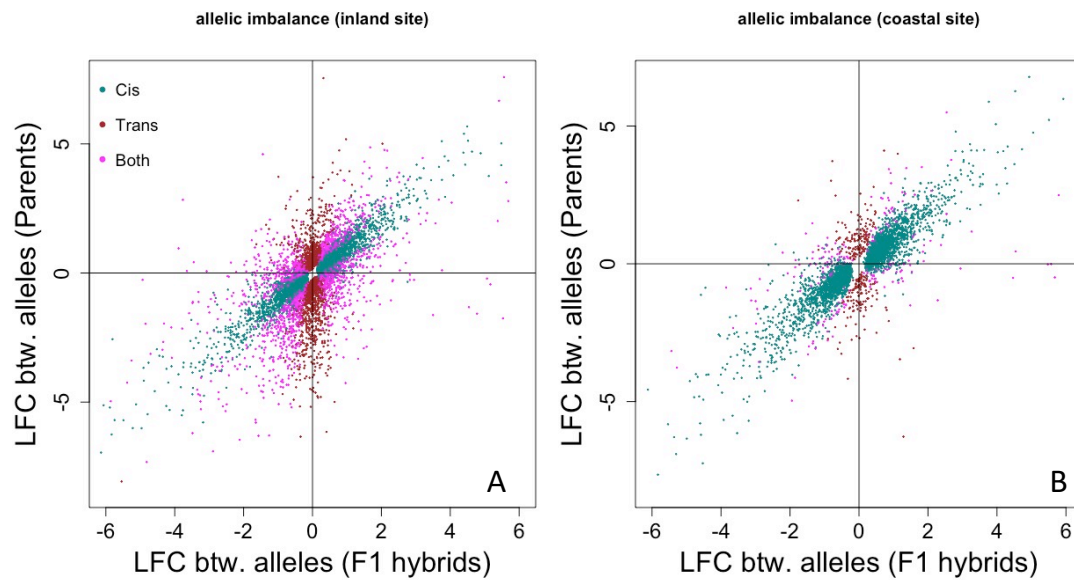
Fig.3. *Cis* and *trans* regulated transcripts detected at the coastal (A) and inland (B) field sites. Average log2-fold change (LFC) ratios are between inland and coastal alleles in the parental (y-axis) and F1 (x-axis) genetic backgrounds.
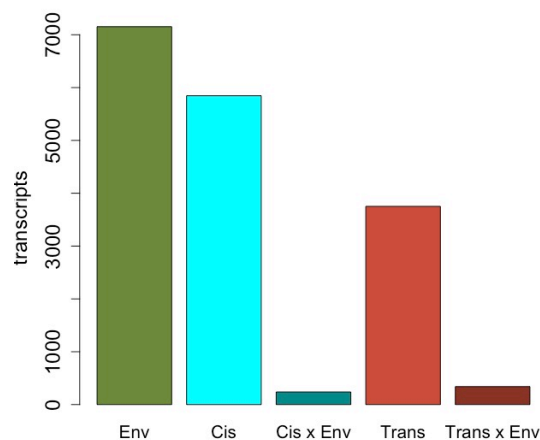


Fig.4. The frequency of significant model effects among all expressed transcripts.
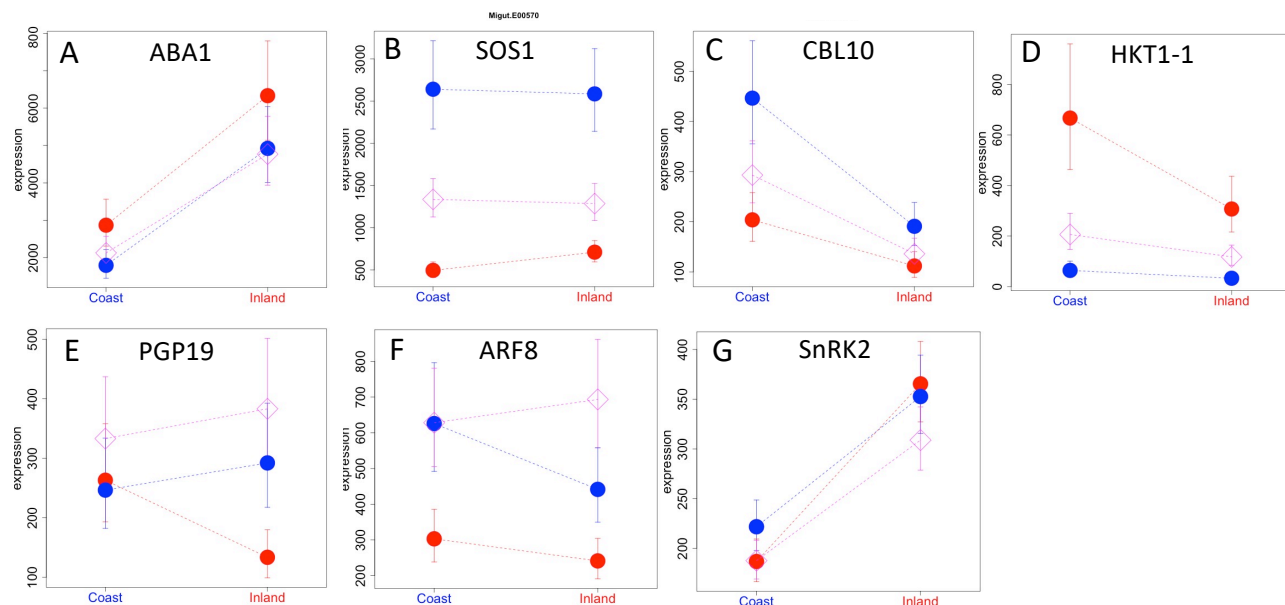
Fig.5 Reaction norms of expression at two field sites for seven candidate adaptive genes. A) ABA1 – abscisic acid deficient 1; B) SOS1 – salt overly-sensitive 1; C) CBL10 -calcineurin B-like protein 10; D) HKT1-1, high-affinity K+ transporter (functions in salt tolerance); E) PGP19, p-glycoprotein 19 (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.