# Gene Regulatory Divergence Between Locally Adapted Ecotypes in Their Native Habitats

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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 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). 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 potentially locally adaptive regulatory variation, 4) quantify gene
119 expression heterosis across habitats, and 5) identify candidate genes underlying local adaptation
120 in this system. The results of our study provide new insights into the gene regulatory evolution
121 and 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*, which was generated from an
187 inland annual line (IM62; Hellsten et al. 2013). Reference bias is of particular concern in *M.*
188 *guttatus* because pairwise polymorphism is generally high ($\pi_S$ = 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
197 four libraries constructed from field collected leaf tissue from the reciprocal transplant
198 experiment (one sample per parental line per field site). These libraries were included in the
199 transcriptome assembly pipeline to capture sequences from genes that may only be expressed
200 under field conditions. Completed libraries were quality controlled and quantified using a
201 combination of Qubit dsDNA HS, Caliper LabChipGX HS DNA, and Kapa Biosystems Illumina
202 Library Quantification qPCR assay kits. A total of 10 libraries were barcoded with dual index
203 barcodes and pooled together. The entire pool was sequenced using 125 bp paired-end reads on a
204 single 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 ($N$ = 16,243 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. Within each environment, we tested a model with the
309 following factors: allele (genotype), generation (F0/F1), and the interaction term. Any transcript
310 with any *cis* regulation had a significant allele effect (measured in the F1 generation only). Any
311 transcript with only *trans* regulation had a significant difference between generations, but a non-
312 significant allele effect in the F1 generation. Any transcript with both *cis* and *trans* regulation
313 had a significant generational effect and a significant allele effect in the F1.
314
315 To gauge the significance of *cis* x environment interactions, we tested the interaction term of a
316 model using only F1 data and the factors allele, environment, and interaction. To gauge the
317 significance of trans x environment interactions we modeled all the data (F0 and F1) and
318 measured the significance of a three-way interaction term: allele x generation x environment.
319

320 ***Gene Ontology Enrichment and Expression Networks***

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

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

365 cross between different inland and coastal lines (Holeski et al. 2014). High differentiation
366 between the SS and LL parental lines in both inversion regions has been demonstrated by an
367 allele frequency outlier analysis (Gould et al. 2017).
368
369 ***Expression Heterosis***
370 We analyzed heterosis of gene expression separately for each field site using a model with a
371 single factor for genotype (SS/LL/SL). Data from the inland site was down-sampled to 12 lines
372 for each parental genotype to equalize power to detect effects between sites. Specific contrasts of
373 the F1 genotype (SL) were made to each parental genotype (LL/SS) using makeContrasts in
374 limma. Contrasts were judged significant if they fell below a Benjamini-Hoechberg false-
375 discovery rate corrected *P*-value of 0.05. Heterosis of gene expression for each transcript was
376 classified into 7 categories depending on contrasts between the parental genotype with lower
377 expression (LP), parental genotype with higher expression (HP), and the F1:
378 i. additive expression (no heterosis): LP<F1<HP
379 ii. low parent heterosis: F1<LP<HP
380 iii. below mid-parent heterosis: F1=LP<HP
381 iv. above mid-parent heterosis: LP<HP=F1
382 v. high parent heterosis: LP<HP<F1
383 vi. ambiguous: LP<HP, but LP=F1=HP
384 vii. no differential expression: LP=HP
385
386 Transcripts with ambiguous expression patterns were excluded from enrichment tests (Table S1).
387

388 ***Patterns of Candidate Gene Expression***

389 In a study of species-wide genomic variation, Gould et al. (2017) identified a set of 667
390 candidate adaptation genes. These genes had unusually high differentiation in allele frequencies
391 between a set of 47 coastal and 50 inland *Mimulus* populations. High differentiation was detected
392 in either the genic or 1 kb upstream promoter regions of the genes, or both. We tested for
393 enrichment of differential expression patterns and heterosis among the expressed candidate genes
394 in the current study. Enrichment was tested against all expressed non-candidate genes using
395 Fisher's Exact tests. The magnitude of model effects among genes with significant effects were
396 compared using Wilcox rank sum tests in R.

397

398 **RESULTS**

399

400 ***Reference Transcriptome Assembly***

401 From the tissues of the two *M. guttatus* parental lines we were able to reconstruct both parental
402 alleles for 25,893 transcripts, of which 708 pairs were removed because they contained no
403 polymorphism between lines. Eighty-two percent of transcripts could be matched back to
404 previously annotated *M. guttatus* genes (www.phytozome.org). Our transcriptome reconstructed
405 54.0% of *M. guttatus* primary transcripts at 50% coverage or greater. On average, there were 43
406 polymorphisms (SNPs or indels) between each pair of alleles in the reference (Fig. S3B).

407

408 ***Differential Expression Analyses***

409 RNA-sequencing of plants from the field experiments produced approximately 25.9 million raw
410 reads per library (range 9.2-35.9 million reads). We were able to evaluate gene expression for

411 10,122 transcripts corresponding to 9,326 genes, after transcriptome reconstruction and filtering.
412 Significant expression differences (FDR = 0.05) between the inland and coastal field sites were
413 common (6765 genes; 73% of genes). Expression differences between parental genotypes within
414 field sites were also common (7213 genes; 77%; Table 1 and 2). We found 54% more genes
415 were differentially expressed between parental lines at the inland site than at the coastal site
416 (Table 2). We can attribute only part of this discrepancy to differences in power at the two sites.
417 Average power to detect DE genes was only slightly higher at the inland site than the coastal site
418 (75% vs. 68% for a minimum fold-change of 2X).
419
420 Genotype x site (GxE) interactions were relatively common. In total, 1837 transcripts
421 (corresponding to 19% of expressed genes, Table 1, Table S3) had significant GxE interactions,
422 where the magnitude of expression differences between parental ecotypes depended on the field
423 site in which they were grown (Fig. 2A). Only 94 transcripts had crossing reaction norms (green
424 points, Fig. 2B). More often, expression differences between genotypes were significant at one
425 site and non-significant at the other (1137 transcripts, 11%, blue and red points, Fig. 2).
426
427 Most of the expression differences between the coastal and inland lines could be attributed to *cis*-
428 regulatory variation: 79% (5532 genes) of differentially expressed genes had *cis* regulatory
429 differences, while about half (3611 genes) of the differentially expressed genes had detectable
430 *trans* effects (Fig. 3A-C, Table 1). At both coastal and inland field sites, the magnitudes of *cis*
431 and *trans* regulatory differences were similar (Table S4). However, we detected far more
432 significant *trans* effects in the inland field site than the coastal field site (Fig. 3A-C, Table S4).
433 Overall, there was only moderate overlap between the sets of *cis*-regulated transcripts from the

434 two environments (Fig. S6). The same was true for the sets of *trans*-regulated transcripts from
435 the two environments.
436
437 ***Gene Ontology Enrichment and Expression Networks***
438 We explored the functional significance of genes with differential expression between
439 environments and between parental ecotypes. Differentially expressed genes in the coastal (SS)
440 parent between sites were enriched for glycosinolate/glucosinolate/S-glycoside biosynthesis and
441 metabolism, response to starvation, and others (Table S1). Differentially expressed genes in the
442 inland parent (LL) between sites were enriched for GO categories related to cuticle development,
443 meristem and floral development, and pigment production. Differentially expressed genes
444 between the parental lines (across sites) were enriched for genes involved with cell wall
445 formation, meristem initiation, and responses to viral pathogens. Genes with genotype x site
446 (GxE) interactions had the most significantly enriched GO terms ($N = 69$), including
447 brassinosteroid biosynthesis/metabolism, cell wall formation, regulation of development, and
448 pigment accumulation.
449
450 We used network clustering analysis to examine correlated expression of gene clusters (modules)
451 within habitats for the coastal and inland lines (Pearson's correlation, $r > 0.75$, Fig. S5). The
452 coastal perennial line (SS) preferentially expressed modules related to root and epidermal
453 development at its home site, while oxidative stress response, chlorophyll metabolism, and
454 circadian rhythm modules were highly expressed in inland habitat (Table S2). The inland annual
455 line preferentially expressed gene clusters related to nucleoside catabolism, sugar and starch

456 metabolism, pigment accumulation, and photoperiodism at the inland field site, but had no
457 modules strongly associated with the coastal field site.
458
459 ***Chromosomal Inversion Analyses***
460 Chromosomal inversions are thought to act as adaptation supergenes that hold together long
461 haplotypes containing multiple adaptive polymorphisms through suppressed recombination
462 (Kirkpatrick 2010; Joron et al. 2011; Thompson & Jiggins 2014). We were thus interested in
463 evaluating the hypothesis of whether the known inversions in this system hold together
464 haplotypes of differentially expressed genes. There were significantly more differentially
465 expressed genes inside the chromosome 5 inversion than expected by chance and there were
466 significantly more GxE interactions for genes in this inversion (Table 1). The adaptive (Lowry &
467 Willis 2010) chromosome 8 inversion also had significantly more differentially expressed genes,
468 but only at the coastal site (Table 2). Interestingly, the chromosome 8 inversion had a significant
469 enrichment of *cis*-eQTLs (Table 1), suggesting that it may maintain a higher level regulatory
470 divergence caused by the evolution of *cis*-regulatory elements.
471
472 ***Expression Heterosis***
473 Heterosis is commonly found in crosses among locally-adapted populations and can potentially
474 facilitate gene flow between those populations (Lowry et al. 2008; Charlesworth & Willis 2009;
475 Baack et al. 2016). Heterosis is typically quantified at the level of an individual trait, including
476 expression traits by comparing the trait value of hybrids to the trait values of the parents
477 (Springer & Stupar 2007a,b; Niemi et al. 2015; Lovell et al. 2016). Consistent with previous
478 studies (Lowry et al. 2008; Lowry & Willis 2010), we have found robust heterosis for multiple

479 phenotypic traits in F1 hybrids between coastal and inland lines (Fig. 1F). In the current study,
480 we also found nearly 73% of differentially expressed transcripts had heterotic (non-additive)
481 expression in the F1 (Fig. 3D, Table S5). The percentage of transcripts with heterotic expression
482 did not vary strongly by site (Fig 3D), although only 54% of heterotic genes were shared
483 between sites. Heterotic genes were enriched for functional terms associated with multiple
484 growth and development terms at both field sites (Table S6).
485
486 ***Patterns of Candidate Gene Expression***
487 To identify genes putatively linked to local adaptation, we cross-referenced our results with a
488 recent genomic outlier analysis, which identified 667 genes with a high level of allelic
489 differentiation between coastal and inland populations (Lowry et al. 2010). We found that the
490 magnitude of differential expression between lines and between field sites was significantly
491 elevated for candidate genes with highly differentiated SNPs (from Gould et al. 2017) in their
492 promoter regions (Table S7).
493
494 We were able to examine gene expression in seven top candidate genes highlighted by the outlier
495 analysis and/or previous QTL mapping studies (Fig. 3E-G, Fig. S7). The candidate gene *ABA1*
496 mediates production of abscisic acid, which controls responses to osmotic stress, including
497 drought (Xiong et al. 2002). An *ABA1* homolog, Migut.H00431, is located within the
498 chromosome 8 inversion and may contribute to inland ecotype's adaptation to low soil water
499 availability. *ABA1* was always more highly expressed in the inland line and was upregulated in
500 both lines at the inland field site (Fig. 3E). Previously, we showed that coastal *M. guttatus*
501 populations have evolved leaf tissue tolerance to oceanic salt spray (Lowry et al. 2009). Leaf salt

502 tolerance is generally mediated by a $Na^+/H^+$ antiporter (*SOS1*; Qiu et al. 2002), which is
503 activated by a calcium sensor (*CBL10*; Kim et al. 2007). Both *SOS1* (Migut.E00570) and *CBL10*
504 (Migut.A00138) were more highly expressed in the coastal line than the inland line across
505 habitats (Fig. 3G, F), a pattern consistent with elevated salt tolerance of coastal populations.
506

507 **DISCUSSION**

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

519 ***Sources of Variation in Gene Expression***

520 One of the major outstanding questions in local adaptation research is why the fitness effects of
521 individual loci on local adaptation are so often asymmetric across habitats. Theoretical models
522 (Levene 1953; Hedrick 1976, 1986; Dieckmann & Doebeli 1999) and studies in key model
523 systems (Abzhanov et al. 2004; Hoekstra et al. 2006; Barrett et al. 2009) have suggested trade-
524 offs at individual loci (e.g. antagonistic pleiotropy) to be common. However, field reciprocal

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

547 gene expression and fitness in the field to determine whether asymmetries of fitness effects
548 across habitats are the result of habitat-specific differential gene expression.
549
550 Overall, *cis*-regulatory evolution was responsible for at least 79% of the differentially expressed
551 genes. This is about twice the percentage of *cis*-regulated differentially expressed genes for
552 crosses both within and between *Drosophilia* species (Coolon et al. 2014). However, the
553 relatively high proportion of *cis*-regulatory divergence underlying differentially expressed genes
554 is not too surprising, given the high level of polymorphism among *M. guttatus* accessions
555 (Brandvain et al. 2014; Gould et al. 2017; Puzey et al. 2017). Indeed, the degree of allele
556 frequency differentiation in promoter regions between the coastal and inland ecotypes (Gould et
557 al. 2017) was predictive of differential expression in this study. Further, in terms of the
558 proportion of *cis* regulated genes out of all genes surveyed (59% for our study), our results are
559 not very different from other study systems. Mack et al. (2016) found significant *cis* effects for
560 68% of surveyed genes in crosses between house mice subspecies (*Mus musculus musculus* and
561 *M. m. domesticus*). Steige et al. (2015) found that 33-39% of genes surveyed had significant
562 allele-specific expression effects for hybrids between accessions of the plant *Capsella*
563 *grandiflora*.
564
565 ***Gene Regulatory Evolution and Chromosomal Inversions***
566 Chromosomal inversions have been thought to be involved in evolutionary adaptations since
567 classic studies in *Drosophila* found that inversion polymorphisms are frequently correlated with
568 environmental conditions over geographic space and through time (Dobzhansky 1951, 1970;
569 Kirpatrick & Barton 2006; Hoffman & Rieseberg 2008; Cheng et al. 2012; Ayala et al. 2013,

570 2014; Adrion et al. 2015). Inversions strongly suppress genetic recombination in heterokaryotic
571 (inversion heterozygotes) individuals because recombinant gametes are unbalanced (Dobzhansky
572 1970; Rieseberg 2001). Researchers have thus hypothesized that inversions could act as
573 supergenes by holding together long haplotypes containing multiple adaptive polymorphisms
574 through suppressed recombination (Dobzhansky 1970; Joron et al. 2011; Thompson & Jiggins
575 2014; Kirkpatrick & Barton 2006; Kirkpatrick & Barrett 2015).
576
577 The supergene hypothesis is a major reason why inversions are thought to frequently contribute
578 to the evolution of adaptations (Dobzhansky 1970; Kirkpatrick & Barton 2006; Hoffman &
579 Rieseberg 2008; Faria & Navarro 2009; Thompson & Jiggins 2014). Early researchers,
580 especially Dobzhansky (1970), argued that inversions could hold together co-adapted epistatic
581 complexes of genes through suppressed recombination. However, theoreticians have recently
582 shown that inversions can spread due to purely additive effects, when at least two locally
583 adaptive loci are captured within the inverted region (Kirkpatrick & Barton 2006; Kirkpatrick &
584 Barrett 2015). Regardless of the ultimate genetic mechanisms, these models all predict that
585 suppression of recombination by inversions maintains adaptive divergence at multiple linked
586 loci.
587
588 Global gene expression analyses offer one way to test whether inversions hold together
589 haplotypes of genetic variation potentially responsible for phenotypic divergence between locally
590 adapted populations. Research in a few systems has found evidence of increased levels of
591 expression divergence in inversions (Marquès-Bonet et al. 2004; Cassone et al. 2011; Fuller et al.
592 2016). For example, Marquès-Bonet et al. (2004) found 8.9% greater divergence (fold change) in

593 human versus chimp brain gene expression for chromosomal inversions when compared to the
594 rest of the genome. We did not observe a significant difference in fold change between inverted
595 and non-inverted regions in our study. However, genes within the region of the chromosome 5
596 inversion were 12.5% more likely to be differentially expressed. Genes within the adaptive
597 chromosome 8 inversion were 8.3% more likely to have a *cis*-eQTL.
598
599 The enrichment of gene expression differences in our study is consistent with patterns found in
600 inversions between chimpanzees and humans (Marquès-Bonet et al. 2004) and *Drosophila*
601 (Fuller et al. 2016), but contrasts with results for the plant *Boechera stricta* (Lee et al. 2017).
602 While the finding of an enrichment of differential gene expression for inversions in this study
603 and others is consistent with the supergene hypothesis of inversion evolution, it is by no means
604 definitive proof. Demonstrating that an inversion has evolved as an adaptation supergene
605 requires confirmation that multiple genes within the inversion have effects on adaptive
606 phenotypes (Hoffman & Rieseberg 2008; Kirkpatrick 2010; Kirkpatrick & Kern 2012). There is
607 currently no study that we are aware of which has experimentally confirmed that two or more
608 genes within an inversion contribute additively or epistatically to adaptive phenotypic
609 differences between locally adapted populations, ecotypes, or species (but see Kunte et al. 2014).
610
611 ***Heterosis of Gene Expression***
612 We have previously found that heterosis is common in F1 hybrids between coastal perennial and
613 inland annual accessions (Lowry et al. 2008; Lowry & Willis 2010). In this study, we observed a
614 high level of above mid-parent heterosis for gene expression. However, only a small percentage
615 (<1%) of genes had above high-parent or below low-parent heterosis. Heterosis often causes

616 increased growth and vigor (reviewed in Hochholdinger & Hoecker 2007; Charlesworth & Willis
617 2009). Consistent with this pattern, heterotic genes were enriched for functional terms associated
618 with growth and development at both field sites (Table S1). The enrichment of heterotic genes
619 involved with growth and development likely reflects the downstream responses of the ultimate
620 genetic causes of heterosis.
621
622 Even though heterosis is a well-documented phenomenon occurring in hybrids, the ultimate
623 causes of heterosis are still not well characterized (Hochholdinger & Hoecker 2007;
624 Charlesworth & Willis 2009). The dominance hypothesis postulates that heterosis is the result of
625 masking of deleterious recessive loci through complementation genome-wide in hybrids.
626 Alternatively, the overdominance hypothesis proposes that heterosis is the result of heterozygote
627 advantage at multiple loci across the genome. A third major alternative hypothesis is that
628 heterosis is conferred by epistatic interactions.
629
630 Some readers may question our use of the term heterosis to describe the observation of non-
631 additive gene expression in F1 hybrid individuals. Many in the field have used other terms to
632 describe non-additive gene expression in allele-specific expression studies, often labeling above
633 mid-parent heterosis as "overdominance" and below mid-parent heterosis as "underdominance"
634 (Gibson et al. 2004; Swanson-Wagner et al. 2006; Rockman & Kruglyak 2006; Bell et al. 2013).
635 However, the use of overdominance in this way conflates the phenomena of heterosis with one of
636 the hypotheses for its ultimate cause (i.e. heterosis is conflated with the overdomiance
637 hypothesis; Hochholdinger & Hoecker 2007; Niemi et al. 2015). Further, it is important to
638 recognize that the quantification of heterosis is not limited to fitness and yield traits. In the plant

639 biology and crop literature, heterosis is routinely quantified for any trait measured in F1 hybrids,
640 including leaf shape, root morphology, and flowering time (Blum et al. 1977; Barth et al. 2003).
641 Gene expression is also a trait. Thus, heterosis can be quantified as transcript levels for
642 individual genes, as has been done in previous studies (Huang et al. 2006; Stupar & Springer
643 2006; Ji et al. 2014; Niemi et al. 2015; Lovell et al. 2016).
644
645 ***Caveats, Limitations, and Future Directions***
646 There are important caveats and concerns to consider in the interpretation of the results of our
647 study. One of the foremost caveats of the study is its narrow scope. We only evaluated gene
648 expression differences between one inbred line per ecotype, at two field sites, and for gene
649 expression at one time point. Despite these drawbacks, this is the first study that we are aware of
650 to evaluate allele-specific expression in the field between known locally adapted populations
651 (Voelckel et al. 2017), which allowed us to detect novel *cis* x habitat and *trans* x habitat
652 interaction (Fig. 3; Table S8). The major outstanding questions include how variable gene
653 expression is across populations, field sites, and tissue types? In the future, it will be critical to
654 evaluate gene expression at different time points and across tissue types to gain a more
655 comprehensive understanding of how ecotypic differences in gene expression manifest
656 throughout development under natural field conditions.
657
658 Perhaps, the greatest challenge for interpreting gene expression results from reciprocal transplant
659 experiments is understanding how patterns of expression actually contribute to phenotypes and
660 fitness across habitats. Fitness trade-offs that are associated with local adaptation could
661 potentially be caused by any type of gene expression pattern. Constitutive expression differences

662 between ecotypes could cause stable phenotypic differences that are beneficial in one habitat but
663 deleterious in another. For example, in our study, expression of *SOS1* does not appear to be
664 plastic in either ecotype and it may be a major contributor to the fitness trade-offs observed
665 between habitats (Fig. 3F). Gene expression characterized by genotype x environment
666 interactions could cause fitness trade-offs, but just as likely could mitigate trade-offs by helping
667 ecotypes maintain homeostasis across habitats.
668
669 Until recently, studies of gene regulation have for the most part been limited to the laboratory
670 setting. Coupling those studies with reciprocal transplant experiments to better understand local
671 adaptation is still rare (Kenkel & Matz 2016; Lohman et al. 2017; Voelckel et al. 2017). Allele-
672 specific expression is clearly an important tool for understanding the evolution of regulatory
673 differences between locally adapted populations, ecotypes, and species. However, we hope that
674 future reciprocal transplant experiments will go beyond RNA-sequencing by also examining
675 genotypic differences in genome-wide patterns of chromatin remodeling and protein translation
676 across divergent habitats (Rodgers-Melnick et al. 2016; Voelckel et al. 2017).
677
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679
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686

687 **REFERENCES**

688

689 Abzhanov A, Protas M, Grant BR *et al.* (2004) Bmp4 and morphological variation of beaks in
690 Darwin's finches. *Science,* **305,** 1462-1465.

691

692 Adrion JR, Hahn MW, Cooper BS (2015) Revisiting classic clines in *Drosophila melanogaster*
693 in the age of genomics. *Trends in Genetics*, **31**, 434-444.

694

695 Aguilar-Rangel MR, Montes RA, González-Segovia E *et al.* (2017) Allele specific expression
696 analysis identifies regulatory variation associated with stress-related genes in the Mexican
697 highland maize landrace Palomero Toluqueño. *PeerJ,* **23**, 5e3737.

698

699 Ågren, J, Oakley CG, McKay JK *et al.* (2013) Genetic mapping of adaptation reveals fitness
700 trade-offs in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences,* **110**,
701 21077-21087.

702

703 Alexa A, Rahnenführer J (2015) Gene set enrichment analysis with topGO. http://www.mpi-
704 sb.mpg.de/~alexa

705

706 Anderson JT, Willis JH, Mitchell-Olds T (2011) Evolutionary genetics of plant adaptation.
707 *Trends in Genetics,* **27**, 258-266.
708
709 Anderson JT, Lee CR, Rushworth C *et al.* (2013) Genetic tradeoffs and conditional neutrality
710 contribute to local adaptation. *Molecular Ecology*, **22**, 699-708.
711
712 Andrews S (2015) FastQC v0.11.3. A quality control tool for high throughput sequence data.
713 http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
714
715 Ayala D, Guerrero RF, Kirkpatrick M (2013) Reproductive isolation and local adaptation
716 quantified for a chromosome inversion in a malaria mosquito. *Evolution,* **67**, 946-958.
717
718 Baack E, Melo MC, Rieseberg LH, *et al.* (2016) The origins of reproductive isolation in plants.
719 *New Phytol,* **207,** 207, 968-984.
720
721 Barrett RD, Rogers SM, Schluter D (2009) Environment specific pleiotropy facilitates
722 divergence at the Ectodysplasin locus in threespine stickleback. *Evolution*, **63**, 2831-2837.
723
724 Barth S, Busimi AK, Utz HF, Melchinger AE (2003) Heterosis for biomass yield and related
725 traits in five hybrids of *Arabidopsis thaliana* L. Heynh. *Heredity,* **1**, 36-42.
726
727 Bi R, Liu P (2016) Sample size calculation while controlling false discovery rate for differential
728 expression analysis with RNA-sequencing experiments. BMC Bioinformatics, **17**, 146.

729

730 Bell GD, Kane NC, Rieseberg LH, Adams KL (2013) RNA-seq analysis of allele-specific
731 expression, hybrid effects, and regulatory divergence in hybrids compared with their parents
732 from natural populations. *Genome Biology and Evolution,* **5**, 1309-1323.

733

734 Blum A, Jordan WR, Arkin GF (1977) Sorghum root morphogenesis and growth. II.
735 Manifestation of heterosis. *Crop Science*, **17**, 153-157.

736

737 Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence
738 data. *Bioinformatics,* **30**, 2114-2120.

739

740 Bono LM, Smith LB, Pfennig DW, Burch CL (2017) The emergence of performance trade-offs
741 during local adaptation: insights from experimental evolution. *Molecular Ecology*, **26**, 1720-
742 1733.

743

744 Brandvian Y, Kenney AM, Flagel L *et al.* (2014) Speciation and introgression between *Mimulus*
745 *nasutus* and *Mimulus guttatus*. *PLoS Genetics,* **10**, e1004410.

746

747 Cassone BJ, Molloy MJ, Cheng C *et al.* (2011) Divergent transcriptional response to thermal
748 stress by *Anopheles gambiae* larvae carrying alternative arrangements of inversion 2La.
749 *Molecular Ecology,* **20**, 2567-2580.

750

751 Charlesworth D, Willis JH (2009) The genetics of inbreeding depression. *Nature Review*
752 *Genetics,* **10**, 783-796.
753
754 Cheng C, White BJ, Kamdem C *et al.* (2012) Ecological genomics of *Anopheles gambiae* along a
755 latitudinal cline: a population-resequencing approach. *Genetics,* **190**, 1417-1432.
756
757 Colosimo PF, Hosemann KE, Balabhadra S *et al.* (2005) Widespread parallel evolution in
758 sticklebacks by repeated fixation of ectodysplasin alleles. *Science,* **307**, 1928-1933.
759
760 Coolon JD, McManus CJ, Stevenson KR *et al.* (2014) Tempo and mode of regulatory evolution
761 in Drosophila. *Genome Research,* **24**, 797-808.
762
763 Clausen J (1951) Stages in the evolution of plant species. *Cornell University Press*. Ithaca NY,
764 USA.
765
766 Corbin JD, Thomsen MA, Dawson TE, D'Antonio CM (2005) Summer water use by California
767 coastal prairie grasses: fog, drought, and community composition. *Oecologia*, **145**, 511–521.
768
769 Cowles CR, Hirschhorn JN, Altshuler D *et al.* (2002) Detection of regulatory variation in mouse
770 genes. *Nature Genetics,* **32**, 432-437.
771
772 Cubillos FA, Stegle O, Grondin C *et al.* (2014) Extensive *cis*-regulatory variation robust to
773 environmental perturbation in *Arabidopsis*. *Plant Cell,* **26**, 4298-4310.

774

775 Dayan DI, Crawford DL, Oleksiak MF (2015) Phenotypic plasticity in gene expression
776 contributes to divergence of locally adapted populations of *Fundulus heteroclitus*. *Molecular*
777 *Ecology*, **24**, 3345-3359.

778

779 de Meaux J, Goebel U, Pop A *et al.* (2005) Allele-specific assay reveals functional variation in
780 the chalcone synthase promoter of *Arabidopsis thaliana* that is compatible with neutral
781 evolution. *Plant Cell,* **17**, 676-690.

782

783 Des Marais DL, Hernandez KM, Juenger TE (2013) Genotype-by-environment interaction and
784 plasticity: exploring genomic responses of plants to the abiotic environment. *Annual Review of*
785 *Ecology, Evolution, and Systematics*, **44**, 5-29.

786

787 Des Marais DL, Guerrero RF, Lasky JR, Scarpino SV (2017) Topological features of a gene co-
788 expression network predict patterns of natural diversity in environmental response. *Proceedings*
789 *of the Royal Society B*, **284**, 20170914.

790

791 Dieckmann U, Doebeli M (1999) On the origin of species by sympatric speciation. *Nature*, **400**,
792 354–357.

793

794 Dobzhansky T. 1951. Genetics and the Origin of Species (3rd ed.). *Columbia University Press.*
795 New York, NY, USA.

796

797 Dobzhansky T. 1970. Genetics of the Evolutionary Process. *Columbia University Press*. New
798 York, NY, USA.
799
800 Faria R, Navarro A (2010) Chromosomal speciation revisited: rearranging theory with pieces of
801 evidence. *Trends in Ecology & Evolution,* **25**, 660-669.
802
803 Fuller ZL, Haynes GD, Richards S *et al.* (2016) Genomics of natural populations: how
804 differentially expressed genes shape the evolution of chromosomal inversions in *Drosophila*
805 *pseudoobscura*. *Genetics,* **204**, 287-301.
806
807 Friedman J (2014) Genetic determinants and epistasis for life history trait differences in the
808 common monkeyflower, *Mimulus guttatus*. *Journal of Heredity,* **105**, 816-827.
809
810 Friedman J, Twyford AD, Willis JH *et al.* (2015) The extent and genetic basis of phenotypic
811 divergence in life history traits in *Mimulus guttatus*. *Molecular Ecology*, **24**, 111-122.
812
813 Gardner KM, Latta RG (2006) Identifying loci under selection across contrasting environments
814 in *Avena barbata* using quantitative trait locus mapping. *Molecular Ecology,* **15,** 1321-1333.
815
816 Ghalambor CK, McKay JK, Carroll SP *et al.* (2007) Adaptive versus non-adaptive phenotypic
817 plasticity and the potential for contemporary adaptation in new environments. *Functional*
818 *Ecology,* **21**, 394-407.
819

820 Gibson G (2008) The environmental contribution to gene expression profiles. *Nature Reviews*
821 *Genetics,* **9**, 575-581.
822
823 Gibson G, Riley-Berger R, Harshman L *et al.* (2004) Extensive sex-specific nonadditivity of
824 gene expression in *Drosophila melanogaster*. *Genetics,* **167**, 1791-1799.
825
826 Gould BA, Chen Y, Lowry DB (2017) Pooled ecotype sequencing reveals candidate genetic
827 mechanisms for adaptive differentiation and reproductive isolation. *Molecular Ecology,* **26**, 163-
828 177.
829
830 Grabherr MG, Haas BJ, Yassour M *et al.* (2011) Full-length transcriptome assembly from RNA-
831 Seq data without a reference genome. *Nature Biotechnology,* **29**, 644-652.
832
833 Hall MC, Willis JH (2006) Divergent selection on flowering time contributes to local adaptation
834 in *Mimulus guttatus* populations. *Evolution,* **60**, 2466-2477.
835
836 Hall MC, Basten C, Willis JH (2006) Pleiotropic quantitative trait loci contribute to population
837 divergence in traits associated with life-history variation in *Mimulus guttatus*. *Genetics,* **172**,
838 1829-1844.
839
840 Hall MC, Lowry DB, Willis JH (2010) Is local adaptation in *Mimulus guttatus* caused by
841 tradeoffs at individual loci? *Molecular Ecology* **19**, 2739-2753.
842

843 Hedrick PW, Ginevan ME, Ewing EP. 1976. Genetic polymorphism in heterogeneous
844 environments. *Annual Review of Ecology and Systematics*, **7**, 1-32.
845
846 Hedrick PW (1986) Genetic polymorphism in heterogeneous environments: a decade later.
847 *Annual Review of Ecology and Systematics*, **17**, 535-566.
848
849 Hellsten U, Wright KM, Jenkins J *et al.* (2013) Fine-scale variation in meiotic recombination in
850 *Mimulus* inferred from population shotgun sequencing. *Proceedings of the National Academy of*
851 *Sciences,* **110**, 19478-19482.
852
853 Hereford J (2009) A quantitative survey of local adaptation and fitness trade-offs. *American*
854 *Society of Naturalists,* **173**, 579-588.
855
856 Hoekstra HE, Hirschmann RJ, Bundey RA *et al.* (2006) A single amino acid mutation
857 contributes to adaptive beach mouse color pattern. *Science,* **313**, 101-104.
858
859 Hochholdinger F, Hoecker N (2007) Towards the molecular basis of heterosis. *Trends in Plant*
860 *Science*, **12**, 427-432.
861
862 Holeski L, Monnahan P, Koseva B *et al.* (2014) A high-resolution genetic map of yellow
863 monkeyflower identifies chemical defense QTLs and recombination rate variation. *G3: Genes,*
864 *Genomes, Genetics,* **4**, 813-821.
865

866 Hoffmann AA, Rieseberg LH (2008) Revisiting the impact of inversions in evolution: From
867 population genetic markers to drivers of adaptive shifts and speciation? *Annual Review of*
868 *Ecology, Evolution, and Systematics,* **39**, 21-42.
869
870 Huang Y, Zhang L, Zhang J *et al.* (2006) Heterosis and polymorphisms of gene expression in an
871 elite rice hybrid as revealed by a microarray analysis of 9198 unique ESTs. *Plant Molecular*
872 *Biology,* **62**, 579-591.
873
874 Ji H, Pardo JM, Batelli G *et al.* (2013) The Salt Overly Sensitive (SOS) pathway: established and
875 emerging roles. *Molecular Plant,* **6**, 275-286.
876
877 Joron M, Frezal L, Jones RT *et al.* (2011) Chromosomal rearrangements maintain a polymorphic
878 supergene controlling butterfly mimicry. *Nature,* **477**, 203-206.
879
880 Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecology Letters,* **7**, 1225-
881 1241.
882
883 Kenkel CD, Matz MV (2016) Gene expression under chronic heat stress in populations of the
884 mustard hill coral (*Porites astreoides*) from different thermal environments. *Nature Ecology and*
885 *Evolution,* **1,** 0014.
886

887 Kim BG, Waadt R, Cheong YH, Pandey GK, Dominguez-Solis JR, Schültke S, Lee SC, Kudla J,
888 Luan S. (2007) The calcium sensor CBL10 mediates salt tolerance by regulating ion homeostasis
889 in *Arabidopsis*. *The Plant Journal*, **52**, 473-484.
890
891 Kirkpatrick M, Barton NH (2006) Chromosome inversions, local adaptation and speciation.
892 *Genetics,* **173**, 419-434.
893
894 Kirkpatrick M, Barrett B (2015) Chromosome inversions, adaptive cassettes and the evolution of
895 species' ranges. *Molecular Ecology,* **24**, 2046-2055.
896
897 Kirkpatrick M, Kern A (2012) Where's the money? Inversions, genes, and the hunt for genomic
898 targets of selection. *Genetics*, **190**, 1153-1155.
899
900 Kirkpatrick M (2010) How and why chromosome inversions evolve. *PLOS Biology*, **8**, e1000501
901
902 Langfelder P, Horvath S (2008) WGCNA: an R package for weighted correlation network
903 analysis. *BMC Bioinformatics,* **9**, 559.
904
905 Kunte K, Zhang W, Tenger-Trolander A et al. (2014) Doublesex is a mimicry supergene. *Nature*,
906 **507**, 229-232.
907
908 Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nature Methods,* **9**,
909 357-359.

910

911 Langmead B, Trapnell C, Pop M *et al.* (2009) Ultrafast and memory-efficient alignment of short
912 DNA sequences to the human genome. *Genome Biology,* **10**, R25.
913

914 Law CW, Chen Y, Shi W *et al.* (2014) Precision weights unlock linear model analysis tools for
915 RNA-seq read counts. *Genome Biology,* **15**, R29.
916

917 Lee CR, Wang B, Mojica JP *et al.* (2017) Young inversion with multiple linked QTLs under
918 selection in a hybrid zone. *Nature Ecology and Evolution,* **1**, 0119.
919

920 Leinonen PH, Remington DL, Leppala *et al.* (2013) Genetic basis of local adaptation and
921 flowering time variation in *Arabidopsis lyrata*. *Molecular Ecology,* **22,** 709-723.
922

923 Levene H (1953) Genetic equilibrium when more than one ecological niche is available. *The*
924 *American Naturalist*, **87**, 331-333.
925

926 Lohman B, Stutz W, Bolnick D (2017) Gene expression stasis and plasticity following migration
927 into a foreign environment. *bioRxiv*, 121608.
928

929 Ji T, Liu P, Nettleton D (2014) Estimation and testing of gene expression heterosis. *Journal of*
930 *Agricultural, Biological, and Environmental Statistics,* **19**, 319.
931

932 Lowry DB, Rockwood JH, Willis JH (2008) Ecological reproductive isolation of coast and
933 inland races of *Mimulus guttatus*. *Evolution,* **62**, 2196-2214.
934
935 Lowry DB, Hall MC, Salt DE *et al.* (2009) Genetic and physiological basis of adaptive salt
936 tolerance divergence between coastal and inland *Mimulus guttatus*. *New Phytol,* **183**, 776-788.
937
938 Lowry DB, Willis JH (2010) A widespread chromosomal inversion polymorphism contributes to
939 a major life-history transition, local adaptation, and reproductive isolation. *PLOS Biology,* **8**,
940 e1000500.
941
942 Lovell JT, Schwartz S, Lowry DB *et al.* (2016) Drought responsive gene expression regulatory
943 divergence between upland and lowland ecotypes of a perennial C4 grass. *Genome Research,* **26**,
944 510-518.
945
946 Mack KL, Campbell P, Nachman MW (2016) Gene regulation and speciation in house mice.
947 *Genome Research*, **26**, 451-461.
948
949 Marquès-Bonet T, Cáceres M, Bertranpetit J *et al.* (2004) Chromosomal rearrangements and the
950 genomic distribution of gene-expression divergence in humans and chimpanzees. *Trends in*
951 *Genetics,* **20**, 524-529.
952

953 Niemi J, Mittman E, Landau W *et al.* (2015) Empirical Bayes analysis of RNA-seq data for
954 detection of gene expression heterosis. *Journal of Agricultural, Biological, and Environmental*
955 *Statistics,* **20**, 614-628.
956
957 Oneal E, Lowry DB, Wright KM *et al.* (2014) Divergent population structure and climate
958 associations of a chromosomal inversion polymorphism across the *Mimulus guttatus* species
959 complex. *Molecular Ecology,* **23**, 2844-2860.
960
961 Puzey JR, Willis JH, Kelly JK (2017) Population structure and local selection yield high
962 genomic variation in *Mimulus guttatus*. *Molecular Ecology*, **26**, 519-535.
963
964 Qiu QS, Guo Y, Dietrich MA, Schumaker KS, Zhu JK (2002) Regulation of SOS1, a plasma
965 membrane Na+/H+ exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. *Proceedings of the*
966 *National Academy of Sciences*, **99**, 8436-8441.
967
968 R. Core Team (2012) R: A language and environment for statistical computing. (available at
969 http://www.r-project.org/.le).
970
971 Rieseberg LH (2001) Chromosomal rearrangements and speciation. *Trends in Ecology &*
972 *Evolution,* **16**, 351-358.
973
974 Ritchie ME, Phipson B, Wu D *et al.* (2015) limma powers differential expression analyses for
975 RNA-sequencing and microarray studies. *Nucleic Acids Research,* **43**, e47.

976

977 Robinson M, Oshlack A (2010) A scaling normalization method for differential expression
978 analysis of RNA-seq data. *Genome Biology,* **11**, R25.

979

980 Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential
981 expression analysis of digital gene expression data. *Bioinformatics,* **26**, 139-140.

982

983 Rockman MV, Kruglyak L (2006) Genetics of global gene expression. *Nature Reviews Genetics,*
984 **7,** 862-872.

985

986 Rodgers-Melnick E, Vera DL, Bass HW, Buckler ES (2016) Open chromatin reveals the
987 functional maize genome. *Proceedings of the National Academy of Sciences*, **113**, E3177-3184.

988

989 Rutherford SL (2003) Between genotype and phenotype: protein chaperones and evolvability.
990 *Nature Review Genetics,* **4**, 263-274.

991

992 Selby JP, Jeong AL, Toll K, Wright KM, Lowry DB (2014) Methods and discoveries in the
993 pursuit of understanding the genetic basis of adaptation to harsh environments in *Mimulus*. In
994 *Plant Ecology and Evolution in Harsh Environments* (pp. 243-265). *NOVA Science Publishers*.
995 Hauppauge, NY, USA.

996

997 Shen Y, Garcia T, Pabuwal V, et al. (2013) Alternative strategies for development of a reference
998 transcriptome for quantification of allele specific expression in organisms having sparse genomic
999 resources. *Comp. Biochem. Physiol. - Part D Genomics Proteomics*, **8**, 11–16.
1000
1001 Sobel JM, Chen GF, Watt LR *et al.* (2010) The biology of speciation. *Evolution,* **64**, 295-315.
1002
1003 Smyth GK, Ritchie M, Thorne N (2015) Linear models for microarray and RNA-Seq data user's
1004 guide. R, doi:10.1093/nar/gkv007.
1005
1006 Stupar RM, Springer NM (2006) *Cis*-transcriptional variation in maize inbred lines B73 and
1007 Mo17 leads to additive expression patterns in the F1 hybrid. *Genetics*, **173**, 2199-2210.
1008
1009 Springer NM, Stupar RM (2007a) Allelic variation and heterosis in maize: how do two halves
1010 make more than a whole? *Plant Cell,* **19**, 2391-2402.
1011
1012 Springer NM, Stupar RM (2007b) Allele-specific expression patterns reveal biases and embryo-
1013 specific parent-of-origin effects in hybrid maize. *Plant Cell,* **19**, 2391-2402.
1014
1015 Steige KA, Reimegård J, Koenig D *et al.* (2015) *Cis*-regulatory changes associated with a recent
1016 mating system shift and floral adaptation in *Capsella*. *Molecular Biology and Evolution,* **32**,
1017 2501-2514.
1018

1019 Steige KA, Laenen B, Reimegård J (2017) Genomic analysis reveals major determinants of cis-
1020 regulatory variation in *Capsella grandiflora*. *Proceedings of the National Academy of Sciences,*
1021 **114**, 1087-1092.
1022
1023 Stevenson KR, Coolon JD, Wittkopp PJ (2013) Sources of bias in measures of allele-specific
1024 expression derived from RNA-seq data aligned to a single reference genome. *BMC genomics,* **14**,
1025 536.
1026
1027 Swanson-Wagner R, Jia Y, De Cook R *et al.* (2006) All possible modes of gene action are
1028 observed in a global comparison of gene expression in a maize f1 hybrid and its inbred parents.
1029 *Proceedings of the National Academy of Sciences* **103,** 6805–6810.
1030
1031 Thompson MJ, Jiggins CD 2014. Supergenes and their role in evolution. *Heredity*, **113**, 1–8.
1032
1033 Thorvaldsdóttir H, Robinson JT, Mesirov JP (2015) Integrative Genomics Viewer (IGV): high-
1034 performance genomics data visualization and exploration. *Briefings in Bioinformatics,* **14**, 1476-
1035 1486.
1036
1037 Tung J, Primus A, Bouley AJ, Severson TF, Alberts SC, Wray GA (2009) Evolution of a malaria
1038 resistance gene in wild primates. *Nature*, **460**, 388-391.
1039
1040 Twyford AD, Friedman J (2015) Adaptive divergence in the monkey flower *Mimulus guttatus* is
1041 maintained by a chromosomal inversion. *Evolution,* **69**, 1476-1486.

1042

1043 Twyford AD, Streisfeld MA, Lowry DB *et al.* Genomic studies on the nature of species:
1044 adaptation and speciation in *Mimulus*. *Molecular Ecology,* **24**, 2601-2609.

1045

1046 Verhoeven KJF, Poorter H, Nevo E *et al.* (2008) Habitat-specific natural selection at a
1047 flowering-time QTL is a main driver of local adaptation in two wild barley populations.
1048 *Molecular Ecology,* **17,** 3416-3424.

1049

1050 Voelckel C, Gruenheit N, Lockhart P (2017) Evolutionary transcriptomics and proteomics:
1051 Insight into plant adaptation. *Trends in Plant Science*, **22**, 462-471.

1052

1053 Wadgymar S, Lowry DB, Gould BA *et al.* (2017) Identifying targets and agents of selection:
1054 innovative methods to evaluate the processes that contribute to local adaptation. *Methods in*
1055 *Ecology and Evolution*, **8**, 738-749.

1056

1057 Wang M, Uebbing S, Ellegren H (2017) Bayesian inference of allele-specific gene expression
1058 indicates abundant *cis*-regulatory variation in natural flycatcher populations. *Genome Biology*
1059 *and Evolution,* **9**, 1266-1279.

1060

1061 Wittkopp PJ, Haerum BK, Clark AG (2004) Evolutionary changes in *cis* and *trans* gene
1062 regulation. *Nature*, **430**, 85-88.

1063

1064 Wu CA, Lowry DB, Nutter LI *et al.* (2010) Natural variation for drought-response traits in the
1065 *Mimulus guttatus* species complex. *Oecologia,* **162**, 23-33.
1066
1067 Xiong L, Lee H, Ishitani M *et al.* (2002) Regulation of osmotic stress-responsive gene
1068 expression by the LOS6/ABA1 Locus in *Arabidopsis*. *The Journal of Biological Chemistry,* **277**,
1069 8588–8596.
1070
1071 Zhang X, Borevitz JO (2009) Global analysis of allele-specific expression in *Arabidopsis*
1072 *thaliana*. *Genetics,* **182**, 943-954.
1073
1074 **DATA ACCESSIBILITY**
1075 RNA-sequence data are deposited are available through the NCBI Sequence Read Archive under
1076 project identifier XX.
1077
1078 **AUTHOR CONTRIBUTIONS**
1079 DBL designed and conducted the field experiment. YC conducted the laboratory work. BAG
1080 analyzed the data. DBL and BAG wrote the manuscript.
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1087 **TABLES**

1088

1089 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 |

1090 *significantly greater percentage than non-inversion regions at p<0.05, **p<0.01.
1091 LFC: average magnitude (abs) log2 fold-change of genes with significant effects
1092

1093

1094

1095 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*** ** |

1096 *significantly greater than non-inversion regions at p<0.05
1097 *** significantly greater than the coastal field site, p<0.001
1098 LFC: average magnitude (abs) log2 fold-change of genes with significant effects
1099

1100

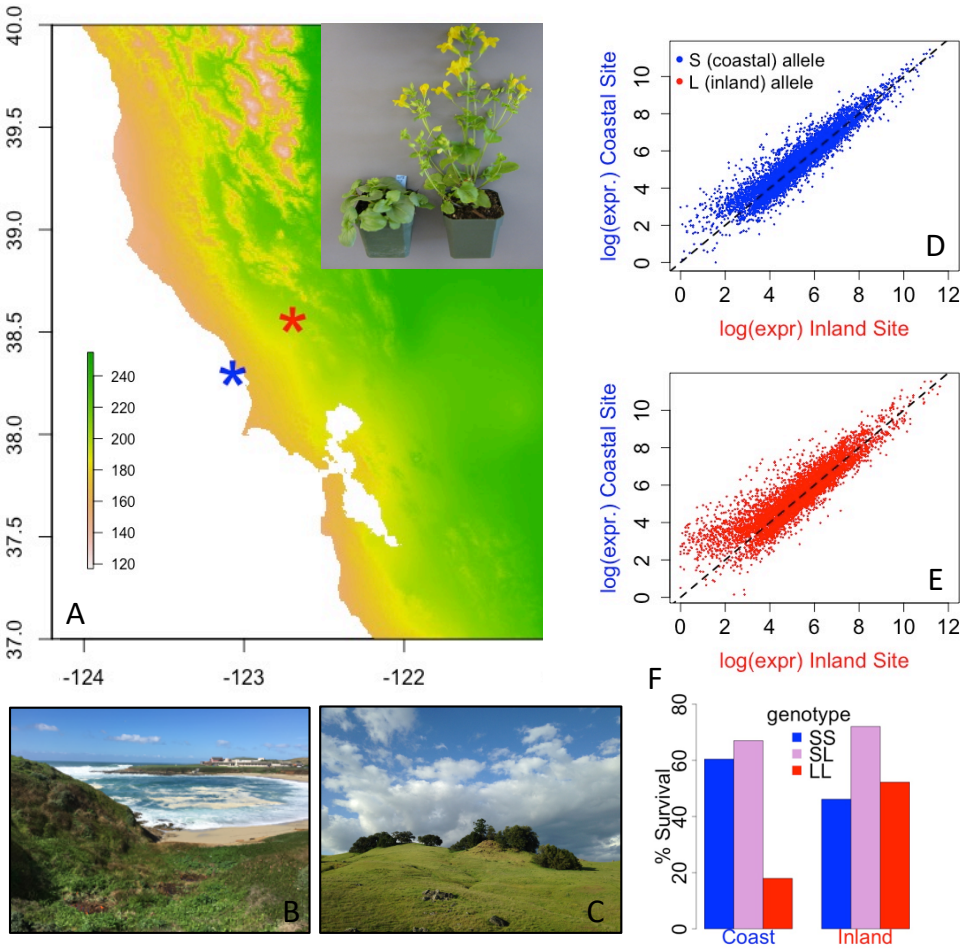


**Figure 1.** A) Map of Northern California with the location of two field reciprocal transplant sites. Colors represent mean temperature of the warmest quarter in °C x 10. Inset: left, coastal ecotype; right, inland ecotype. B) The coastal field site. C) The inland field site. D) SS parental gene expression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

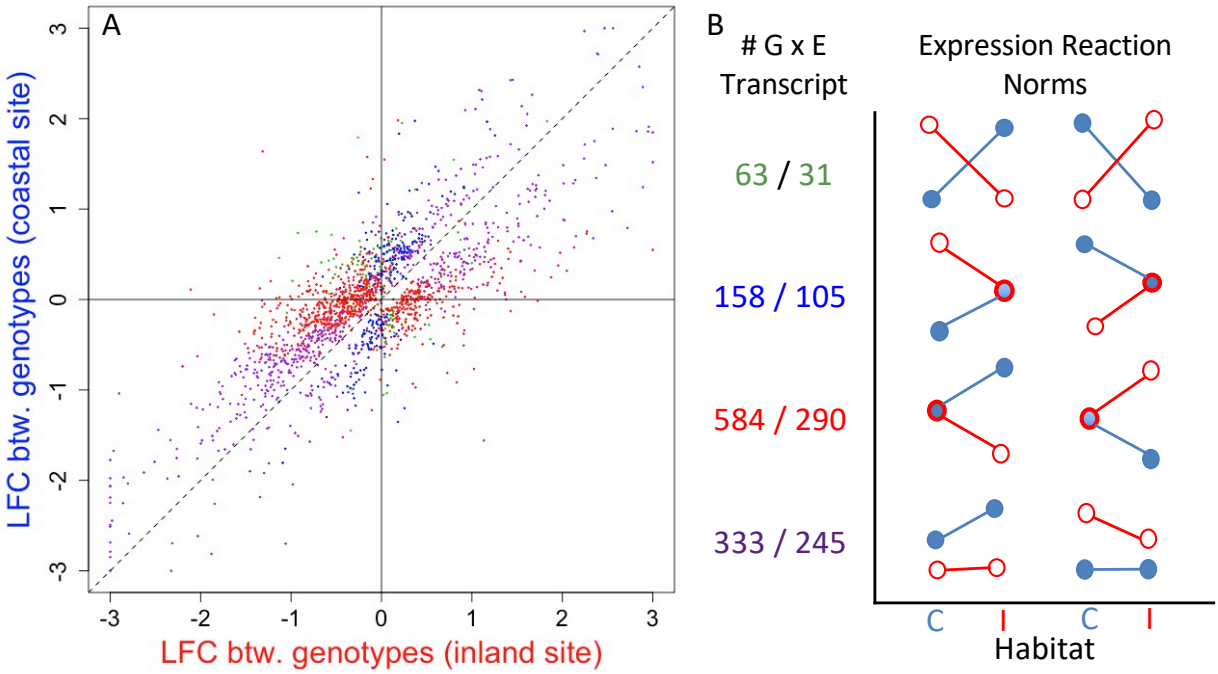


**Figure 2.** A) Average log2 fold-change (LFC) differences between parental genotypes for expressed transcripts at two field sites. B) Expression reaction norms corresponding to colored points in A and B. LFC has been restricted to <3 and <-3 for graphing purposes. Note, 28 transcripts with ambiguous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

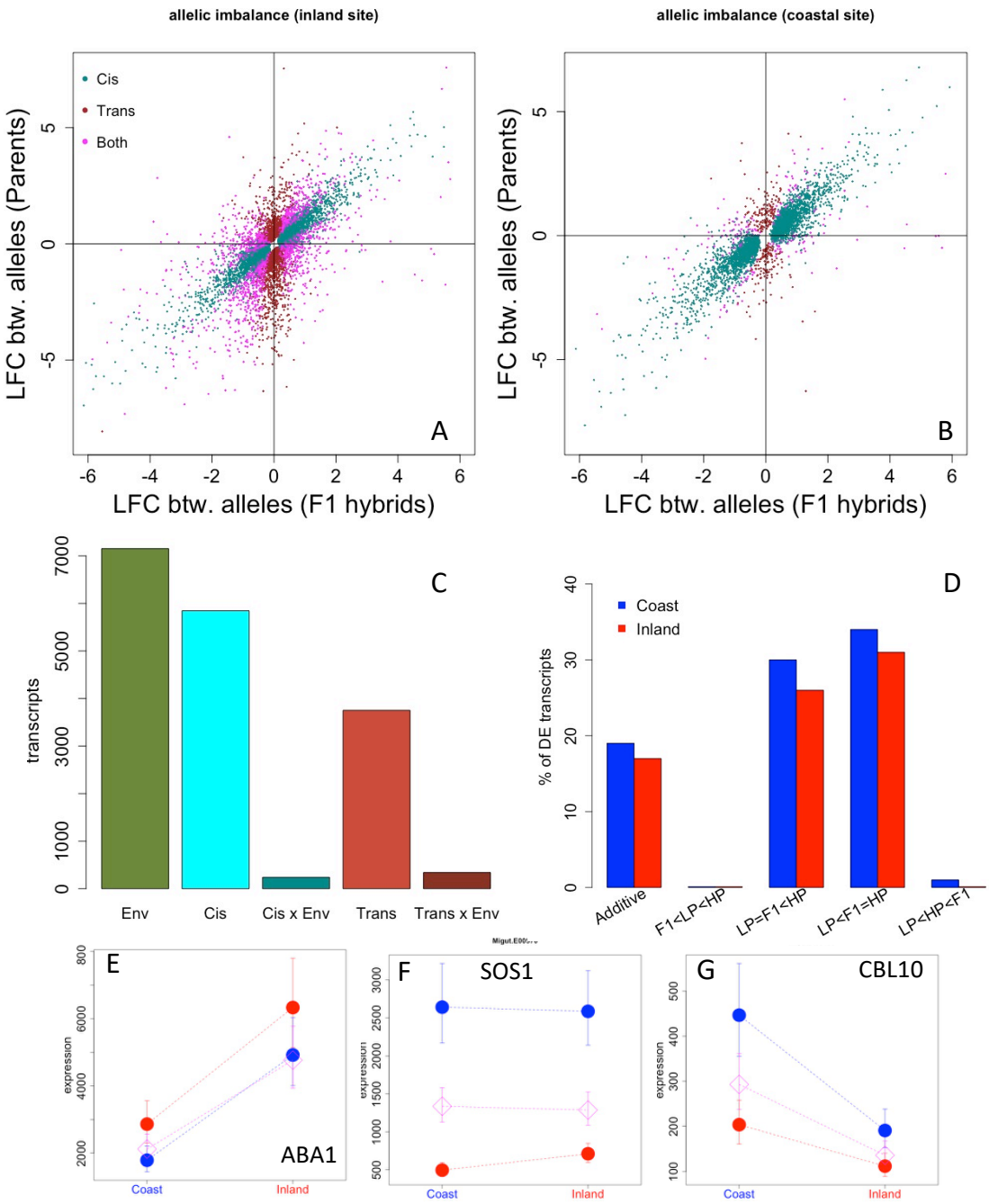


**Figure 3.** *Cis* and *trans* regulated transcripts detected at the coastal (A) and inland (B) field sites. Average log2-fold change (LFC) ratios are between inland and coastal alleles in the parental (y-axis) and F1 (x-axis) genetic backgrounds. C) The frequency of significant model effects among 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 .