i. Mechanisms of a locally adaptive shift in allocation among growth, reproduction, and herbivore resistance in *Mimulus guttatus*

ii. Running Title: Trade-offs underlying local adaptation

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Mechanisms of a locally adaptive shift in allocation among growth, reproduction, and herbivore resistance in *Mimulus guttatus*

ABSTRACT

Environmental gradients can drive adaptive evolutionary shifts in plant resource allocation among growth, reproduction, and herbivore resistance. However, few studies have attempted to connect these adaptations to underlying physiological and genetic mechanisms. Here, we evaluate potential mechanisms responsible for a coordinated locally adaptive shift between growth, reproduction, and herbivore defense in the yellow monkeyflower, *Mimulus guttatus.* Through manipulative laboratory experiments we found that gibberellin (GA) growth hormones may play a role in the developmental divergence between perennial and annual ecotypes of *M. guttatus*. Further, we detected an interaction between a locally adaptive chromosomal inversion, *DIV1*, and GA addition. This finding is consistent with the inversion contributing to the evolutionary divergence between inland annual and coastal perennial ecotypes by reducing GA biosynthesis/activity in perennials. Finally, we found evidence that the *DIV1* inversion is partially responsible for a coordinated shift in the divergence of growth, reproduction, and herbivore resistance traits between coastal perennial and inland annual *M. guttatus*. The inversion has already been established to have a substantial impact on the life-history shift between long-term growth and rapid reproduction. Here, we demonstrate that the *DIV1* inversion also has sizable impacts on both the total abundance and composition of phytochemical compounds involved in herbivore resistance.

Key Words: Chromosomal Inversion, Ecotype, Gibberellin, *Mimulus guttatus*, Supergene, Trade-offs

INTRODUCTION

One of the fundamental tenets of evolutionary biology is that adaptation of organisms to specific environmental conditions inevitability results in a fitness trade-off. Trade-offs often manifest in the form of a cost, such that organisms that become adapted to one set of environmental conditions will be at a disadvantage in alternative environments (Futuyma and Moreno 1988; Whitlock 1996). The idea of trade-offs involved in adaptation and ecological specialization has been borne out in a wide range of evolutionary scenarios, including predator-prey relationships and host-races formation in insect herbivores (Futuyma and Moreno 1988; Kawecki 1998; Svanback and Eklov 2003; Forister et al. 2012). A common source of ecological specialization and consequent trade-offs is the process of local adaptation across environmental gradients (Kawecki and Ebert 2004; Hereford 2009).

Local adaption across environmental gradients can lead to shifts in the allocation of resources to long-term growth (survival) and reproduction (fecundity; Clausen and Hiesey 1958; Lowry 2012; Friedman and Rubin 2015). Those shifts in life-history strategy along environmental gradients can also have major impacts on allocation to herbivore defense (Hahn and Maron 2016). However, there appears to be key differences in how resources are allocated across environmental gradient for interspecific and intraspecific comparisons. Interspecific variation in plant species typically fits well with the resource allocation hypothesis (Coley et al. 1985), where low resource environments tend to be composed of slower growing better defended species while high resource environments promote faster growing poorly defended plants (Endara and Coley 2011). In contrast, intraspecific variation along environmental gradients is far less consistent and more often than not contradicts the predictions of the resource allocation hypothesis (Hahn and

47 Maron 2016). One common pattern for intraspecific plant variation is a positive relationship
48 between the length of the growing season along an environmental gradient and the level of
49 herbivore resistance (Hahn and Maron 2016; Kooyers et al. 2017). This pattern could be driven
50 by plants having more time to allocate resources to leaf production and defense, greater
51 herbivore pressure in habitats with longer growing seasons, and/or a greater apparency of plants
52 with longer growing seasons (Feeny 1976; Mason and Donovan 2015; Hahn and Maron 2016;
53 Kooyers et al. 2017). Another important factor to consider is allocation to reproduction
54 (fecundity), which frequently trades off with constitutive and/or induced herbivore resistance
55 (Agren and Schemske 1993; Heil and Baldwin 2002; Strauss et al. 2002; Stowe and Marquis
56 2011; Cipollini et al. 2014).
57
58 Achieving an evolutionary optimum in how resources are allocated to growth, reproduction, and
59 defense will depend on the nature of all environmental challenges faced by each local population
60 (Rhoades 1979; Rausher 1996; Hamilton et al. 2001; Strauss et al. 2002; Stamp 2003; Karban
61 2011; Cipollini et al. 2014; Jensen et al. 2015; Smilanich et al. 2016). Despite the development
62 of multiple ecological and evolutionary hypotheses that postulate a relationship between growth,
63 reproduction, and resistance to herbivores (Feeny 1976; Coley et al. 1985; Rhoades 1979; Herms
64 and Mattson 1992; Strauss et al. 2002; Stamp 2003, Fine et al. 2006; Agrawal et al. 2010;
65 Cipollini et al. 2014; Hahn and Maron 2016), these hypotheses do not make any predictions
66 about the underlying molecular mechanisms that mediate these relationships. The genetic
67 mechanisms responsible for trade-offs among growth, reproduction, and resistance are just
68 beginning to be elucidated in model systems (Lorenzo et al. 2004; Yang et al. 2012; Kerwin et al.

69 2015; Campos et al. 2016; Havko et al. 2016; Major et al. 2017; Howe et al. 2018; Rasmann et
70 al. 2018), but have yet to be evaluated in the evolutionary context of local adaptation.
71 Recent studies have shown that changes in the allocation of resources to growth versus resistance
72 are made through a set of interacting gene networks (Karzan and Manners 2012; Huot et al.
73 2014; Campos et al. 2016; Havko et al. 2016). Jasmonates (JA) are key regulatory hormones
74 involved in the response of plants to herbivore attack (Zhang and Turner 2008; Havko et al.
75 2016). While JA production increases herbivore defenses, it also inhibits plant growth through
76 interactions with other gene networks (Zhang and Turner 2008; Yan et al. 2007; Karzan and
77 Manners 2012; Yang et al. 2012). For example, the critical cross-talk between downstream genes
78 in the JA pathway (JAZ genes) and Gibberellin (GA) pathway (DELLA genes) are thought to
79 play a pivotal role in mediating resource allocation (Yang et al. 2012; Hou et al. 2013; Havko et
80 al. 2016).
81
82 Here, we focus on understanding the physiological and genetic mechanisms underlying shifts in
83 allocation to growth, reproduction, and defense for local adapted populations of the yellow
84 monkeyflower *Mimulus guttatus*. The availability of soil water is a key driver of local adaptation
85 in the *M. guttatus* species complex (Hall and Willis 2006; Lowry et al. 2008; Ferris et al. 2017).
86 The coastal habitats of California and Oregon have many wet seeps and streams that are
87 maintained year-round as a result of persistent summer oceanic fog and cool temperatures (Hall
88 and Willis 2006; Lowry et al. 2008; Lowry and Willis 2010). All coastal populations of *M.*
89 *guttatus* that reside in those habitats have a late-flowering life-history strategy. These coastal
90 perennial populations thus, make a long-term investment in growth over reproduction (Hall and
91 Willis 2006; Lowry et al. 2008; Hall et al. 2010; Baker and Diggle 2011; Baker et al. 2012). That

92 investment in growth manifests through the production of many vegetative lateral stolons,
93 adventitious roots, and leaves in coastal perennial plants. In contrast, the vast majority of nearby
94 inland populations of *M. guttatus* in the coastal mountain ranges reside in habitats that dry out
95 completely during summer months. These inland populations have evolved a rapid growth,
96 drought escape annual life-history strategy. Instead of investing in vegetative lateral stolons, the
97 axillary branches of inland plants are mostly upcurved and typically produce flowers quickly
98 (Lowry et al. 2008; Lowry and Willis 2010; Friedman et al. 2015). Further, inland plants invest
99 less into the production of leaves before flowering than coastal perennials (Friedman et al. 2015).
100 It should be noted that a smaller number of inland populations in the coastal mountain ranges do
101 reside in rivers and perennials seeps and have a perennial life-history. Farther inland, perennial
102 populations are more common, especially in high elevation streams and hot springs of the Sierra
103 and Cascade Mountains (Oneal et al. 2014). The inland perennial plants are similar to coastal
104 perennials in overall morphology, especially in that they are more prostrate in growth habit, with
105 the production many stoloniferous branches. However, the coastal perennials differ from inland
106 perennial by being more robust in their growth form and having evolved tolerance to oceanic salt
107 spray (Lowry et al. 2009).
108
109 While perennial populations invest more into vegetative growth than reproductive growth, they
110 also invest more heavily in defending their vegetative tissues. Perennial populations of *M.*
111 *guttatus* have higher levels of both constitutive and induced defensive phenylpropanoid
112 glycoside (PPG) compounds than the annual populations when grown in a common environment
113 (Holeski et al. 2013). PPG levels have a negative relationship with the performance of multiple
114 generalist herbivores in *M. guttatus* (Rotter et al. 2018). This pattern of highly defended plants in

115 wetter habitats with long growing seasons versus poorly defended plants in dry habitats with
116 short growing seasons is consistent with an optimal defense strategy: Greater allocation of
117 resources to herbivore resistance is favored in habitats with a long growing season by both a
118 greater abundance of herbivores and a lower physiological cost of producing defensive
119 compounds due to high soil water availability (Kooyers et al. 2017).
120
121 Two major QTLs (*DIV1* and *DIV2*) and many minor QTLs control key traits involved in local
122 adaptation to perennial and annual habitats within the *M. guttatus* species complex (Hall et al.
123 2006; Lowry and Willis 2010; Hall et al. 2010; Friedman and Willis 2013; Friedman et al. 2015).
124 *DIV1* has the largest effect on the most traits and has thus been more extensively studied than
125 *DIV2*. *DIV1* is a large paracentric chromosomal inversion that plays a pivotal role in the annual
126 versus perennial life-history divergence described above (Lowry and Willis 2010). The inversion
127 is at minimum 6.3 Mbp in length along linkage group 8 (LG8) and contains at least 785
128 annotated genes. In hybrids, *DIV1* has a major effect on growth rate including the adaptive
129 flowering time phenotype, explaining 21% to 48% of the divergence between inland annual and
130 coastal perennial parents (Lowry and Willis 2010). In addition to flowering time, the *DIV1*
131 inversion has major effects on multiple traits involved in the evolutionary shift from more
132 allocation of resources to long-term growth versus reproduction (Lowry and Willis 2010). Plants
133 with the perennial (PE) orientation of the inversion have a more prostrate growth habitat,
134 produce more lateral stolons and adventitious roots, have thicker shoots, and larger flowers than
135 plants with the annual (AN) orientation of the inversion (Lowry and Willis 2010; Friedman et al.
136 2015). A recent allele frequency outlier analysis of coastal perennial and inland annual
137 populations identified candidate genes in the gibberellin pathway that may underlie a pleiotropic

138 shift in allocation between growth and reproduction (Gould et al. 2017). That study also found
139 evidence of recent selection on a key GA biosynthetic gene, *GA20-oxidase2* (*GA20ox2*), within
140 the *DIV1* inversion and for the *Gibberellic Acid Insensitive* (*GAI*) in the vicinity of *DIV2.*
141
142 Given the recent outlier analysis identifying divergence among genes in the GA pathway and the
143 GA pathways interaction with the JA herbivore resistance pathway, we evaluate the role of GA
144 in the divergence of growth morphology and herbivore resistance between perennial and annual
145 ecotypes of *M. guttatus.* We hypothesized that perennial accessions would display a greater
146 morphological response to GA addition than annual accession, as their prostrate morphology was
147 consistent with lower GA biosynthesis and/or downstream signaling activity. Further, we
148 hypothesized that the adaptive *DIV1* inversion contributes to the lower GA biosynthesis/activity
149 in coastal perennial plants. To test this hypothesis, we utilized near-isogenic lines (NILs) for the
150 *DIV1* locus and evaluated whether there was interaction between the *DIV1* inversion and GA
151 addition. If the GA biosynthesis/activity is down regulated by the perennial orientation of the
152 inversion, then we predict that the NILs containing the perennial inversion orientation will
153 respond more to the GA addition than the NILs containing the annual orientation. Finally, we
154 tested the hypothesis that the *DIV1* inversion is partially responsible for the evolution of locally
155 adaptive trade-offs in allocation between reproduction and defense that has been broadly
156 observed for populations of *M. guttatus* that vary in growing season length (Lowry et al. 2008;
157 Holeski et al. 2013; Kooyers et al. 2017). To test this hypothesis, we compared the
158 concentrations of PPGs between NILs containing coastal perennial and inland annual
159 orientations of the *DIV1* inversion.
160

161 **METHODS**

162

163 ***Plant material***

164 For comparisons among ecotypes, we utilized single family population accessions derived from
165 coastal perennial, inland annual, and inland perennial populations of *M. guttatus* (Fig. 1). The
166 locations from where population accessions were collected are listed in Table S1. Previous
167 population structure analyses found that coastal perennial populations of *M. guttatus* are more
168 closely related to each other than they are to the inland annual populations (Lowry et al. 2008;
169 Twyford and Friedman 2015). Thus, the coastal populations collectively constitute a distinct
170 locally adapted ecotype (Lowry 2012). In contrast, population structure between inland annuals
171 and inland perennial populations is generally low (Twyford and Friedman 2015). However,
172 particular regions of the genome, including an adaptive chromosomal inversion (*DIV1*, discussed
173 below) are more differentiated between inland annuals and inland perennials (Oneal et al. 2014;
174 Twyford and Friedman 2015). We therefore consider inland annuals and inland perennials as
175 different ecotypes as well.

176

177 To understand the phenotypic effects of the *DIV1* inversion, Lowry and Willis (2010) previously
178 created near-isogenic lines (NILs) for the inversion. The NILs are the product of crosses between
179 inbred lines from the coastal perennial SWB population and the nearby inland annual LMC
180 population. F1 hybrids were recurrently backcrossed to both of their respective parents for four
181 generations. Heterozygous fourth generation backcrosses were then self-fertilized to produce two
182 types of NILs: 1) Individuals that were homozygous for the introgressed allele of *DIV1*
183 (Introgression-NILs) and 2) Individuals that were homozygous for the *DIV1* allele of the genetic

184 background (henceforth referred to as Control-NILs). Comparisons between Introgression-NILs
185 and Control-NILs are ideal for testing inversion function because their genetic backgrounds are
186 nearly identical, but they are homozygous for opposite *DIV1* alleles.
187
188 ***The effects of GA application on plant growth among ecotypes***
189 To evaluate whether perennial and annual plants differ in their response to GA addition, we
190 conducted a greenhouse experiment with accessions derived from five coastal perennial, four
191 inland annual, and two inland perennial populations (Table S1). Seeds were sown in Suremix soil
192 (Michigan Grower Products Inc., Galsburg, MI) and stratified at 4° C for two weeks. After
193 stratification, pots were moved to the Michigan State University greenhouses. Temperature was
194 set in the greenhouse room to 22° C days/18° C nights. Plants were grown in 16-hour days and 8-
195 hour nights, where supplemental lighting was used during the full day period. Seedlings were
196 transplanted to individual 3.5-inch square pots filled with Suremix soil. Transplanted seedlings
197 were randomized across the experiment and randomly assigned to a GA treatment group or a
198 mock control group. After transplantation, plants were sprayed five times each, every other day,
199 with 100mM GA3 (GA treatment) or DI water (mock). This daily spray volume amounts to ~3.5
200 mL.
201
202 To standardize the developmental time point at which plant traits were quantified, we measured
203 the following traits on the day of first flowering: Total number of nodes on the primary shoot,
204 lengths and widths of the first three internodes, length and width of the corolla of the first open
205 flower, plant height, the total number of adventitious roots at the first node of all branches, total
206 number of stoloniferous nodes sprouting adventitious roots, total number of aerial branches, total

207 number of stolons, length of the longest aerial branch, length of the longest stolon, and the length
208 and width of the longest leaf at the second node. Ten days after first flower, we quantified the
209 same traits as at first flower, with the following exceptions: Length and width of corollas were
210 not quantified, but we did count the total number of flowers.
211
212 Results were analyzed with JMP 14.0.0 (SAS Institute, Cary, NC). To test the hypothesis that the
213 perennials respond morphologically more to the addition of GA, we conducted principal
214 components analyses using all of the measured traits. Since many of these traits were highly
215 correlated (Table S1), we reduced the dimensionality of the data with a principal components
216 (PC) analysis. We conducted the PC analysis on correlations in JMP. We used a Scree Test
217 (Cattell 1966) to determine how many principal components to retain. To understand the effects
218 of accession, ecotype, GA treatment, and the interactions of these effects on each PC, we fit the
219 default standard least squares ANOVA model with the Fit Model platform in JMP. Each of the
220 PCs were modeled as response variables to the following fixed-effect factors and interactions:
221 accession (nested within ecotype), ecotype (coastal perennial, inland annual, inland perennial),
222 treatment (GA vs. Mock), accession x treatment, and ecotype x treatment. A significant ecotype
223 x treatment interaction would indicate that the ecotypes vary in their response to GA. Further, if
224 perennials responded more to the GA treatment it would support the hypothesis that the
225 perennials have less GA biosynthesis/activity than the annuals.
226
227 ***Interactions of GA application with the adaptive DIV1 inversion***
228 We grew coastal (SWB-S1) and inland (LMC-L1) parental inbred lines along with the NILs
229 derived from those lines in a fully randomized design in the Michigan State University

230 greenhouses, with 16-hours of supplemental lighting. We focused on the effect of the inversion
231 in the coastal perennial genetic background, as a previous study had shown that the effect of the
232 inversion had the greatest effect in the perennial genetic background (Friedman 2014). Following
233 transplantation, seedlings were sprayed with GA or a mock water treatment every other day and
234 traits were quantified in the same way as for the comparing population accessions.
235
236 To establish how trait variation of the coastal and inland parental lines was influenced by the GA
237 treatment, we conducted a PC analysis of all plants in the experiment in JMP 14.0.0. As for the
238 analysis with multiple population accessions (above), we conducted principal components
239 analyses using all traits and used a Scree Test (Cattell 1966) to determine how many principal
240 components to retain. Models were fit for each PC to test the effect of the following fixed-effect
241 factors: line, treatment, and the line x treatment interaction. We fit models independently for the
242 parental lines and the NILs. The analysis of the parental lines was conducted to confirm that the
243 responses to GA were similar as for the population survey (above). The analysis of the NILs
244 allowed us to test whether there was a significant interaction of the inversion with the GA
245 treatment (i.e. line x treatment interaction). Line x treatment least square means were compared
246 with Tukey HSD post hoc analyses for both parental lines and NILs. A significant line x
247 treatment interaction for the NILs, with a greater response for the introgression NILs, would
248 support the hypothesis that the perennial orientation of the *DIV1* inversion reduces GA
249 biosynthesis/activity.
250
251 ***Effects of the DIV1 inversion on resistance compound concentrations***

252 To evaluate the effects of the *DIV1* inversion on the production of herbivore resistance
253 compounds, we conducted an experiment using the inversion NILs. Seeds were stratified,
254 germinated, and transplanted following the same protocols as in the previous two experiments. In
255 contrast to the GA NIL experiment, we grew outbred NILs which were created by intercrossing
256 independently derived NILs because we did not have any prior data on the impacts of inbreeding
257 on PPG production. As for the GA experiment, we focused our study on the effect of the
258 inversion in the perennial genetic background. Here, we used the outbred NILs made by
259 intercrossing SWB-S2 and SWB-S3 derived coastal genetic background NILs. We used
260 intercrosses between LMC-L2 andLMC-L3 for the inland parents and between SWB-S2 and
261 SWB-S3 for the coastal perennial parent comparisons. See Lowry & Willis (2010) for full
262 description of outbred NIL generation.
263
264 To ensure that enough leaf tissue was available for analyses, outbred NILs and outbred parents
265 were allowed to flower prior to the collections for PPG quantification. Collected leaf tissue was
266 lyophilized for two days and then shipped to Northern Arizona University for analyses. We
267 ground the leaf tissue using a 1600 MiniG (Spex, Metuchen, New Jersey). Extractions were
268 conducted in methanol, as described in Holeski et al. 2013, 2014. We quantified PPGs using high
269 performance liquid chromatography (HPLC), via an Agilent 1260 HPLC (Agilent Technologies,
270 Santa Clara, California) with a diode array detector and Poroshell 120 EC-C18 analytical column
271 (4.6 x 250mm, 2.7µm particle size) maintained at 30°C. HPLC run conditions, were conducted
272 as described in Kooyers et al. (2017). We calculated concentrations of PPGs as verbascoside
273 equivalents, using a standard verbascoside solution (Santa Cruz Biotechnology, Dallas, Texas),
274 as described in Holeski et al. (2013, 2014). We compared the concentrations of total PPGs and

275 individual PPGs with one-way ANOVAs fit in JMP 12.2.0. Post-hoc Tukey HSD Tests were
276 used to compare means of parental and NIL classes.
277
278 **RESULTS**
279 ***The effects of GA application on plant growth among ecotypes***
280 Consistent with previous studies (Hall et al. 2006; Lowry et al. 2008; Lowry and Willis 2010;
281 Oneal et al. 2014), there were large differences in morphology between coastal perennial, inland
282 annual, and inland perennial ecotypes. The perennials, especially the coastal ones, were generally
283 larger overall across a suite of traits. The perennials also all had a prostrate growth habit with
284 many lateral stolon branches that produced adventitious roots. In contrast, the inland annuals had
285 thinner shoots, smaller flowers, and primarily produced upcurved aerial branches lacking
286 adventitious roots. We reduced the dimensionality of the data using a principal components
287 analysis, retaining the first 4 PCs based on a Scree Test (Figure S1; Cattell 1966). The first four
288 PCs collectively explained 70.3% of the phenotypic variation. Most traits (32 out of 37) heavily
289 loaded (Loadings > 0.40) onto the first PC (Eigenvalue = 14.698; Table S2). Ecotype divergence
290 accounted for for much of the variation of PC1 ($F_{2,225}$ =211.04, $P < 0.0001$; Figure 2A; Table 1).
291 Within ecotype, there was a significant effect of accession on PC1 ($F_{8,225}$ = 23.70, $P < 0.0001$),
292 and there was a significant effect of the GA treatment on PC1 ($F_{1,225}$ = 15.22, $P = 0.0001$). While
293 there was a significant accession x treatment effect for PC1 ($F_{8,225}$ = 2.59, $P = 0.0101$), the
294 treatment x ecotype effect was not significant ($P = 0.1930$). In contrast to PC1, both of the
295 interactions were significant for PC2 (Eigenvalue = 5.632; accession x treatment: $F_{8,225}$ = 7.61, P
296 < 0.0001; ecotype x treatment: $F_{2,225}$ = 6.16, $P = 0.0025$; Fig. 2A) and PC3 (Eigenvalue = 3.443;
297 accession x treatment: $F_{8,225}$ =4.03; $P = 0.0002$, ecotype x treatment: $F_{2,225}$ = 42.07, $P < 0.0001$;

298 Fig. 2B). Accession x treatment and ecotype x treatment interactions were not significant for
299 PC4 (Table 1).
300
301 Since there were significant ecotype x treatment interactions for PC2 and PC3, indicating a role
302 of the GA pathway in ecotype divergence, we were interested in which traits might be driving
303 these patterns. Second and third internode length, plant height, total number of stoloniferous
304 nodes sprouting adventitious roots, total number of aerial branches, total number of stolons, and
305 the length of the longest aerial branch all heavily loaded (> 0.40) onto PC2 (Table S2). The total
306 number of aerial branches, the length of the longest aerial branch, number of nodes with
307 adventitious roots, and leaf width heavily loaded onto PC3 (Table S2).
308
309 It should be noted that the effect of GA varied across accessions within ecotype. For example,
310 the coastal PGR accession is the tallest coastal accession with the fewest stolons. This difference
311 in overall morphology likely explains why its response to GA differed from the other four
312 coastal accessions (Fig. S2). The inland perennials showed a similar level of response to GA as
313 the coastal perennials for PC2, but not for PC3 (Fig. 2, S2).
314
315 ***Interactions of GA application with the adaptive DIV1 inversion***
316 Based on a Scree test (Fig. S3), we retained the first three PCs for analyses of the parental
317 genotypes and NILs. Consistent with previous studies (Lowry and Willis 2010; Friedman 2014),
318 the coastal perennial (SWB-S1) and inland annual (LMC-L1) lines were highly divergent in
319 morphological traits and the two lines were differentiated strongly along PC1 ($F_{1,163}$ = 314.39, P
320 < 0.0001; Fig. 3A; Table 2). Similar to the accession analysis (above), the line x treatment

321 interaction was not significant for PC1, but was highly significant for PC2 ($F_{1,163}$ = 262.49, P <
322 0.0001; Table 2). The coastal perennial line (SWB-S1; N = 31 GA treatment, 42 Mock treatment)
323 responded more strongly (Fig. 3B) to GA treatment for PC2 than the inland annual line (LMC-
324 L1; N = 47 GA treatment, 47 Mock treatment), just as we found across coastal and inland
325 populations more generally (above). The set of traits heavily loading (>0.40) onto PC2 was very
326 similar to that for PC2 of the accession analysis (above): Third internode length, plant height,
327 total number of stoloniferous nodes sprouting adventitious roots, number of adventitious roots at
328 the first node, total number of aerial branches, total number of stolons, and the length of the
329 longest aerial branch (Table S3).
330
331 The *DIV1* chromosomal inversion is one of many loci responsible for divergence between the
332 annual and perennial ecotypes (Hall et al. 2006; Lowry & Willis 2010; Friedman 2014). Thus,
333 main effects and interactions in the NILs were expected to be subtler than for the parental lines.
334 As in previous studies (Lowry and Willis 2010; Friedman 2014), the inversion had major effects
335 on traits associated with ecotype divergence, with highly significant main effects on PC1 ($F_{1,166}$
336 = 51.23, P < 0.0001; Table 3). The GA treatment had significant effects on PC1 ($F_{1,166}$ = 10.86,
337 P = 0.0012) and PC2 ($F_{1,166}$ = 606.48, P < 0.0001). There was a significant, line x treatment
338 interactions for PC2 ($F_{1,166}$ = 24.91, P < 0.0001; Fig. 3E), where Introgression-NILs (N = 39 GA
339 treatment, 48 Mock treatment) containing perennial inversion orientation responded more to the
340 GA treatment than the Control-NILs (N = 39 GA treatment, 44 Mock treatment) containing the
341 annual orientation. None of the effects were significant for PC3 after Bonferroni correction for
342 multiple testing (Table 3).
343

344 ***Effects of the DIV1 inversion on resistance compound concentrations***

345 We quantified the concentrations of seven PPGs (Table 4). Consistent with our previous
346 observations (Holeski et al. 2013), the coastal perennial parental (SWB) plants produced 2.5
347 times more total PPGs than the inland annual parental (LMC) plants ($F_{1,31}$ = 51.03; P < 0.0001;
348 Table 4; Fig. 4). There were also significant differences for six out of seven of the PPGs between
349 the coastal perennial (SWB) and inland annual (LMC) parental lines.

350

351 Analysis of the *DIV1* NILs revealed that the introgressed region containing the inversion had
352 major effects on foliar concentrations of PPGs. Control NILs that were homozygous for the
353 coastal orientation of the *DIV1* inversion produced 35% higher concentrations of total PPGs than
354 the introgression NILs, which were homozygous for the inland *DIV1* orientation ($F_{1,87}$ = 22.70; P
355 < 0.0001). In addition, the *DIV1* locus had significant effects on four out of the seven individual
356 PPGs. Interestingly, the control NILs had higher concentrations of conandroside and
357 mimuloside, but lower concentrations of calceolarioside A and unknown PPG10, than the
358 introgression NILs (Table 4). Thus, the *DIV1* inversion influences both the total concentration of
359 PPGs as well as the composition of suites of these PPGs.

360

361 **DISCUSSION**

362 In this study, we identified a potential mechanism underlying a coordinated evolutionary shift
363 between growth, reproduction, and herbivore resistance in the *M. guttatus* species complex. Our
364 finding that perennial accessions were more responsive to the GA treatment along the second
365 principal component axis (PC2; Fig. 2B) is consistent with the hypothesis that perennial
366 populations have less GA biosynthesis and/or activity. This pattern was replicated for the coastal

367 perennial and inland annual parents of the NILs (Fig. 3B). Further, the finding that the
368 introgression NILs had less response to GA addition along the PC2 axis than the control NILs
369 (Fig. 3E) is consistent with the hypothesis that the perennial orientation of the *DIV1* inversion
370 reduces GA biosynthesis/activity. The morphological traits most likely impacted through this
371 mechanism include plant height, adventitious root production, and whether lateral branches
372 develop as vegetative stolons or upcurved reproductive shoots. Finally, we found that coastal
373 perennial plants and NILs containing the perennial orientation of the inversion produced higher
374 concentration of defensive PPG compound than inland annual plants and NILs containing the
375 inland orientation of the inversion. Therefore, the coastal perennial orientation of the *DIV1*
376 inversion promotes allocation to long-term growth and herbivore resistance, while the inland
377 orientation promotes allocation of resources to traits associated with rapid reproduction. We
378 discuss these findings in the context of the broader literature below.
379
380 ***Environmental gradients and the evolution of growth, reproduction, and defense traits***
381 Studies of intraspecific variation among natural populations adapted to different soil water
382 availability regimes provide an excellent opportunity to understand how the abiotic environment
383 influences the relative allocation of resources by plants to growth and constitutive/induced
384 resistance. Soil water is one of the most limiting factors for plants on Earth (Whittaker 1975;
385 Bohnert et al. 1995; Bray 1997) and can drastically differ in availability among seasons
386 (Cowling et al. 1996), which in turn influences plant resource allocation (Juenger 2013). The
387 timing of soil water availability can dictate the length of the growing season. One major
388 evolutionary strategy for seasonally low water availability is to allocate resources primarily to
389 growth and reproduction to achieve an early flowering, drought escape life-history (Ludlow

390 1989; Juenger 2013; Kooyers 2015). Beyond selection on plants, soil moisture gradients can
391 drive the abundance of herbivores, which in turn exert their own selective pressures (Kooyers et
392 al. 2017).
393
394 In *M. gutattus*, evolutionary shifts across a soil moisture gradient drives changes in the allocation
395 not only between growth and reproduction, but also for herbivore resistance (Lowry et al. 2008;
396 Holeski et al. 2013; Kooyers et al. 2017). The phenotypic differences between coastal perennial
397 and inland annual populations is likely driven by multiple selective pressures that are tied to the
398 divergence in soil water availability between coastal and inland habitats. Inland annual habitats
399 generally dry out very quickly at the end of the spring, which leaves little time for a plant to
400 reproduce before being killed by the summer drought. Further, the short growing season may
401 also prevent the establishment of sizable herbivore populations, which would explain the low
402 level of leaf damage in fast drying inland annual habitats (Lowry et al. 2008; Kooyers et al.
403 2017). In contrast, the year-round soil water availability of coastal habitats means that plants
404 growing there have much more time to allocate resources to vegetative growth and herbivore
405 resistance. In addition, wet coastal habitats can build up a considerable load of herbivores, which
406 is likely contribute to the higher incidences of leaf damage and early season mortality at the coast
407 (Lowry et al. 2008; Lowry and Willis 2010; Popovic & Lowry 2019). For intraspecific
408 differences among populations, the strength of herbivore pressure is thought to be a key driver of
409 plant resistance (Hahn and Maron 2016). It should be noted that a fair amount of leaf damage in
410 coastal habitats may also be due to oceanic salt spray (Boyce 1954; Ahmed and Wainwright
411 1976; Griffiths 2006; Lowry et al. 2009; Popovic & Lowry 2019). However, a recent

412 manipulative field experiment did find much greater levels of herbivory of *M. guttatus* plants in
413 common gardens in coastal habitat than inland habitat (Popovic & Lowry 2019).
414
415 Our findings in *M. guttatus* are likely to have implications for intraspecific variation in many
416 other plant species as well. There are many studies that have found similar developmental
417 differences between coastal and inland populations as we have found for *M. guttatus* (reviewed
418 in Lowry 2012). Given the commonality of coastal plants investing more heavily in lateral
419 vegetative branches versus inland populations investing primarily in upright flowering branches,
420 we predict that coastal population of plants will generally be more highly defended than inland
421 populations, particularly in Mediterranean climates with steep soil water availability gradients.
422
423 ***The role of pleiotropy and linkage***
424 The results of this study and previous studies (Lowry and Willis 2010; Friedman 2014; Friedman
425 et al. 2015) collectively demonstrate that adaptive chromosomal inversion *DIV1* contributes to
426 the shift in allocation between long-term growth, short-term fecundity, and herbivore resistance.
427 An outstanding question is whether this coordinated shift is due to genetic linkage or pleiotropy.
428 Chromosomal inversions are thought to evolve as adaptation "supergenes," which can trap
429 multiple linked adaptive loci through their suppression of meiotic recombination (Dobzhansky
430 1970; Kirkpatrick and Barton 2006; Schwander et al. 2014; Wellenreuther and Bernatchez 2018).
431 Thus, the fact that the *DIV1* inversion contributes to the evolution of multiple phenotypes could
432 be result of adaptive alleles at multiple linked loci being held together in tight linkage by the
433 chromosomal inversion. Alternatively, a single gene within the inversion could have pleiotropic
434 effects on all of the phenotypic changes. Functional studies involving transformation or gene

435 editing of candidate genes within the inversion will be necessary to distinguish between these
436 alternative hypotheses.
437
438 Two other recent studies have also found potential pleiotropic effects of genes on allocation to
439 reproduction and herbivore resistance. Rasmann et al. (2018) found that NILs containing genetic
440 variants of the *Flowering Locus C* (*FLC*) gene in *Cardamine hirsute* are responsible for a trade-
441 off between early flowering and herbivore resistance in terms of glucosinolate production.
442 Kerwin et al. (2015) found that there was a positive correlation in *Arabidopsis thaliana* between
443 glucosinalate production and flowering time for mutant alleles of genes in the glucosinolate
444 biosynthetic pathway. Overall, both of these studies identified the same trade-off of rapid
445 reproduction versus herbivore resistance that we found in our study, although mediated through
446 independent genetic mechanisms.
447
448 ***A hormonal basis of a coordinated shift in the evolution of growth, reproduction, and***
449 ***herbivore resistance?***
450 The finding that coastal perennial and inland annual plants respond differentially to GA is
451 consistent with the role of this hormone playing a role in their evolutionary divergence. A recent
452 outlier analysis of coastal perennial and inland annual populations of *M. gutattus* found that the
453 gene *GA20-oxidase2* (*GA20ox2*) was a major allele frequency outlier between the ecotypes
454 within the vicinity of the *DIV1* inversion (Gould et al. 2017). This gene is a strong potential
455 candidate gene for a pleiotropic shift in allocation between growth and reproduction. GA-
456 oxidases are involved in the evolution of dwarfed coastal populations of *A. thaliana* (Barboza *et*
457 *al*. 2013) and played a key role in the development of dwarfed Green Revolution rice and barley

458 (Sasaki et al. 2002; Jia et al. 2009). Further, the DELLA gene, *GAI*, is located within the vicinity
459 of another growth regulatory QTL (*DIV2*). *GAI* was also an allele frequency outlier between
460 coastal and inland populations (Gould et al. 2017). Friedman (2014) found that the *DIV1* and
461 *DIV2* loci interact epistatically. Thus, it would not be surprising if the genetic changes that
462 underlie both QTL are in the same molecular pathway. Further, the negative antagonism between
463 the GA and JA hormone pathways via the DELLA-JAZ signaling node (Havko et al. 2016; Guo
464 et al. 2018; Howe et al. 2018) suggests a direct mechanism by which trade-offs between growth,
465 reproduction, and resistance could easily evolve. Future functional genetic studies will be needed
466 to determine whether these genes in fact are involved in adaptive shifts between growth,
467 reproduction, and defense underlying local adaptation in this system.
468
469 While we saw a greater response to GA addition in coastal plants and observed an interaction
470 between GA addition and the inversion, other hormones could also play a role or even be the
471 ultimate cause of the divergence between the coastal perennial and inland annual ecotypes. Three
472 major classes of hormones, Auxins, Brassinosteroids and Gibberellins, are all associated with
473 growth phenotypes like those that differ between coastal perennial and inland annual ecotypes of
474 *M. guttatus* (Ross and Quittenden 2016; Unterholzner et al. 2016). These hormones interact in
475 multiple ways, which have yet to be fully elucidated, to result in shifts in growth/reproduction
476 phenotypes.
477
478 ***Conclusions and future directions***
479 There are numerous evolutionary and ecological models that make predictions on the evolution
480 of relationships between growth, reproduction, and herbivore resistance. While recent meta-

481 analyses have found that some models have moderate support (Endara and Coley 2011), there
482 are many exceptions and some models appear to not be well supported at all (Stamps et al. 2003;
483 Hahn and Maron 2016; Smilanich et al. 2016). The reasons that these models do not hold up are
484 often attributed to vast variation in the extrinsic environmental factors that exert selective
485 pressures on plant populations, broad variation in life-history among plant species, differences
486 between interspecific and intraspecific variation, and variation in herbivore abundances across
487 environmental gradients (Stamps et al. 2003; Hahn and Maron 2016; Smilanich et al. 2016; Hahn
488 et al. 2019). Less well appreciated are the molecular genetic mechanisms that underlie shifts in
489 allocation between growth, reproduction, and herbivore resistance (Kerwin et al. 2015; Rasmann
490 et al. 2018). The nature of the gene networks responsible for shifts in allocation may also be very
491 important for whether or not particular systems will conform to a given evolutionary or
492 ecological model. Future research should focus on uncovering the molecular mechanisms that
493 underlie the evolution of growth, reproduction, and defense trade-offs in natural populations and
494 integrate predictions from those mechanisms into ecological and evolutionary models.
495

496 **LITERATURE CITED**

497 Agrawal, A. A., J. K. Conner, and S. Rasmann. 2010. Tradeoffs and adaptive negative
498 correlations in evolutionary ecology. Pp. 243-268 in M. A. Bell, et al., eds. Evolution after
499 Darwin: The first 150 years. Sinaur Press, Sunderland, MA.
500

501 Agren, J., and D. W. Schemske. 1993. The cost of defense against herbivores: an experimental
502 study of trichome production in *Brassica rapa*. Am. Nat. **141**:338-350.
503

504 Ahmad, I., and S. E. Wainwright. 1976. Ecotype differences in leaf surface properties of *Agrostis*
505 *stolonifera* from salt marsh, spray zone and inland habitats. New Phytol. **76**:361-366.
506
507 Baker, R. L., and P. K. Diggle. 2011. Node-specific branching and heterochronic changes
508 underlie population-level differences in *Mimulus guttatus* (Phrymaceae) shoot architecture. Am.
509 J. Bot. **98**:1924-1934.
510
511 Baker, R. L., L. C. Hileman, and P. K. Diggle. 2012. Patterns of shoot architecture in locally
512 adapted populations are linked to intraspecific differences in gene regulation. New Phytol.
513 **196**:271-281.
514
515 Barboza, L., S. Effgen, C. Alonso-Blanco, R. Kooke, J. J B. Keurentjes, M. Koornneef, and R.
516 Alcázar. 2013. *Arabidopsis* semidwarfs evolved from independent mutations in GA20ox1,
517 ortholog to green revolution dwarf alleles in rice and barley. Proc. Natl. Acad. Sci. 201314979.
518
519 Bohnert, H. J., Nelson D. E., and Jensen R. G. 1995. Adaptations to environmental stresses. Plant
520 Cell **7**:1099–1111.
521
522 Boyce, S. G. 1954. The salt spray community. Ecol. Monograph. **24**:29–67.
523
524 Bray, E. A. 1997. Plant responses to water deficit. Trends Plant Sci. **2**:48–54.
525

526 Campos, M. L., Y. Yoshida, I. T. Major, D. de Oliveira Ferreira, S. M. Weraduwage, J. E.
527 Froehlich, B. F. Johnson, D. M. Kramer, G. Jander, T. D. Sharkey, and G. A. Howe. 2016.
528 Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs.
529 Nat. Commun. **7**:12570.
530
531 Cipollini, D., D. Walters, C. Voelckel. 2014. Costs of resistance in plants: from theory to
532 evidence. Annu. Plant Rev. 47:263-307.
533
534 Clausen, J., and W. H. Hiesey. 1958. Experimental studies on the nature of species. IV. Genetic
535 structure of ecological races. Carnegie Institution of Washington, Washington, DC.
536
537 Coley, P. D., J. P Bryant, F. F. S. Chapin III. 1985. Resource availability and plant antiherbivore
538 defense. Science **230**:895-899.
539
540 Cowling, R. M., P. W Rundel., B. B. Lamont, M. K. Arroyo, and M. Arianoutsou. 1996. Plant
541 diversity in Mediterranean-climate regions. Trends Ecol. Evol. **11**:362-366.
542
543 Defossez, E., L. Pellissier, and S. Rasmann. 2018. The unfolding of plant growth form-defense
544 syndromes along elevational gradients. Ecol. Lett. **21**:609-618.
545
546 Dobzhansky, T. 1970. Genetics of the Evolutionary Process. Columbia University Press, New
547 York, NY.
548

549 Endara, M. J., and P. D. Coley. 2011. The resource availability hypothesis revisited: A meta-
550 analysis. Funct. Ecol. **25**:389-398.
551
552 Feeny, P. 1976 Plant apparency and chemical defense. Pp. 1-40 in J. W. Wallace and R. L.
553 Mansell, eds. Biochemical interaction between plants and insects. Springer, Boston, MA.
554
555 Ferris, K. G., L. L. Barnett, B. K. Blackman, J. H. Willis. 2017. The genetic architecture of local
556 adaptation and reproductive isolation in sympatry within the *Mimulus guttatus* species complex.
557 Mol. Ecol. **26**:208-224.
558
559 Fine, P. V, Z. J. Miller, I. Mesones, S. Irazuzta, H. M. Appel, M. H. Stevens, I. Sääksjärvi, J. C.
560 Schultz, and P. D. Coley. 2006. The growth–defense trade-off and habitat specialization by
561 plants in Amazonian forests. Ecology **87**:S150-S162.
562
563 Forister, M. L., L. A. Dyer, M. S. Singer, J. O. Stireman, and J. T. Lill. 2012. Revisiting the
564 evolution of ecological specialization, with emphasis on insect–plant interactions. Ecology
565 **93**:981-991.
566
567 Friedman, J. 2014. Genetic determinants and epistasis for life history trait differences in the
568 common monkeyflower, *Mimulus guttatus*. J. Heredity **105**:910-921.
569

570 Friedman, J., and J. H. Willis. 2013. Major QTLs for critical photoperiod and vernalization
571 underlie extensive variation in flowering in the *Mimulus guttatus* species complex. New Phytol.
572 **199**:571-583.
573
574 Friedman, J., A. D. Twyford, J. H. Willis, and B. K. Blackman. 2015. The extent and genetic
575 basis of phenotypic divergence in life history traits in *Mimulus guttatus*. Mol. Ecol. **24**:111-122.
576
577 Friedman, J., and M. J. Rubin. 2015. All in good time: understanding annual and perennial
578 strategies in plants. American Journal of Botany **102**:497-499.
579
580 Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. Annu. Rev.
581 Ecol. Syst. **19**:207-233.
582
583 Gould, B. A., Y. Chen, and D. B. Lowry. 2017. Pooled ecotype sequencing reveals candidate
584 genetic mechanisms for adaptive differentiation and reproductive isolation. Mol. Ecol. **26**:163-
585 177.
586
587 Griffiths, M.E., and C. M. Orians. 2003. Salt spray differentially affects water status, necrosis,
588 and growth in coastal sandplain heathland species. Am. J. Bot. **90**:1188–1196.
589
590 Hahn, P. G., and J. L. Maron. 2016. A framework for predicting intraspecific variation in plant
591 defense. Trends Ecol. Evol. **31**:646-656.
592

593 Hahn, P. G., Agrawal, A. A., Sussman, K. I., and J. L. Maron. 2019. Population variation,
594 environmental gradients, and the evolutionary ecology of plant defense against herbivory. Am.
595 Nat. **193**:20-34.
596
597 Hall, M. C., and J. H. Willis. 2006. Divergent selection on flowering time contributes to local
598 adaptation in *Mimulus guttatus* populations. Evolution **60**:2466-2477.
599
600 Hall, M. C., D. B. Lowry, and J. H. Willis. 2010. Is local adaptation in *Mimulus guttatus* caused
601 by trade-offs at individual loci? Mol. Ecol. **19**:2739-2753.
602
603 Hamilton, J. G., A. R. Zangerl, E. H. DeLucia, and M. R. Berenbaum. 2001. The carbon–nutrient
604 balance hypothesis: its rise and fall. Ecol. Lett. **4**:86-95.
605
606 Havko, N. E., I. T. Major, J. B. Jewell, E. Attaran, and G. A. Howe. 2016. Control of carbon
607 assimilation and partitioning by jasmonate: An accounting of growth–defense tradeoffs. Plants
608 **5**:7.
609
610 Heil, M., and I. T. Baldwin. 2002. Fitness costs of induced resistance: emerging experimental
611 support for a slippery concept. Trends Plant Sci. **7**:61-67.
612
613 Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. Am. Nat.
614 **173**:579-588.
615

616 Herms, D. A., and W. J. Mattson. 1992. The dilemma of plants: to grow or defend. Q. Rev. Biol.
617 **67**:283-335.
618
619 Holeski, L. M., K. Keefover-Ring, M. D. Bowers, Z. T. Harnenz, and R. L. Lindroth. 2013.
620 Patterns of phytochemical variation in *Mimulus guttatus* (yellow monkeyflower). J. Chem. Ecol.
621 **39**:525–536.
622
623 Holeski, L. M., P. Monnahan, B. Koseva, N. McCool, R. L. Lindroth, and J. K. Kelly. 2014. A
624 high-resolution genetic map of yellow monkeyflower identifies chemical defense QTLs and
625 recombination rate variation. Genes Genom. Genet. **4**:813–821.
626
627 Huot, B., J. Yao, B. L. Montgomery, and S. Y. He. 2014. Growth–defense tradeoffs in plants: a
628 balancing act to optimize fitness. Mol. Plant. **7**:1267-1287.
629
630 Howe, G. A., I. T. Major, and A. J. Koo. 2018. Modularity in jasmonate signaling for multistress
631 resilience. Annu. Rev. Plant Biol. **69**:387-415.
632
633 Hou, X., L. Ding, and H. Yu. 2013. Crosstalk between GA and JA signaling mediates plant
634 growth and defense. Plant Cell Rep. **32**:1067-1074.
635
636 Jensen, L.M., H. K. Jepsen, B. A. Halkier, D. J. Kliebenstein, and M. Burow. 2015 Natural
637 variation in cross-talk between glucosinolates and onset of flowering in *Arabidopsis*. Front. Plant
638 Sci. **6**:697.

639

640 Jia, Q., J. Zhang, S. Westcott, X-Q. Zhang, M. Bellgard, R. Lance, C. Li. 2009. GA-20 oxidase
641 as a candidate for the semidwarf gene sdw1/denso in barley. Funct. Integr. Genomics. **9**:255–262

642

643 Juenger, T. E. 2013 Natural variation and genetic constraints on drought tolerance. Curr. Opin.
644 Plant Biol. **16**:274-281.

645

646 Karban, R. 2011. The ecology and evolution of induced resistance against herbivores. Funct.
647 Ecol. 25:339-347.

648

649 Kazan, K., and J. M. Manners. 2012. JAZ repressors and the orchestration of phytohormone
650 crosstalk. Trends Plant Sci. **17**:22-31.

651

652 Kawecki, T. J. 1988. Red queen meets Santa Rosalia: arms races and the evolution of host
653 specialization in organisms with parasitic lifestyles. Am. Nat. **152**:635-651.

654

655 Kawecki, T. J., and Ebert D. 2004. Conceptual issues in local adaptation. Ecol. Lett. **7**:1225-
656 1241.

657

658 Kerwin, R., J. Feusier, J. Corwin, M. Rubin, C. Lin, A. Muok, B. Larson, B. Li, B. Joseph, M.
659 Francisco, and D. Copeland. 2015. Natural genetic variation in *Arabidopsis thaliana* defense
660 metabolism genes modulates field fitness. eLife. **4**:e05604.

661

662 Kirkpatrick, M., and N. H. Barton. 2006. Chromosome inversions, local adaptation and
663 speciation. Genetics **173**:419-434.
664
665 Kooyers, N. J. 2015. The evolution of drought escape and avoidance in natural herbaceous
666 populations. Plant Sci. 234:155-162.
667
668 Kooyers, N. J., B. K. Blackman, and L. M. Holeski. 2017 Optimal defense theory explains
669 deviations from latitudinal herbivory defense hypothesis. Ecology **98**:1036-1048.
670
671 Lowry, D. B., R. C. Rockwood, and J. H. Willis. 2008. Ecological reproductive isolation of coast
672 and inland races of *Mimulus guttatus*. Evolution **62**:2196-2214.
673
674 Lowry, D. B., M. C. Hall, D. E. Salt, and J. H. Willis. 2009. Genetic and physiological basis of
675 adaptive salt tolerance divergence between coastal and inland *Mimulus guttatus*. New Phytol.
676 **183**:776-788.
677
678 Lowry, D. B., and J. H. Willis. 2010. A widespread chromosomal inversion polymorphism
679 contributes to a major life-history transition, local adaptation, and reproductive isolation. PLoS
680 Biol. **8**:e1000500.
681
682 Lowry, D. B. 2012. Ecotypes and the controversy over stages in the formation of new species.
683 Biol. J. Linnean Soc. **106**:241-257.
684

685 Ludlow, M. M. 1989. Strategies of response to water stress. Pp. 509-519 in K. H. Kreeb et al.
686 (Eds.), Structural and functional responses to environmental stresses. SPB Academic, The
687 Hague.
688
689 Lorenzo, O., J. M. Chico, J. J. Sanchez-Serrano, and R. Solano. 2004. JASMONATE-
690 INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different
691 jasmonate-regulated defense responses in *Arabidopsis*. Plant Cell **16**:1938–1950.
692
693 Major, I. T., Y. Yoshida, M. L. Campos, G. Kapali, X. F. Xin, K. Sugimoto, D. Oliveira Ferreira,
694 S. Y. He, and G. A. Howe. 2017. Regulation of growth–defense balance by the JASMONATE
695 ZIM-DOMAIN (JAZ)-MYC transcriptional module. New Phytol. **215**:1533-1547.
696
697 Mason, C. M., and L. A. Donovan. 2015. Evolution of the leaf economics spectrum in herbs:
698 evidence from environmental divergences in leaf physiology across Helianthus (Asteraceae).
699 Evolution **69**:2705-2720.
700
701 Moreira, X., R. Zas, A. Solla, and L. Sampedro. 2015. Differentiation of persistent anatomical
702 defensive structures is costly and determined by nutrient availability and genetic growth-defence
703 constraints. Tree Physiol. **35**:112-123.
704
705 Oneal, E., D. B. Lowry, K. M. Wright, Z. Zhu, and J. H. Willis. 2014. Divergent population
706 structure and climate associations of a chromosomal inversion polymorphism across the *Mimulus*
707 *guttatus* species complex. Mol. Ecol. **23**:2844-2860.

708

709 Popovic, D., and D. B. Lowry. 2019. Oceanic salt spray and herbivore pressure contribute to
710 local adaptation of coastal perennial and inland annual ecotypes of the Seep Monkeyflower
711 (*Mimulus guttatus*). BioRxiv. 523902.

712

713 Rasmann, S., J. Sánchez Vilas, G. Glauser, M. Cartolano, J. Lempe, M. Tsiantis, and J. R.
714 Pannell. 2018. Pleiotropic effect of the Flowering Locus C on plant resistance and defence
715 against insect herbivores. J. Ecol. **106**:1244-1255.

716

717 Rausher, M. D. 1996. Genetic analysis of coevolution between plants and their natural enemies.
718 Trends in Genetics **12**:212-217.

719

720 Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. P.p. 3–54 in G. A.
721 Rosenthal and D. H. Janzen, editors. Herbivores: their interaction with secondary plant
722 metabolites. Academic Press, Orlando, Florida, USA.

723

724 Ross J. J., and L. J. Quittenden. 2016. Interactions between brassinosteroids and gibberellins:
725 Synthesis or Signaling? Plant Cell **28**:829-832.

726

727 Rotter, M. C., J. J. Couture, E. M. Rothwell, J. Garcia, and L. M. Holeski. 2018. Evolutionary
728 ecology of plant resistance traits across the herbivore diet spectrum: A test in the model plant
729 *Mimulus guttatus*. Evolutionary Ecology Research **19**:423-440.

730

731 Sasaki A., M. Ashikari, M. Ueguchi-Tanaka, H. Itoh, A. Nishimura, D. Swapan, K. Ishiyama, T.
732 Saito, M. Kobayashi, G. S. Khush, H. Kitano, and M. Matsuoka 2002. Green revolution: a
733 mutant gibberellin-synthesis gene in rice. Nature **416**:701.
734
735 Schwander, T., R. Libbrecht, and L. Keller. 2014. Supergenes and complex phenotypes. Curr.
736 Biol. **24**:R288-R294.
737
738 Smilanich, A. M., R. M. Fincher, and L. A. Dyer. 2016. Does plant apparency matter? Thirty
739 years of data provide limited support but reveal clear patterns of the effects of plant chemistry on
740 herbivores. New Phytol. **210**:1044-1057.
741
742 Stamp, N. 2003 Out of the quagmire of plant defense hypotheses. Q. Rev. Biol. **78**:23-55.
743
744 Strauss, S.Y., J.A. Rudgers, J.A. Lau, and R.E. Irwin. 2002. Direct and ecological costs of
745 resistance to herbivory. Trends Ecol. Evol. **17**:278-285.
746
747 Stowe, K. A., and R. J. Marquis. 2011. Costs of defense: correlated responses to divergent
748 selection for foliar glucosinolate content in *Brassica rapa*. Evol. Ecol. **25**:763-775.
749
750 Svanbäck, R., and P. Eklöv. 2003. Morphology dependent foraging efficiency in perch: a trade-
751 off for ecological specialization? Oikos **102**:273-284.
752

753 Twyford, A. D., and J. Friedman. 2015. Adaptive divergence in the monkey flower Mimulus
754 guttatus is maintained by a chromosomal inversion. Evolution **69**:1476-1486.
755
756 Uesugi, A., T. Connallon, A. Kessler, and K. Monro, 2018. Relaxation of herbivore-mediated
757 selection drives the evolution of genetic covariances between plant competitive and defense
758 traits. Evolution **71**:1700-1709.
759
760 Unterholzner, S. J., W. Rozhon, and B. Poppenberger. 2016. Reply: Interaction between
761 Brassinosteroids and Gibberellins: Synthesis or Signaling? In Arabidopsis, Both! Plant Cell.
762 **28**:836-839.
763
764 van Dam, N. M., and I. T. Baldwin. 1998. Costs of jasmonate-induced responses in plants
765 competing for limited resources. Ecol. Lett. **1**:30-33.
766
767 Wellenreuther, M., and L. Bernatchez. 2018. Eco-evolutionary genomics of chromosomal
768 inversions. Trends Ecol. Evol. **33**:427-440.
769
770 Whitlock, M. C. 1996. The red queen beats the jack-of-all-trades: the limitations on the evolution
771 of phenotypic plasticity and niche breadth. Am. Nat. **148**:S65-S77.
772
773 Whittaker, R. H. 1975. Communities and Ecosystems. McMillan, New York, NY.
774

775 Yan, Y., S. Stolz, A. Chetelat, P. Reymond, M. Pagni, L. Dubugnon, and E. E. Farmer. 2007. A
776 downstream mediator in the growth repression limb of the jasmonate pathway. Plant Cell
777 **19**:2470–2483.
778
779 Yang, D. L., J. Yao, C. S. Mei, X. H. Tong, L. J. Zeng, Q. Li, L. T. Xiao, T. P. Sun, J. Li, X. W.
780 Deng, C. M. Lee, M. F. Thomashow, Y. Yang, Z. He, S. Y. He. 2012. Plant hormone jasmonate
781 prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc. Nat.
782 Acad. Sci. **109**:E1192-1200.
783
784 Zhang, Y., and J. Turner. 2008. Wound-induced endogenous jasmonates stunt plant growth by
785 inhibiting mitosis. PLoS ONE **3**:e3699.
786
787
788
789
790
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798 **TABLES**

799 **Table 1.** Effects of individual factors and their interactions on the first four principal components

800 of 37 morphological traits quantified in the experiment comparing ecotypes and accessions.

Trait	Source	DF	SS	F	P
PC1	Accession[Ecotype]	8	730.32	23.70	<0.0001*
PC1	Ecotype	2	1626.14	211.04	<0.0001*
PC1	Treatment	1	58.63	15.22	0.0001*
PC1	Accession*Treatment[Ecotype]	8	79.71	2.59	0.0101*
PC1	Ecotype*Treatment	2	12.77	1.66	0.1930
PC2	Accession[Ecotype]	8	366.72	20.82	<0.0001*
PC2	Ecotype	2	54.38	12.35	<0.0001*
PC2	Treatment	1	173.77	78.92	<0.0001*
PC2	Accession*Treatment[Ecotype]	8	134.09	7.61	<0.0001*
PC2	Ecotype*Treatment	2	27.12	6.16	0.0025*
PC3	Accession[Ecotype]	8	169.98	14.15	<0.0001*
PC3	Ecotype	2	5.76	1.92	0.1492
PC3	Treatment	1	51.18	34.08	<0.0001*
PC3	Accession*Treatment[Ecotype]	8	48.37	4.03	0.0002*
PC3	Ecotype*Treatment	2	126.35	42.07	<0.0001*
PC4	Accession[Ecotype]	8	143.39	16.28	<0.0001*
PC4	Ecotype	2	109.37	49.66	<0.0001*
PC4	Treatment	1	0.51	0.46	0.4981
PC4	Accession*Treatment[Ecotype]	8	15.28	1.73	0.0916
PC4	Ecotype*Treatment	2	0.42	0.19	0.8247

*Significant after Bonferroni correction for multiple testing ($P < 0.0125$)

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809 **Table 2.** Effects of line (SWB-S1 vs. LMC-L1), treatment (GA vs. Mock), and the interaction on

810 the first three principal of 37 morphological traits quantified for the parental inbred lines.

Trait	Source	SS	F	P
PC1	Line	2027.14	314.39	<0.0001*
PC1	Treatment	145.75	22.6	<0.0001*
PC1	Line*Treatment	7.06	1.10	0.2969
PC2	Line	42.25	15.61	0.0001*
PC2	Treatment	257.86	95.24	<0.0001*
PC2	Line*Treatment	262.49	96.95	<0.0001*
PC3	Line	2.12	0.63	0.4271
PC3	Treatment	32.51	9.74	0.0021*
PC3	Line*Treatment	15.73	4.71	0.0314

*Significant after Bonferroni correction for multiple testing ($P < 0.0083$)

811

812 **Table 3.** Effects of line (Introgression-NIL vs. Control-NIL), treatment (GA vs. Mock), and the

813 interaction on the first three principal of 37 morphological traits quantified for the parental

814 inbred lines.

Trait	Source	SS	F	P
PC1	Line	206.86	51.23	<0.0001*
PC1	Treatment	43.84	10.86	0.0012*
PC1	Line*Treatment	28.17	6.98	0.0090
PC2	Line	0.02	0.01	0.9072
PC2	Treatment	606.48	389.98	<0.0001*
PC2	Line*Treatment	24.91	16.02	<0.0001*
PC3	Line	21.12	5.50	0.0202
PC3	Treatment	3.90	1.02	0.315
PC3	Line*Treatment	10.15	2.64	0.1059

*Significant after Bonferroni correction for multiple testing ($P < 0.0083$)

815

816

817 **Table 4.** Means (standard errors) and Tukey HSD post-hoc results for the concentrations of
818 phenylpropanoid glycosides in coastal perennial (SWB) and inland annual (LMC) parents as well
819 as near-isogenic lines of the *DIV1* inversion in the coastal perennial genetic background.

Trait	Coastal Parent (*N*=25)	Inland Parent (*N*=8)	Control NIL (*N*=54)	Introgression NIL (*N*=35)
Total PPGs	164.24 (7.74)A	65.65 (13.69)C	161.32 (5.27)A	119.67 (6.55)B
Conandroside	105.11 (6.65)A	42.26 (11.75)B	106. 98 (4.52)A	55.71 (5.62)B
Calceolarioside A	37.15 (2.13)B	12.79 (3.77)C	32.77 (1.45)B	46.30 (1.80)A
Mimuloside	7.93 (0.64)A	3.62 (1.13)B	7.54 (0.43)A	4.24 (0.55)B
Verbascoside	6.91 (0.36)A	1.90 (0.63)B	6.89 (0.24)A	5.90 (0.30)A
Calceolarioside B	0.98 (0.08)A	0.33 (0.13)B	0.66 (0.06)B	0.62 (0.06)B
Unknown PPG 16	5.52 (0.44)A	4.56 (0.77)A	5.87 (0.30)A	6.09 (0.37)A
Unknown PPG10	0.64 (0.04)B	0.19 (0.07)C	0.60 (0.03)B	0.81 (0.03)A

Mean (SE) and Tukey HSD post-hoc results. Sample sizes in parentheses.

820

821

822

823 **FIGURE LEGENDS**

824 **Figure 1.** A) Map of coastal perennial (blue), inland annual (yellow), and inland perennial
825 (green) populations from where accessions for experiment were derived. B) Effects of the
826 introgression of the inland *DIV1* inversion orientation into the coastal perennial genetic
827 background. Left: Coastal perennial parental inbred line. Center: Control Near Isogenic Line
828 (NIL) containing the coastal orientation of the inversion. Right: Introgression NIL containing the
829 inland orientation of the inversion. All three plants germinated on the same day. C) Differential
830 responses of inland annual and coastal perennial accessions to gibberellin treatment and water
831 control (mock).

832

833 **Figure 2.** Least square means of ecotype x treatment interactions of the first four principal
834 components for coastal perennial, inland annual, and inland perennial ecotypes in response to
835 mock and gibberellin treatments. Error bars are standard errors.

836

837 **Figure 3.** Least square means of line x treatment interactions of coastal perennial (SWB-S1) and
838 inland annual (LMC-L1) parents and coastal perennial genetic background near-isogenic lines
839 (NILs) to mock and gibberellin treatments. The introgression NILs were homozygous for the
840 inland annual orientation of the *DIV1* inversion, while the control NILs were homozygous for the
841 coastal perennial orientation of the *DIV1* inversion. Lower case letters indicate significant
842 differences of least square means by Tukey HSD *post hoc* analyses. Error bars are standard
843 errors.

844

845 **Figure 4.** Concentration of phenylpropanoid glycosides for coastal perennial (SWB-S2xS3) and
846 inland annual (LMC-L2xL3) parents as well as coastal perennial genetic background near-
847 isogenic lines (NILs). The introgression NILs were homozygous for the inland annual orientation
848 of the *DIV1* inversion, while the control NILs were homozygous for the coastal perennial
849 orientation of the *DIV1* inversion.

850

851 **Supplemental Materials**

852

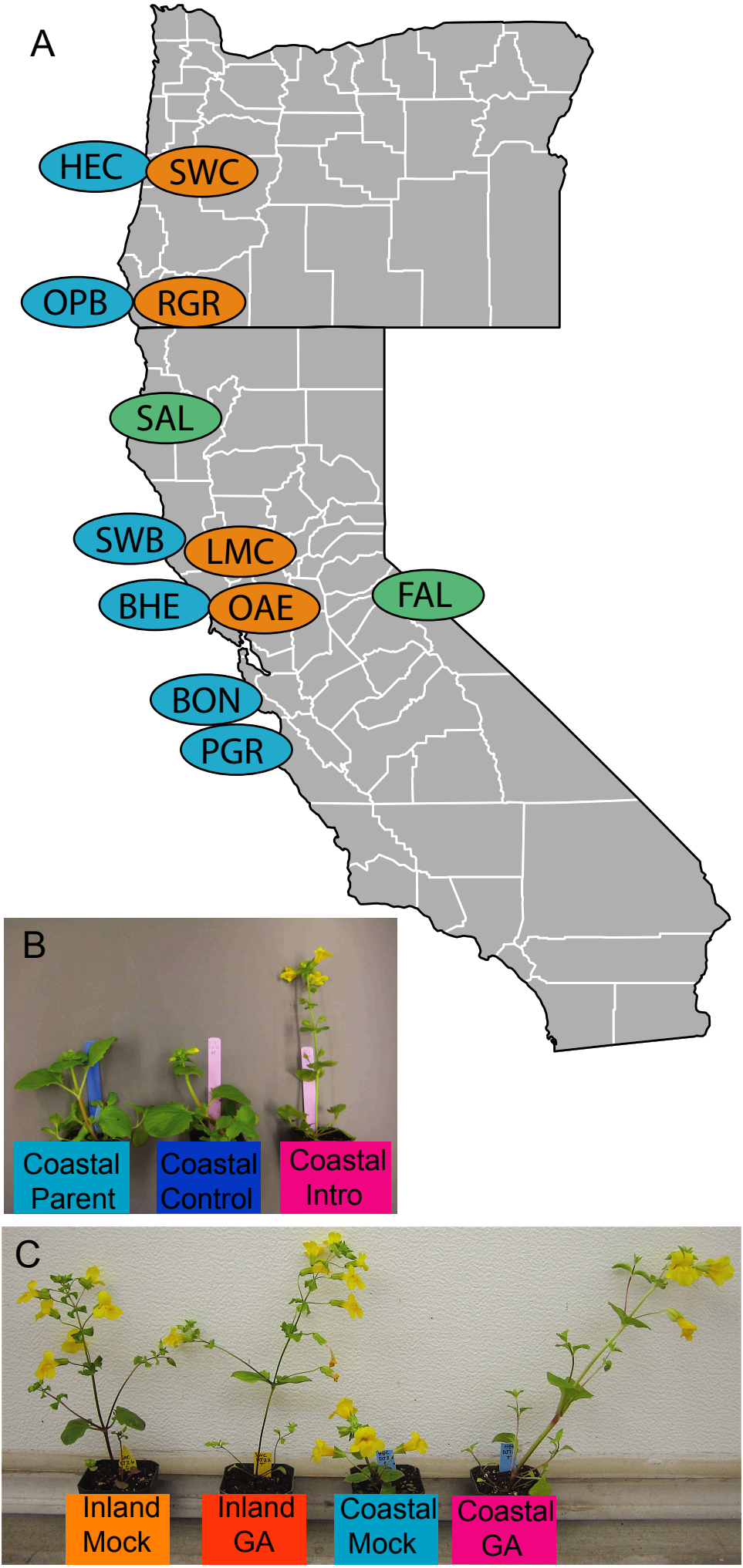
853 **Table S1.** Collection locations of population accessions used in this study, including the number
854 of individuals used for ANOVAs of principal components per each accession.

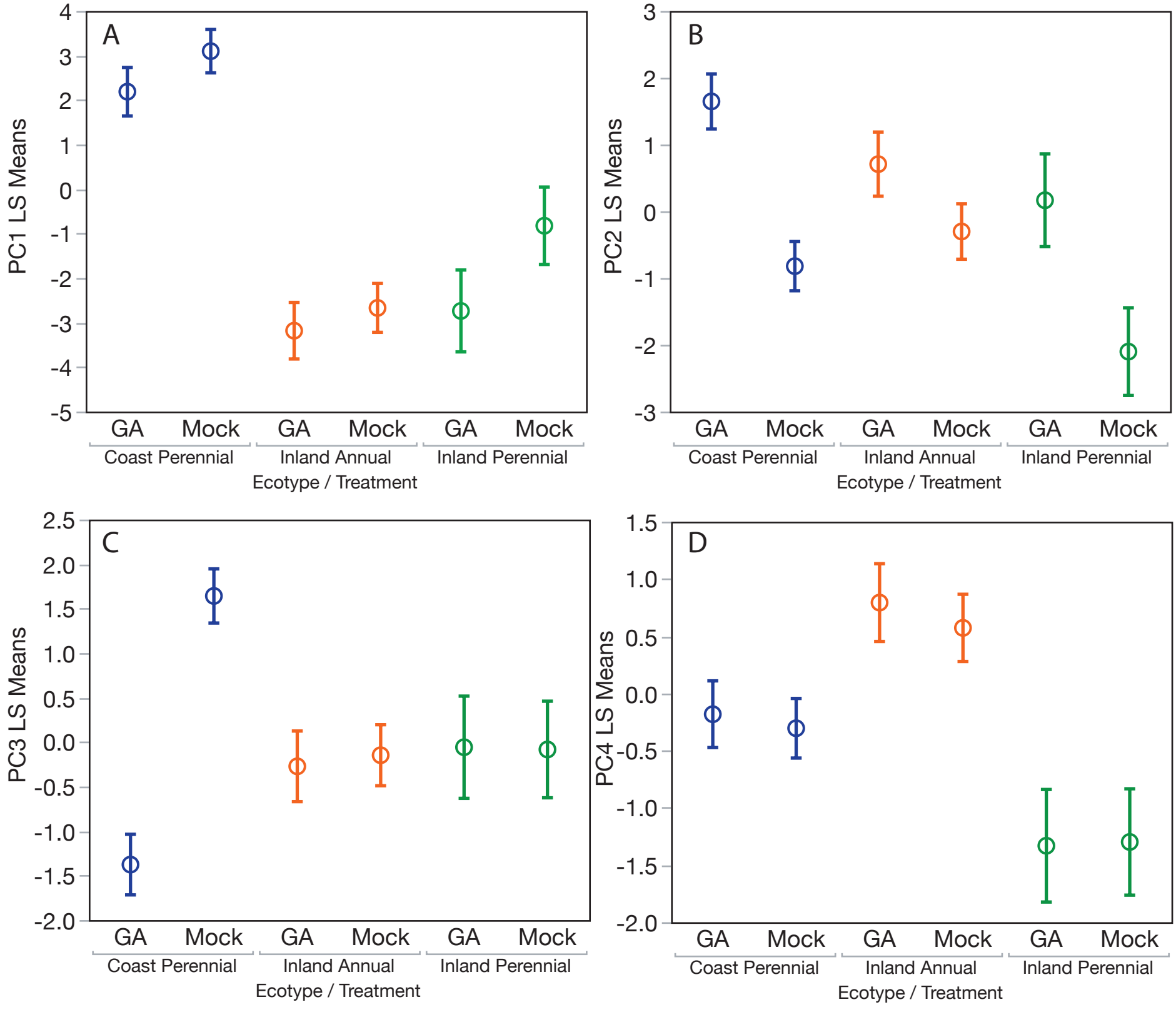
855

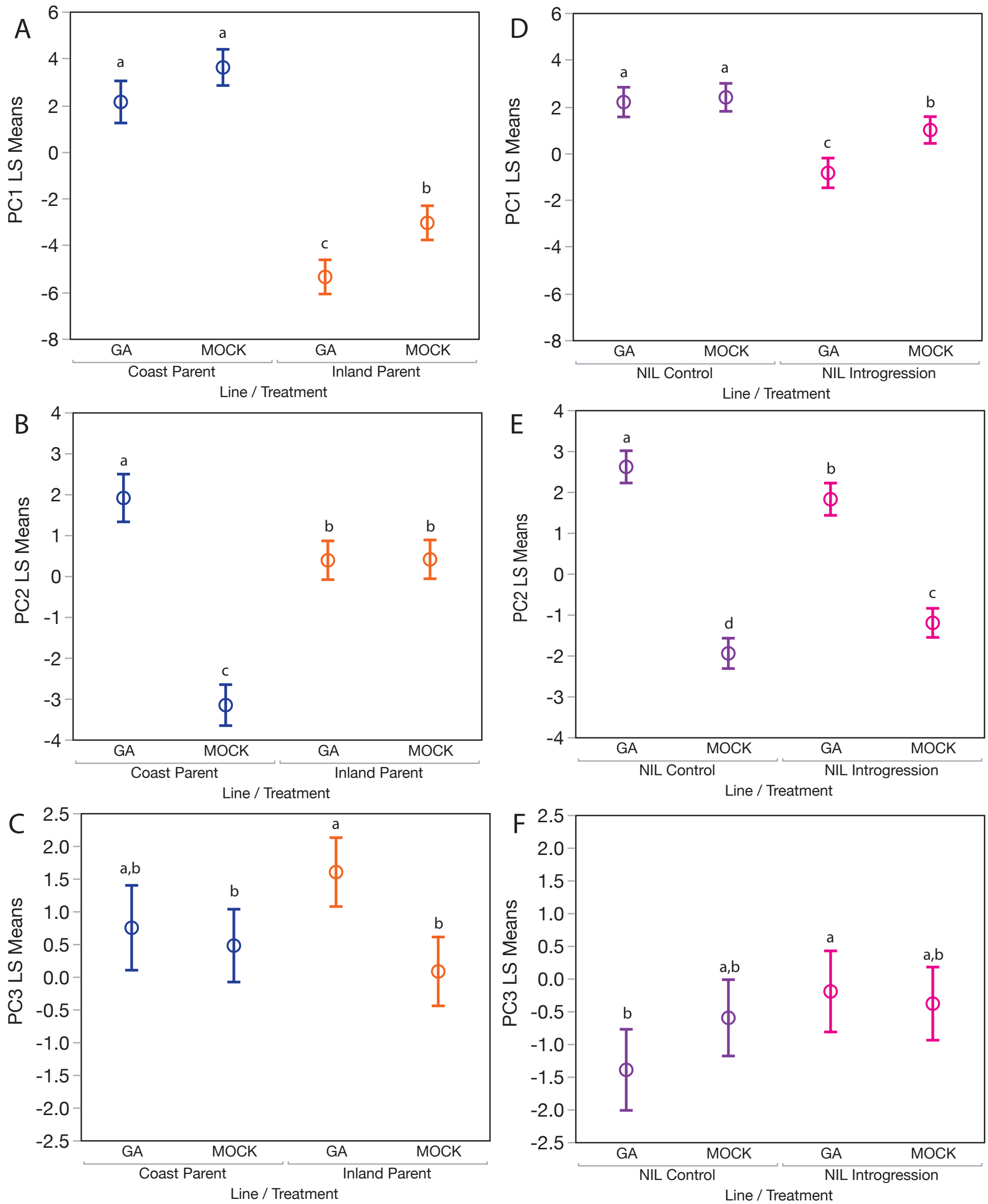
856 **Table S2.** Trait loadings on the first four principle components axes for experiment comparing
857 population accession. Percent of variation explained by each PC included in parentheses.

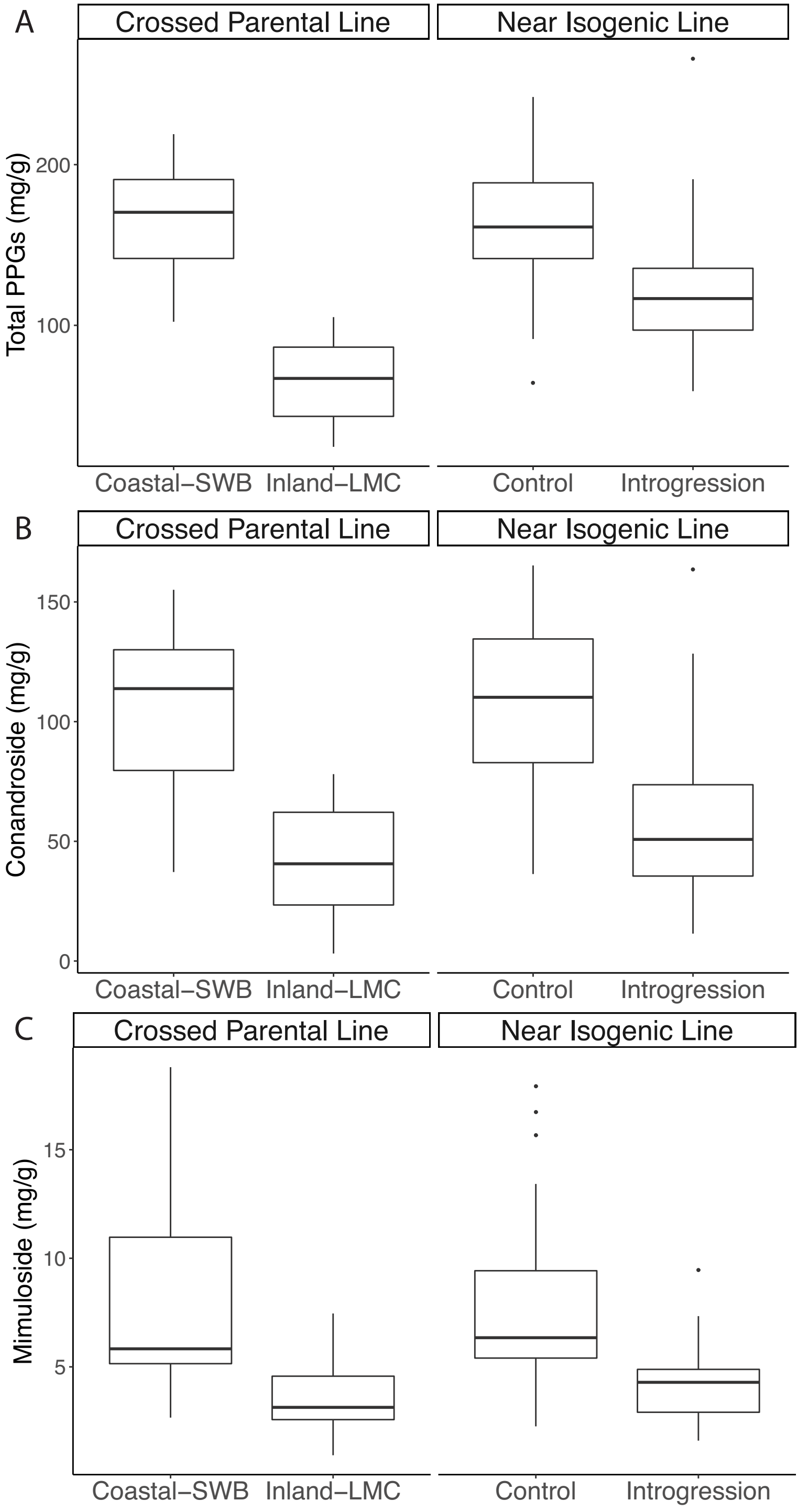
858

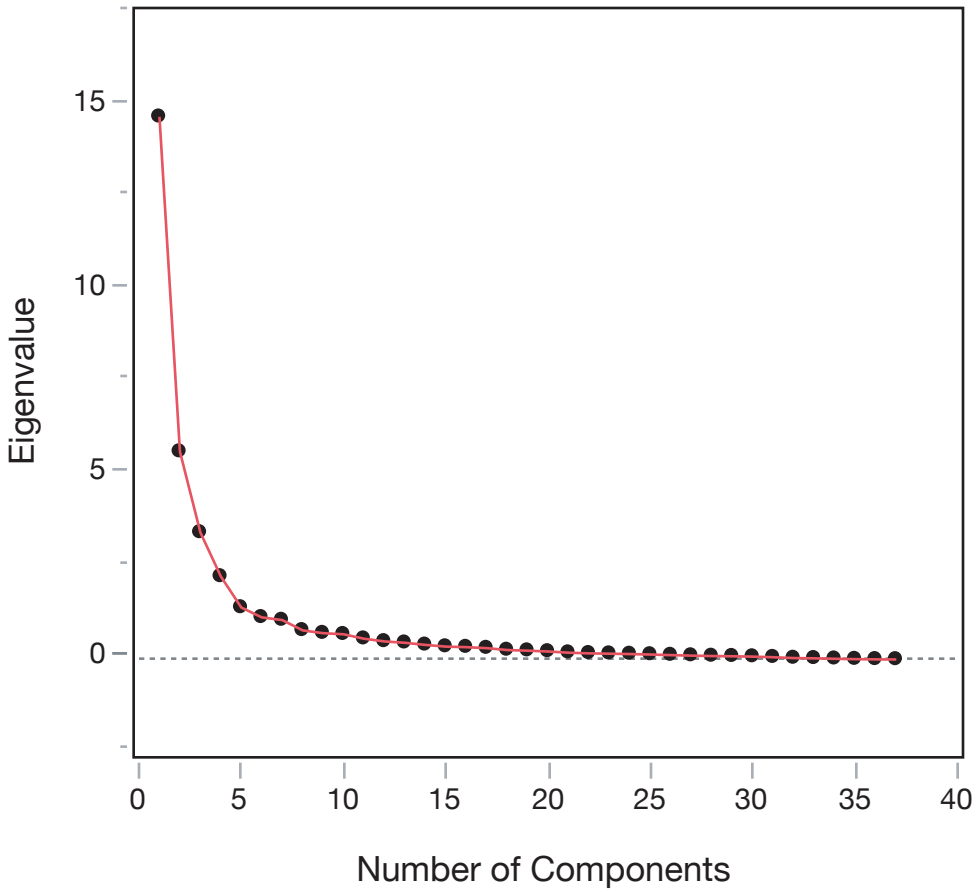
859 **Table S3.** Trait loadings on the first three principle components axes for all plants grown in the
860 experiment with near isogenic lines and the parental genotypes. Percent of variation explained by
861 each PC included in parentheses.
862
863 **Figure S1.** Scree plot of eigenvalues for principal components analysis of trait data collected for
864 population accessions
865
866 **Figure S2.** Least square means of accession x treatment interactions of the first four principal
867 components for coastal perennial, inland annual, and inland perennial accessions in response to
868 mock and gibberellin treatments.
869
870 **Figure S3.** Scree plot of eigenvalues for principal components analysis of trait data collected for
871 experiment with near isogenic lines and parental lines.

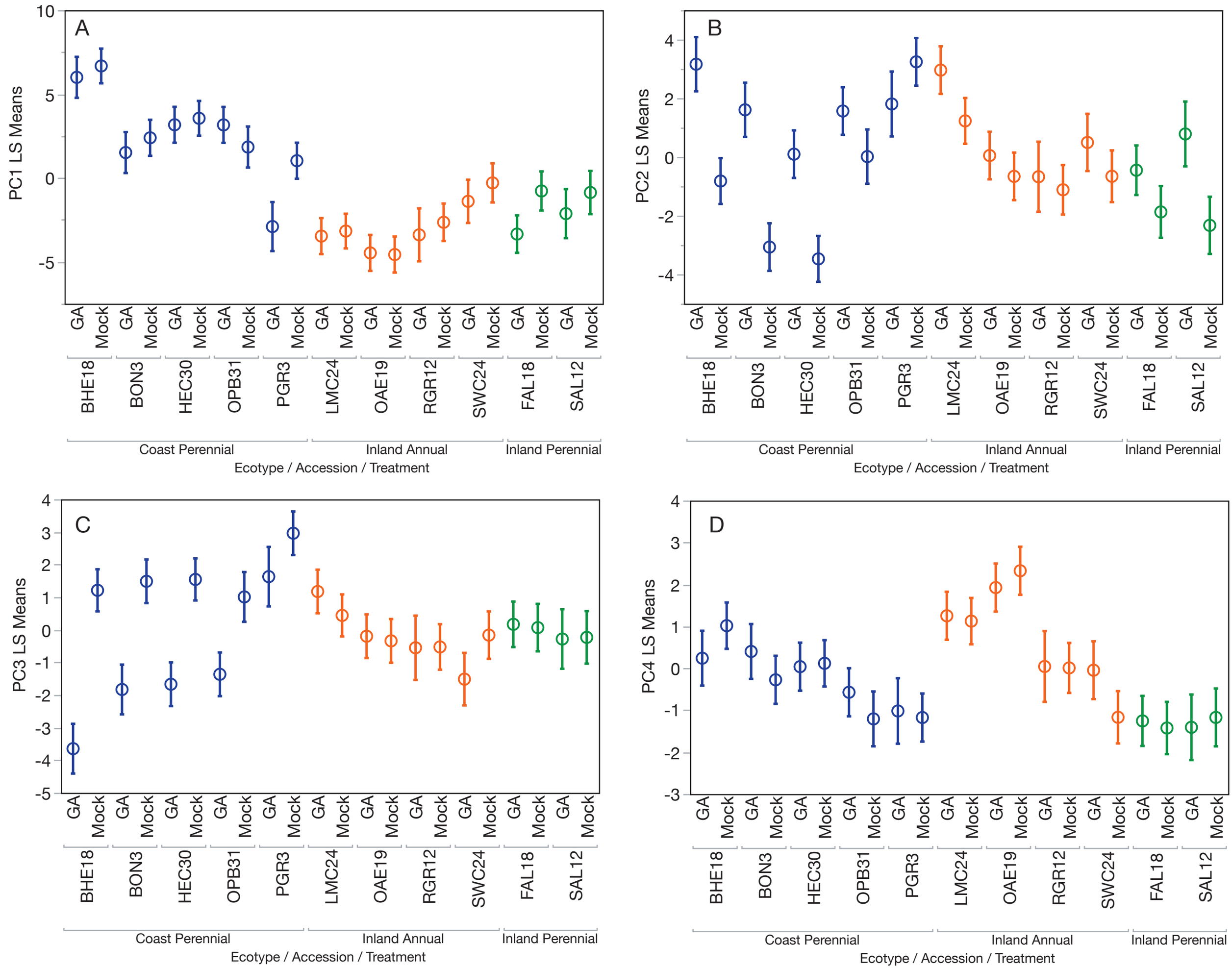

A
HEC
SWC
OPB
RGR
SAL
SWB
LMC
BHE
OAE
FAL
BON
PGR
B
Coastal Parent
Coastal Control
Coastal Intro
C
Inland Mock
Inland GA
Coastal Mock
Coastal GA

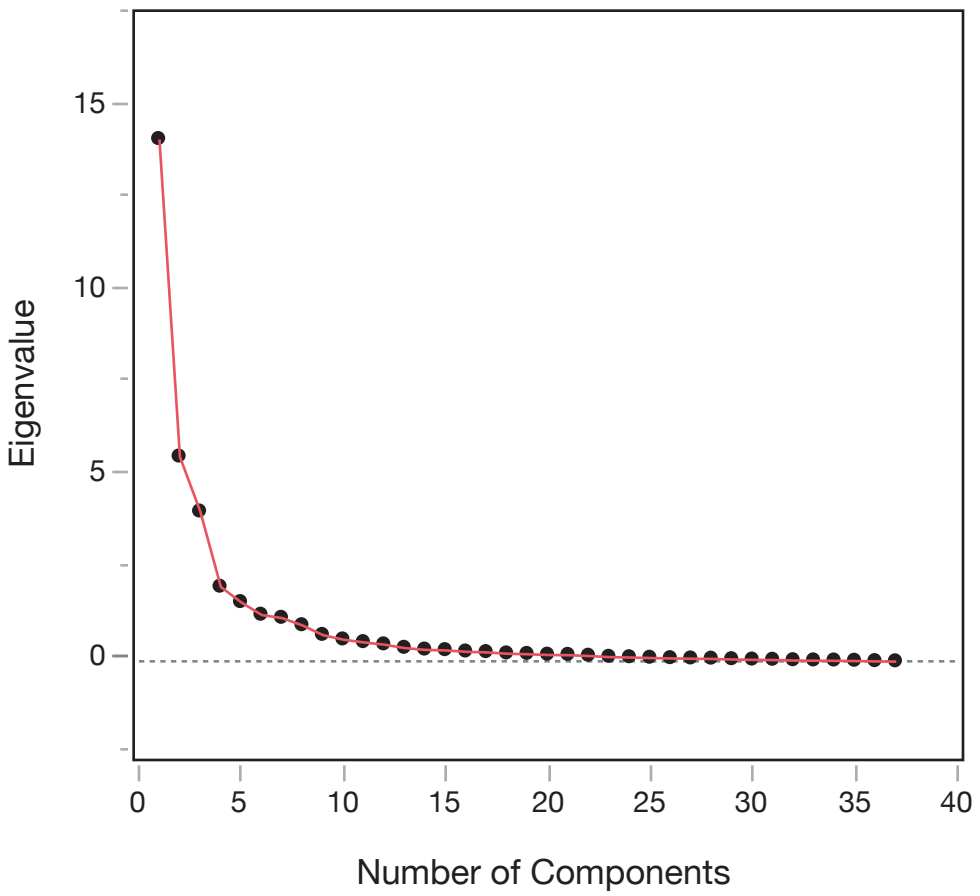

A
PC1 LS Means
4
3
2
1
0
-1
-2
-3
-4
-5
GA
Mock
GA
Mock
GA
Mock
Coast Perennial
Inland Annual
Inland Perennial
Ecotype / Treatment
B
PC2 LS Means
3
2
1
0
-1
-2
-3
GA
Mock
GA
Mock
GA
Mock
Coast Perennial
Inland Annual
Inland Perennial
Ecotype / Treatment
C
PC3 LS Means
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
GA
Mock
GA
Mock
GA
Mock
Coast Perennial
Inland Annual
Inland Perennial
Ecotype / Treatment
D
PC4 LS Means
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
GA
Mock
GA
Mock
GA
Mock
Coast Perennial
Inland Annual
Inland Perennial
Ecotype / Treatment


A
PC1 LS Means
6
4
2
0
-2
-4
-6
-8
a
a
c
b
GA
MOCK
GA
MOCK
Coast Parent
Inland Parent
Line / Treatment
D
PC1 LS Means
a
a
c
b
GA
MOCK
GA
MOCK
NIL Control
NIL Introgression
Line / Treatment
B
PC2 LS Means
4
3
2
1
0
-1
-2
-3
-4
a
c
b
b
GA
MOCK
GA
MOCK
Coast Parent
Inland Parent
Line / Treatment
E
PC2 LS Means
a
d
b
c
GA
MOCK
GA
MOCK
NIL Control
NIL Introgression
Line / Treatment
C
PC3 LS Means
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
a,b
b
a
b
GA
MOCK
GA
MOCK
Coast Parent
Inland Parent
Line / Treatment
F
PC3 LS Means
b
a,b
a
a,b
GA
MOCK
GA
MOCK
NIL Control
NIL Introgression
Line / Treatment


A
Crossed Parental Line
Near Isogenic Line
Total PPGs (mg/g)
200
100
Coastal–SWB
Inland–LMC
Control
Introgression
B
Crossed Parental Line
Near Isogenic Line
Conandroside (mg/g)
150
100
50
0
Coastal–SWB
Inland–LMC
Control
Introgression
C
Crossed Parental Line
Near Isogenic Line
Mimuloside (mg/g)
15
10
5
Coastal–SWB
Inland–LMC
Control
Introgression


Eigenvalue
15
10
5
0
0
5
10
15
20
25
30
35
40
Number of Components


A
PC1 LS Means
10
5
0
-5
B
PC2 LS Means
4
2
0
-2
-4
C
PC3 LS Means
4
3
2
1
0
-1
-2
-3
-4
-5
D
PC4 LS Means
4
3
2
1
0
-1
-2
-3
GA
Mock
BHE18
BON3
HEC30
OPB31
PGR3
LMC24
OAE19
RGR12
SWC24
FAL18
SAL12
Coast Perennial
Inland Annual
Inland Perennial
Ecotype / Accession / Treatment


Eigenvalue
15
10
5
0
0
5
10
15
20
25
30
35
40
Number of Components