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

David B. Lowry[a,b,c], Damian Popovic[a,b], Darlene J. Brennan[a], Liza M. Holeski[d]

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

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

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

[d]Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, 86011

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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 the underlying physiological and genetic mechanisms. Here, we evaluate potential mechanisms underlying 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 coastal perennial and inland 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 evolution of growth form via an interaction with the GA pathway. 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 in this system. Here, we demonstrate that the *DIV1* inversion also has sizable impacts on both the total abundance and profile of phytochemical compounds involved in herbivore resistance.

INTRODUCTION

One of the fundamental tenants 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

70 Maron 2016). One common pattern for intraspecific plant variation is a positive relationship
71 between the length of the growing season along an environmental gradient and the level of
72 herbivore resistance (Hahn and Maron 2016; Kooyers et al. 2017). This pattern could be driven
73 by plants having more time to allocate resources to leaf production and defense, greater
74 herbivore pressure in habitats with longer growing seasons, and/or a greater apparency of plants
75 with longer growing seasons (Feeny 1976; Mason and Donovan 2015; Hahn and Maron 2016;
76 Kooyers et al. 2017). Allocation to reproduction (fecundity) frequently trades off with
77 constitutive and/or induced herbivore resistance (Agren and Schemske 1993; Heil and Baldwin
78 2002; Strauss et al. 2002; Stowe and Marquis 2011; Cipollini et al. 2014).
79
80 Achieving an evolutionary optimum in how resources are allocated to growth, reproduction, and
81 defense will depend on the nature of all environmental challenges faced by each local population
82 (Rhoades 1979; Rausher 1996; Hamilton et al. 2001; Strauss et al. 2002; Stamp 2003; Karban
83 2011; Cipollini et al. 2014; Jensen et al. 2015; Smilanich et al. 2016). Despite the development
84 of multiple ecological and evolutionary hypotheses that postulate a relationship between growth,
85 reproduction, and resistance to herbivores (Feeny 1976; Coley et al. 1985; Rhoades 1979; Herms
86 and Mattson 1992; Strauss et al. 2002; Stamp 2003, Fine et al. 2006; Agrawal et al. 2010;
87 Cipollini et al. 2014; Hahn and Maron 2016), these hypotheses do not make any predictions
88 about the underlying molecular mechanisms that mediate these relationships. The genetic
89 mechanisms responsible for trade-offs among growth, reproduction, and resistance are just
90 beginning to be elucidated in model systems (Lorenzo et al. 2004; Yang et al. 2012; Kerwin et al.
91 2015; Campos et al. 2016; Havko et al. 2016; Major et al. 2017; Howe et al. 2018; Rasmann et
92 al. 2018), but have yet to be evaluated in the evolutionary context of local adaptation.

93 Recent studies have shown that changes in the allocation of resources to growth versus resistance
94 are made through a set of interacting gene networks (Karzan and Manners 2012; Huot et al.
95 2014; Campos et al. 2016; Havko et al. 2016). Jasmonates (JA) are key regulatory hormones
96 involved in the response of plants to herbivore attack (Zhang and Turner 2008; Havko et al.
97 2016). While JA production increases herbivore defenses, it also inhibits plant growth through
98 interactions with other gene networks (Zhang and Turner 2008; Yan et al. 2007; Karzan and
99 Manners 2012; Yang et al. 2012). For example, the interactions of JAZ genes with DELLA
100 genes in the signaling pathway of Gibberellin (GA) growth hormones are thought to play a key
101 role in mediating resource allocation (Yang et al. 2012; Hou et al. 2013; Havko et al. 2016).
102
103 Here, we focus on understanding the physiological and genetic mechanisms underlying shifts in
104 allocation to growth, reproduction, and defense for local adapted populations of the yellow
105 monkeyflower *Mimulus guttatus*. The availability of soil water is a key driver of local adaptation
106 in the *M. guttatus* species complex (Hall and Willis 2006; Lowry et al. 2008; Ferris et al. 2017).
107 The coastal habitats of California and Oregon have many wet seeps and streams that are
108 maintained year-round as a result of persistent summer oceanic fog and cool temperatures (Hall
109 and Willis 2006; Lowry et al. 2008; Lowry and Willis 2010). All coastal populations of *M.*
110 *guttatus* that reside in those habitats have a late-flowering life-history strategy. These coastal
111 perennial populations thus, make a long-term investment in growth over reproduction (Hall and
112 Willis 2006; Lowry et al. 2008; Hall et al. 2010; Baker and Diggle 2011; Baker et al. 2012). That
113 investment in growth manifests through the production of many vegetative lateral stolons,
114 adventitious roots, and leaves in coastal perennial plants. In contrast, the vast majority of nearby
115 inland populations of *M. guttatus* in the coastal mountain ranges reside in habitats that dry out

116 completely during summer months. These inland populations have evolved a rapid growth
117 drought escape annual life-history strategy. Instead of investing in vegetative lateral stolons, the
118 axillary branches of inland plants are mostly upcurved and typically produce flowers quickly
119 (Lowry et al. 2008; Lowry and Willis 2010; Friedman et al. 2015). Further, inland plants invest
120 less into the production of leaves before flowering than coastal perennials (Friedman et al. 2015).
121 It should be noted that a smaller number of inland populations in the Coast Ranges do reside in
122 rivers and perennials seeps and have a perennial life-history. Farther inland, perennial
123 populations are more common, especially in high elevation streams and hot springs of the Sierra
124 and Cascade Mountains (Oneal et al. 2014).
125
126 While perennial populations invest more into vegetative growth than reproductive growth, they
127 also invest more heavily in defending their vegetative tissues. Perennial populations have higher
128 levels of both constitutive and induced defensive phenylpropanoid glycoside (PPG) compounds
129 than the annual populations when grown in a common environment (Holeski et al. 2013). This
130 pattern of highly defended plants in wetter habitats with long growing seasons versus poorly
131 defended plants in dry habitats with short growing seasons is consistent with an optimal defense
132 strategy: Greater allocation of resources to herbivore resistance is favored in long growing
133 season habitats by a greater abundance of herbivores and a lower cost of producing defensive
134 compounds (Kooyers et al. 2017).
135
136 Two major QTLs (*DIV1* and *DIV2*) and many minor QTLs control key traits involved in local
137 adaptation to perennial and annual habitats within the *M. guttatus* species complex (Hall et al.
138 2006; Lowry and Willis 2010; Hall et al. 2010; Friedman and Willis 2013; Friedman et al. 2015).

139 *DIV1* has the largest effect on the most traits and has thus been more extensively studied than
140 *DIV2*. *DIV1* is a large paracentric chromosomal inversion that plays a pivotal role in the annual
141 versus perennial life-history divergence described above (Lowry and Willis 2010). The inversion
142 is at minimum 6.3 Mbp in length along linkage group 8 (LG8) and contains at least 785
143 annotated genes. In hybrids, *DIV1* has a major effect on growth rate including the adaptive
144 flowering time phenotype, explaining 21% to 48% of the divergence between inland annual and
145 coastal perennial parents (Lowry and Willis 2010). In addition to flowering time, the *DIV1*
146 inversion has major effects on multiple traits involved in the evolutionary shift from more
147 allocation of resources to long-term growth versus reproduction (Lowry and Willis 2010). These
148 traits include the production of lateral stolons, adventitious roots, and leaf size (Lowry and Willis
149 2010; Friedman et al. 2015). Recent outlier analysis of coastal perennial and inland annual
150 populations identified candidate genes in the gibberellin pathway that may underlie a pleiotropic
151 shift in allocation between growth and reproduction (Gould et al. 2017).
152
153 In this study, we evaluate the role of GA in the divergence of growth morphology and herbivore
154 resistance between perennial and annual ecotypes of *M. guttatus.* We then test whether there is
155 an interaction between the *DIV1* inversion and GA addition, which would be consistent with the
156 inversion contributing to the evolution of shifts in allocation between long-term growth and
157 reproduction via effects on the GA pathway. Finally, we examined whether the *DIV1* inversion is
158 responsible for the shift in allocation between reproduction and defense that has been broadly
159 observed for populations of *M. guttatus* that vary in growing season length (Lowry et al. 2008;
160 Holeski et al. 2013; Kooyers et al. 2017).
161

162 **METHODS**

163

164 ***Plant material***

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

177

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

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

208 length and width of the longest leaf at the second node. Ten days after first flower, we quantified
209 the same traits as at first flower, with the following exceptions: Length and width of corollas
210 were not quantified, but we did count the total number of flowers.
211
212 Results were analyzed with JMP 12.2.0 (SAS Institute, Cary, NC). To gain a general
213 understanding of the effect of GA addition on coastal perennial, inland annual, and inland
214 perennial ecotypes, we conducted principle components analyses using all traits measured 10
215 days after flowering plus the width and length of corollas measured at flowering. We did not use
216 the other traits measured at first flower to avoid including repeated measures in the principle
217 components analysis. We saved the first three PCs from the analysis of all individuals. To
218 understand the effects of accession, ecotype, and GA treatment on PCs, we fit standard least
219 squares models. Each of the three PCs were modeled as response variables to the following
220 factors and interactions: accession (nested within ecotype), ecotype (coastal perennial, inland
221 annual, inland perennial), treatment, accession x treatment, and ecotype x treatment. Following
222 our PC analysis, we fit the same model for individual traits.
223
224 ***Interactions of GA application with the adaptive DIV1 inversion***
225 We grew coastal (S1) and inland (L1) parental inbred lines along with the NILs derived from
226 those lines in a fully randomized design in the Michigan State University greenhouses, with 16-
227 hours of supplemental lighting. We focused on the effect of the inversion in the coastal perennial
228 genetic background, as a previous study had shown that the effect of the inversion had the
229 greatest effect in the perennial genetic background (Friedman 2014). Following transplantation,

230 seedlings were sprayed with GA or a mock water treatment every other day and traits were
231 quantified in the same way as for the comparing population accessions.
232
233 To establish how trait variation of the coastal and inland parental lines was influenced by the GA
234 treatment, we conducted a PC analysis with the parental lines. As for the analysis with multiple
235 population accessions (above), we conducted principle components analyses using all traits
236 measured 10 days after flowering plus the width and length of corollas measured at flowering.
237 Models were fit with the first three PCs and individual traits as response variables to the
238 following factors: line, treatment, and the line x treatment interaction. Following analysis of the
239 parental lines, we analyzed the effects of the inversion in the perennial genetic background NILs.
240 As above, we first conducted a PC-based analysis and saved the first three PCs. We then fit the
241 first three PCs and individual traits as response variables for the following factors: inversion
242 type, treatment, and the inversion type x treatment interaction.
243
244 ***Effects of the DIV1 inversion on resistance compound concentrations***
245 To evaluate the effects of the *DIV1* inversion on the production of herbivore resistance
246 compounds, we conducted an experiment using the inversion NILs. Seeds were stratified,
247 germinated, and transplanted following the same protocols as in the previous two experiments. In
248 contrast to the GA NIL experiment, we grew outbred NILs which were created by intercrossing
249 independently derived NILs. As for the GA experiment, we focused our study on the effect of the
250 inversion in the perennial genetic background. The details of how outbred NILs were generated
251 can be found in Lowry and Willis (2010). Here, we used the outbred NILs made by intercrossing

252 S2 and S3 derived coastal genetic background NILs. We used intercrosses between L2 and L3
253 for the inland parents and between S2 and S3 for the coastal perennial parent comparisons.
254
255 To ensure that enough leaf tissue was available for analyses, outbred NILs and outbred parents
256 were allowed to flower prior to the collections for PPG quantification. Collected leaf tissue was
257 lyophilized for two days and then shipped to Northern Arizona University for analyses. We
258 ground the leaf tissue using a 1600 MiniG (Spex, Metuchen, New Jersey). Extractions were
259 conducted in methanol, as described in Holeski et al. 2013, 2014. We quantified PPGs using high
260 performance liquid chromatography (HPLC), via an Agilent 1260 HPLC (Agilent Technologies,
261 Santa Clara, California) with a diode array detector and Poroshell 120 EC-C18 analytical column
262 (4.6 x 250mm, 2.7µm particle size) maintained at 30°C. HPLC run conditions, as were described
263 in Kooyers et al. (2017). We calculated concentrations of PPGs as verbascoside equivalents,
264 using a standard verbascoside solution (Santa Cruz Biotechnology, Dallas, Texas), as described
265 in Holeski et al. (2013, 2014). We compared the concentrations of total PPGs and individual
266 PPGs with one-way ANOVAs fit in JMP 12.2.0. Post-hoc Tukey Tests were used to compare
267 means of parental and NIL classes.
268
269 **RESULTS**
270 ***The effects of GA application on plant growth among ecotypes***
271 Consistent with previous studies (Hall et al. 2006; Lowry et al. 2008; Lowry and Willis 2010;
272 Oneal et al. 2014), there were large differences in morphology between coastal perennial, inland
273 annual, and inland perennial ecotypes. There were also differences between the inland perennial
274 ecotype and the two other ecotypes. Most traits (18 out of 20) heavily loaded (Loadings > 0.40)

275 onto the first PC (Eigenvalue = 8.258; Table S2). The ecotype effect was highly significant for
276 PC1 ($F_{2,226}$ = 333.64, P < 0.0001; Table 1). Within ecotype, there was a significant effect of
277 accession on PC1 ($F_{8,226}$ = 20.12, P < 0.0001), and there was a significant effect of the GA
278 treatment on PC1 ($F_{1,226}$ = 25.75, P < 0.0001). While there was a significant accession x
279 treatment effect on PC1 ($F_{8,226}$ = 3.36, P = 0.0012), the treatment x ecotype effect was not
280 significant (P > 0.05). In contrast to PC1, both of the interactions were significant for PC2
281 (Eigenvalue = 3.463; accession x treatment: $F_{8,226}$ = 8.18, P < 0.0001; ecotype x treatment: $F_{2,226}$
282 = 6.20, P = 0.0024; Fig. 2A) and PC3 (Eigenvalue = 2.01; accession x treatment: $F_{8,226}$ = 2.92; P
283 = 0.0040, ecotype x treatment: $F_{2,226}$ = 41.64, P < 0.0001; Fig. 2B). Second and third internode
284 length, plant height, the total number of adventitious roots at the first nodes, total number of
285 stoloniferous nodes sprouting adventitious roots, total number of aerial branches, total number of
286 stolons, and the length of the longest aerial branch all heavily loaded (> 0.40) onto PC2 (Table
287 S2). Third internode length, total number of aerial branches, total number of stolons, and leaf
288 width heavily loaded onto PC3 (Table S2).
289
290 The results of the individual traits analyses were largely consistent with the principle
291 components analyses. Ecotype had a significant effect on all traits except for third internode
292 length. There was a significant accession effect for every trait that we measured in the
293 experiment. The GA treatment had a significant effect on 16 out of 19 traits at first flower and 12
294 out of 18 traits measured at 10 days after first flower (Table 1). The most pronounced changes of
295 the plants in response to the GA treatment was an increase in plant height and a conversion of
296 lateral branches from adventitious root-making stolon branches into unpcurved aerial branches
297 (Fig. 2C). As the perennials typically have more stolons with adventitious roots than the annuals,

298 they were generally more obviously affected by the GA treatment. This effect was captured
299 through the significant ecotype x treatment interactions on plant height. It should be noted that
300 the effect of GA varied across accessions within ecotype. The coastal PGR accession is the
301 tallest coastal accession with the fewest stolons (Table 1; Fig. 2C). Thus, it was affected the least
302 by the GA treatment. In contrast, OPB is a short prostrate coastal accession and was dramatically
303 affected by the GA treatment (Fig. 2C). Among the inland annual accession, SWC showed the
304 greatest response to the GA treatment in terms of height and aerial branch formation.
305
306 ***Interactions of GA application with the adaptive DIV1 inversion***
307 Consistent with previous studies (Lowry and Willis 2010; Friedman 2014), the coastal perennial
308 (SWB S1) and inland annual (LMC L1) lines were highly divergent in morphological traits and
309 the two lines were differentiated strongly along PC1 ($F_{1,168}$ = 416.81, P < 0.0001). Similar to the
310 accession analyses (above), the line x treatment interaction was not significant for PC1, but was
311 highly significant for PC2 ($F_{1,168}$ = 85.57, P < 0.0001) and PC3 ($F_{1,168}$ = 13.54, P < 0.0001). The
312 line x treatment interactions was also significant for 16 out of the 19 traits measured at flowering
313 and 12 out of the 18 traits measured 10 days after flowering. Overall, the coastal perennial line
314 (S1) responded more strongly to GA treatment, just as we found across coastal and inland
315 populations more generally (above).
316
317 The *DIV1* chromosomal inversion is one of many loci responsible for divergence between the
318 annual and perennial ecotypes. Thus, main effects and interactions in the NILs were expected to
319 be subtler than for the parental lines. As in previous studies (Lowry and Willis 2010; Friedman
320 2014), the inversion had significant effects on morphology, with highly significant main effects

321 on PC1 ($F_{1,173}$ = 35.44, P < 0.0001; Table 2, S3) and PC3 ($F_{1,173}$ = 37.55, P < 0.0001). The GA
322 treatment had significant effects on PC1 ($F_{1,173}$ = 16.76, P < 0.0001) and PC2 ($F_{1,173}$ = 489.50, P
323 < 0.0001). There were weak, but significant, line x treatment interactions for PC2 ($F_{1,173}$ = 4.13,
324 P = 0.0437; Fig. 3A) and PC3 ($F_{1,173}$ = 4.17, P = 0.0427). While the line x treatment effect on the
325 morphological PCs was marginal, the effect was greater for some of the individual traits (Fig. 3;
326 Table 2).
327
328 ***Effects of the DIV1 inversion on resistance compound concentrations***
329 We quantified the concentrations of seven PPGs (Table 3). Consistent with our previous
330 observations (Holeski et al. 2013), the coastal perennial parental (SWB) plants produced 2.5
331 times more total PPGs than the inland annual parental (LMC) plants ($F_{1,31}$ = 51.03; P < 0.0001;
332 Table 3; Fig. 4). There were also significant differences for six out of seven of the PPGs between
333 the coastal perennial (SWB) and inland annual (LMC) parental lines.
334
335 Analysis of the *DIV1* NILs revealed that the introgressed region containing the inversion had
336 major effects on foliar concentrations of PPGs. Control NILs that were homozygous for the
337 coastal orientation of the *DIV1* inversion produced 35% higher concentrations of total PPGs than
338 the introgression NILs, which were homozygous for the inland *DIV1* orientation ($F_{1,87}$ = 22.70; P
339 < 0.0001). In addition, the *DIV1* locus had significant effects on four out of the seven individual
340 PPGs. Interestingly, the control NILs had higher concentrations of conandroside and
341 mimuloside, but lower concentrations of calceolarioside A and unknown PPG10, than the
342 introgression NILs (Table 3). Thus, the *DIV1* inversion influences both the total concentration of
343 PPGs as well as the composition of suites of these PPGs.

344

345 **DISCUSSION**

346 In this study, we identified a potential genetic mechanism underlying a coordinated evolutionary
347 shift between growth, reproduction, and herbivore resistance in the *M. guttatus* species complex.
348 We found that GA has the potential to play a role in the divergence between coastal perennial
349 and inland annual ecotypes of *M. guttatus*, with coastal and inland plants responding differently
350 to the addition of GA for a number of phenotypic traits associated with shifts between long-term
351 growth and reproduction. Further, we detected an interaction between the locally adaptive *DIV1*
352 inversion and GA addition, which is consistent with the inversion contributing to the evolution of
353 growth form by modulating the GA pathway. Finally, we found evidence that the *DIV1* inversion
354 contributes to the trade-off between growth, reproduction and resistance. The coastal orientation
355 of the *DIV1* inversion causes plants to allocate more to long-term growth and herbivore
356 resistance over rapid reproduction than for the inland inversion orientation. We discuss these
357 findings in the context of the broader literature below.

358

359 ***Environmental gradients and the evolution of growth, reproduction, and defense traits***

360 Studies of intraspecific variation among natural populations adapted to different soil water
361 availability regimes provide an excellent opportunity to understand how the abiotic environment
362 influences the relative allocation of resources by plants to growth and constitutive/induced
363 resistance. Soil water is one of the most limiting factors for plants on Earth (Whittaker 1975;
364 Bohnert et al. 1995; Bray 1997) and can drastically differ in availability among seasons
365 (Cowling et al. 1996), which in turn influences plant resource allocation (Juenger 2013). The
366 timing of soil water availability can dictate the length of the growing season. One major

367 evolutionary strategy for seasonally low water availability is to allocate resources primarily to
368 growth and reproduction to achieve an early flowering, drought escape life-history (Ludlow
369 1989; Juenger 2013; Kooyers 2015). Beyond selection on plants, soil moisture gradients can
370 drive the abundance of herbivores, which in turn exert their own selective pressures (Kooyers et
371 al. 2017).
372
373 In *M. gutattus*, evolutionary shifts across a soil moisture gradient drives changes in the allocation
374 not only between growth and reproduction, but also for herbivore resistance (Lowry et al. 2008;
375 Holeski et al. 2013; Kooyers et al. 2017). The phenotypic differences between coastal perennial
376 and inland annual populations is likely driven by multiple selective pressures that are tied to the
377 soil water availability gradient between coastal and inland habitats. Inland annual habitats
378 generally dry out very quickly at the end of the spring, which leaves little time for a plant to
379 reproduce before being killed by the summer drought. Further, the short growing season may
380 also prevent the growth of sizable herbivore populations, which would explain the low level of
381 leaf damage in fast drying inland annual habitats (Lowry et al. 2008; Kooyers et al. 2017). In
382 contrast, the year-round soil water availability of coastal habitats means that plants growing there
383 have much more time to allocate resources to vegetative growth and herbivore resistance. In
384 addition, wet coastal habitats can build up a considerable load of herbivores, which is likely
385 reflected by much greater leaf damage and early season mortality in these habitats (Lowry et al.
386 2008; Lowry and Willis 2010). For intraspecific differences among populations, the strength of
387 herbivore pressure is thought to be a key driver of plant resistance (Hahn and Maron 2016). It
388 should be noted that a fair amount of leaf damage in coastal habitats may also be due to oceanic
389 salt spray (Boyce 1954; Ahmed and Wainwright 1976; Griffiths 2006; Lowry et al. 2009). Future

390 manipulative field experiments are needed to partition out the relative contributions of herbivory
391 and salt spray to leaf damage for *M. guttatus* in coastal habitats.
392
393 Our findings in *M. guttatus* are likely to have implications for intraspecific variation in many
394 other plant species as well. There are many studies that have found similar developmental
395 differences between coastal and inland populations as we have found for *M. guttatus* (reviewed
396 in Lowry 2012). Given the commonality of coastal plants investing more heavily in lateral
397 vegetative branches versus inland populations investing primarily in upright flowering branches,
398 we predict that coastal population of plants will generally be more highly defended than inland
399 populations, particularly in Mediterranean climates with steep soil moisture gradients.
400
401 ***The role of pleiotropy and linkage***
402 The results of this study and previous studies (Lowry and Willis 2010; Friedman 2014; Friedman
403 et al. 2015) collectively demonstrate that adaptive chromosomal inversion *DIV1* contributes to
404 the shift in allocation between long-term growth, short-term fecundity, and herbivore resistance.
405 An outstanding question is whether this coordinated shift is due to genetic linkage or pleiotropy.
406 Chromosomal inversions are thought to evolve as adaptation "supergenes," which can trap
407 multiple linked adaptive loci through their suppression of meiotic recombination (Dobzhansky
408 1970; Kirkpatrick and Barton 2006; Schwander et al. 2014; Wellenreuther and Bernatchez 2018).
409 Thus, the fact that the *DIV1* inversion contributes to the evolution of multiple phenotypes could
410 be result of adaptive alleles at multiple linked loci being held together in tight linkage by the
411 chromosomal inversion. Alternatively, a single gene within the inversion could have pleiotropic
412 effects on all of the phenotypic changes.

413

414 Two other recent studies have also found potential pleiotropic effects of genes on allocation to
415 reproduction and herbivore resistance. Rasmann et al. (2018) found that NILs containing genetic
416 variants of the *Flowering Locus C* (*FLC*) gene in *Cardamine hirsute* are responsible for a trade-
417 off between early flowering and herbivore resistance in terms of glucosinolate production.
418 Kerwin et al. (2015) found that there was a positive correlation in *Arabidopsis thaliana* between
419 glucosinalate production and flowering time for mutant alleles of genes in the glucosinolate
420 biosynthetic pathway. Overall, both of these studies identified the same trade-off of rapid
421 reproduction versus herbivore resistance that we found in our study, although mediated through
422 independent genetic mechanisms.

423

424 ***A hormonal basis of a coordinated shift in the evolution of growth, reproduction, and***
425 ***herbivore resistance?***

426 The finding that coastal perennial and inland annual plants respond differentially to GA is
427 consistent with the role of this hormone playing a role in their evolutionary divergence. A recent
428 outlier analysis of coastal perennial and inland annual populations of *M. gutattus* found that the
429 gene *GA20-oxidase2* (*GA20ox2*) was a major allele frequency outlier between the ecotypes
430 within the vicinity of the *DIV1* inversion (Gould et al. 2017). This gene is a strong potential
431 candidate gene for a pleiotropic shift in allocation between growth and reproduction. GA-
432 oxidases are involved in the evolution of dwarfed coastal populations of *A. thaliana* (Barboza *et*
433 *al*. 2013) and played a key role in the development of dwarfed Green Revolution rice and barley
434 (Sasaki et al. 2002; Jia et al. 2009). Further, the DELLA gene, *GAI*, is located within the vicinity
435 of another growth regulatory QTL (*DIV2*). *GAI* was also an allele frequency outlier between

436 coastal and inland populations (Gould et al. 2017). Friedman (2014) found that the *DIV1* and
437 *DIV2* loci interact epistatically. Thus, it would not be surprising if the genetic changes that
438 underlie both QTL are in the same molecular pathway. Further, the negative antagonism between
439 the GA and JA hormone pathways via the DELLA-JAZ signaling node (Havko et al. 2016; Guo
440 et al. 2018; Howe et al. 2018) suggests a direct mechanism by which trade-offs between growth,
441 reproduction, and resistance could easily evolve. Future functional genetic studies will be needed
442 to determine whether these genes in fact are involved in adaptive shifts between growth,
443 reproduction, and defense underlying local adaptation in this system.
444
445 While we saw a greater response to GA addition in coastal plants and observed an interaction
446 between GA addition and the inversion, other hormones could also play a role or even be the
447 ultimate cause of the divergence between the coastal perennial and inland annual ecotypes. Three
448 major classes of hormones, Auxins, Brassinosteroids and Gibberellins, are all associated with
449 growth phenotypes like those that differ between coastal perennial and inland annual ecotypes of
450 *M. guttatus* (Ross and Quittenden 2016; Unterholzner et al. 2016). These hormones interact in
451 multiple ways, which have yet to be fully elucidated, to result in shifts in growth/reproduction
452 phenotypes. Future functional studies in *M. guttatus* will be needed to identify the ultimate
453 causative mechanisms underlying the dramatic shift in allocation between annual and perennial
454 populations.
455
456 ***Conclusions and future directions***
457 There are numerous evolutionary and ecological models that make predictions on the evolution
458 of relationships between growth, reproduction, and herbivore resistance. While recent meta-

459 analyses have found that some models have moderate support (Endara and Coley 2011), there
460 are numerous exceptions and many models appear to not be well supported at all (Stamps et al.
461 2003; Hahn and Maron 2016; Smilanich et al. 2016). The reasons that these models do not hold
462 up are often attributed to vast variation in the extrinsic environmental factors that exert selective
463 pressures on plant populations, broad variation in life-history among plant species, and
464 differences between interspecific and intraspecific variation (Stamps et al. 2003; Hahn and
465 Maron 2016; Smilanich et al. 2016). Less well appreciated are the molecular genetic mechanisms
466 that underlie shifts in allocation between growth, reproduction, and herbivore resistance (Kerwin
467 et al. 2015; Rasmann et al. 2018). The nature of the gene networks responsible for shifts in
468 allocation may also be very important for whether or not particular systems will conform to a
469 given evolutionary or ecological model. Future research should focus on uncovering the
470 molecular mechanisms that underlie the evolution of growth, reproduction, and defense trade-
471 offs in natural populations and integrate predictions from those mechanisms into ecological
472 models.
473

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479

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774 **TABLES**

775 **Table 1.** F-values for main effects and interactions on traits measured across population

776 accessions.

Trait	N	Population	Ecotype	Treatment	Population x Treatment	Ecotype x Treatment
PC1	208	22.33****	333.64****	12.84***	3.27**	1.65
PC2	208	16.18****	1.54	52.41****	9.30****	11.70***
PC3	208	17.41****	2.67	54.93****	3.07**	62.66****
Traits Measured at Flowering						
Total number of nodes on the primary shoot	248	20.63****	249.51****	25.94****	0.89	11.71***
Length of the 1st internode	248	15.51****	40.98****	0.02	0.45	0.53
Length of the 2nd internode	248	35.03****	127.59****	35.70****	2.32*	0.79
Length of the 3rd internode	248	27.67****	0.27	18.47****	1.68	0.01
Combined length first three internodes	248	36.71****	31.14****	35.15****	1.55	0.12
Plant Height	248	32.12****	187.20****	100.61****	5.27****	25.68****
Width 1st internode	248	9.59****	153.67****	0.93	0.30	0.80
Width 2nd internode	248	36.32****	571.28****	5.36*	2.12*	0.12
Width 3rd internode	248	29.73****	538.28****	0.85	5.28****	2.16
Corolla length of the 1st flower	248	56.05****	629.66****	16.95****	1.48	1.36
Corolla width of the 1st flower	248	31.87****	303.07****	17.69****	2.71*	2.15
Number of adventitious roots at 1st nodes	248	4.30***	80.55****	7.68**	1.50	3.77
Number of nodes with adventitious roots	248	4.47****	9.16**	6.75*	1.88	4.00*
Total number of aerial branches	248	12.65****	19.28****	33.91****	8.69****	9.32**
Total number of stolons	248	8.37****	66.82****	30.61****	1.78	21.44****
Length of the longest aerial branch	248	19.02****	68.57****	34.38****	3.80***	18.34****
Length of the longest stolon	248	5.32****	23.80****	4.19*	1.47	3.94*
Length of the longest leaf at the 2nd node	248	3.79***	111.60****	1.91	1.44	0.45
Width of the longest leaf at the 2nd node	248	4.88****	50.95****	28.75****	1.91	4.25*
Traits Measured 10 Days After Flowering						
Total number of nodes on the primary shoot	216	19.75****	167.07****	8.41**	2.44*	0.82
Length of the 1st internode	215	16.88****	92.96****	0.23	2.32*	2.78*
Length of the 2nd internode	215	30.52****	108.15****	36.67****	1.53	0.78
Length of the 3rd internode	215	32.82****	0.00	12.54***	2.17*	0.81
Combined length first three internodes	215	39.46****	31.62****	24.54****	1.77	0.37
Plant Height	215	28.85****	181.38****	56.07****	12.74****	0.31
Width 1st internode	215	3.79*	52.44****	1.75	1.79	0.00
Width 2nd internode	215	19.87****	295.50****	10.51**	5.29****	0.03
Width 3rd internode	215	19.43****	470.47****	0.54	3.55**	0.49
Number of adventitious roots at 1st nodes	214	4.93****	71.24****	34.29****	3.79***	18.39****
Number of nodes with adventitious roots	214	5.76****	43.85****	45.64****	5.02****	40.20****
Total number of aerial branches	215	10.51****	0.03	70.45****	5.39****	30.07****
Total number of stolons	215	8.68****	115.03****	82.04****	5.37****	36.71****
Length of the longest aerial branch	214	7.16****	15.46****	8.13**	9.04****	12.37***
Length of the longest stolon	215	2.78**	34.63****	7.56**	0.79	4.98*
Length of the longest leaf at the 2nd node	215	3.29**	53.75****	3.35	1.97	2.99
Width of the longest leaf at the 2nd node	214	3.01**	21.70****	41.65****	2.17*	10.80**
Number of flowers	212	8.99****	13.01***	0.27	1.80	0.68

P-values: *<0.05, **<0.01, ***<0.001, ****<0.0001

777

778 **Table 2.** F-values for main effects and interactions on traits measured on coastal perennial

779 (SWB) and inland annual (LMC) parents as well as near-isogenic lines of the *DIV1* inversion in

780 the coastal genetic background.

Trait	N	Line	Treatment	Line x Treatment
PC1	176	35.44****	16.76****	3.66
PC2	176	2.55	391.46****	4.13*
PC3	176	37.55****	1.46	4.17*
Traits Measured at Flowering				
Total number of nodes on the primary shoot	253	35.90****	26.61****	5.39*
Length of the 1st internode	251	7.84**	2.95	1.03
Length of the 2nd internode	252	81.97****	68.15****	13.00***
Length of the 3rd internode	252	83.51****	19.07****	0.15
Combined length first three internodes	252	91.13**	48.44****	2.41
Plant Height	252	1.80	100.13****	0.90
Width 1st internode	252	20.13****	13.48***	5.19*
Width 2nd internode	252	47.81****	0.34	7.94**
Width 3rd internode	252	98.32****	0.84	5.76*
Corolla length of the 1st flower	250	24.27****	1.99	1.47
Corolla width of the 1st flower	252	109.77****	87.27****	1.71
Number of adventitious roots at 1st nodes	245	7.94**	27.46****	5.40*
Number of nodes with adventitious roots	245	3.73	10.17**	6.39*
Total number of aerial branches	249	36.33****	132.42****	15.87****
Total number of stolons	249	0.07	286.82****	3.46
Length of the longest aerial branch	249	3.76	35.68****	4.64*
Length of the longest stolon	249	0.33	25.17****	0.08
Length of the longest leaf at the 2nd node	248	11.78***	7.58**	0.61
Width of the longest leaf at the 2nd node	248	1.60	21.35****	0.04
Traits Measured 10 Days After Flowering				
Total number of nodes on the primary shoot	204	6.79**	39.53****	1.78
Length of the 1st internode	205	8.74**	13.46***	5.79*
Length of the 2nd internode	205	132.59****	123.94****	20.97****
Length of the 3rd internode	204	93.44****	8.32**	0.00
Combined length first three internodes	204	95.41****	38.86****	2.80
Plant Height	205	5.42*	136.75****	0.12
Width 1st internode	204	5.89*	1.27	2.35
Width 2nd internode	204	30.62****	1.36	4.16*
Width 3rd internode	204	76.93****	1.09	10.24**
Number of adventitious roots at 1st nodes	204	0.03	76.60****	0.09
Number of nodes with adventitious roots	204	0.11	81.51****	0.04
Total number of aerial branches	203	43.80****	160.41****	28.46****
Total number of stolons	204	3.92*	226.09****	0.00
Length of the longest aerial branch	204	0.05	32.21****	2.36
Length of the longest stolon	204	0.20	23.36****	0.00
Length of the longest leaf at the 2nd node	204	12.11***	14.26***	0.67
Width of the longest leaf at the 2nd node	204	0.88	17.47****	0.05
Number of flowers	200	0.59	0.62	0.59

P-values: *<0.05, **<0.01, ***<0.001, ****<0.0001

781

782 **Table 3.** Means and standard errors for the concentrations of phenylpropanoid glycosides in
783 coastal perennial (SWB) and inland annual (LMC) parents as well as near-isogenic lines of the
784 *DIV1* inversion in the coastal genetic background.

Trait	Coastal Parent	Inland Parent	Control NIL	Introgression NIL
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

785

786

787 **FIGURE LEGENDS**

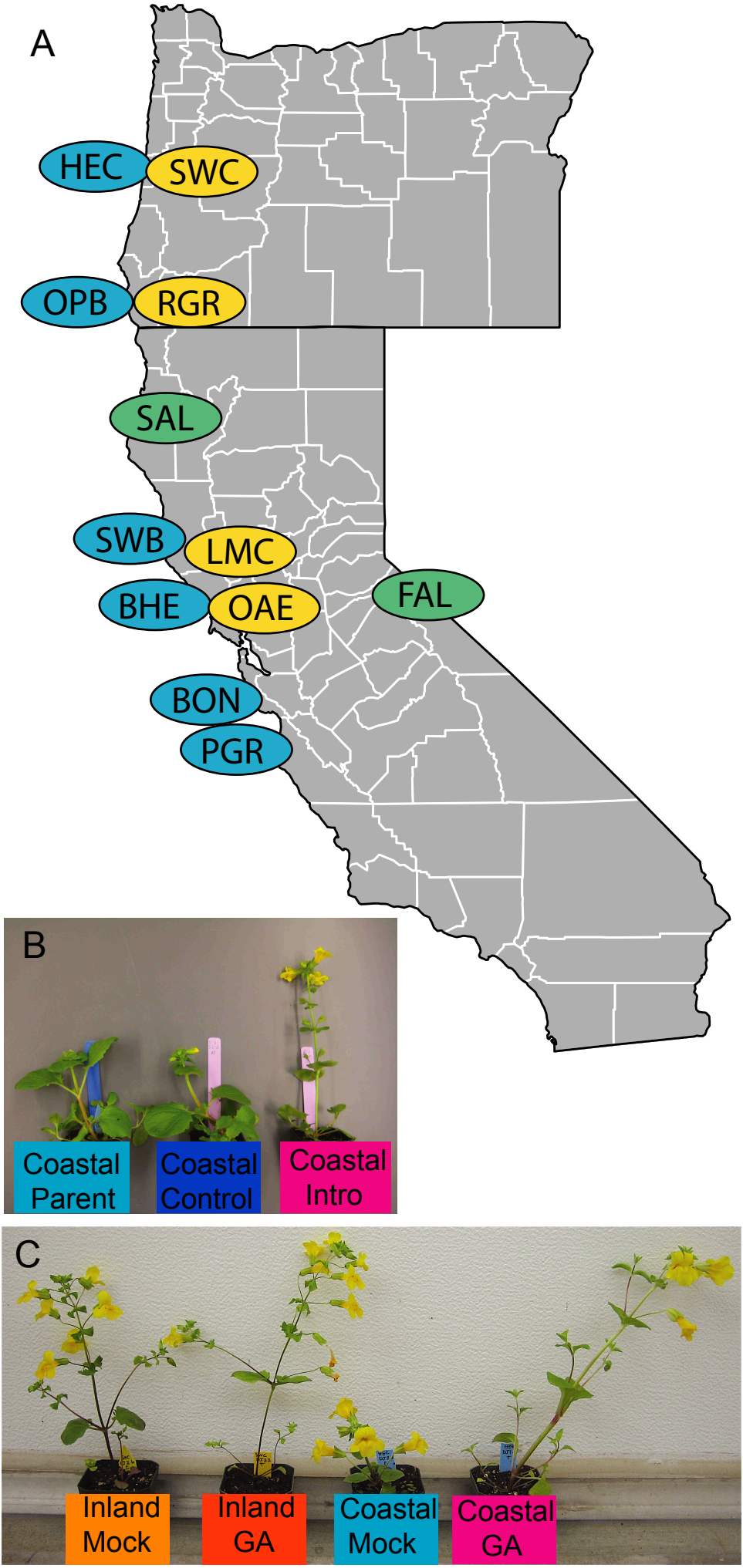
788 **Figure 1.** A) Map of coastal perennial (blue), inland annual (yellow), and inland perennial
789 (green) populations from where accessions for experiment were derived. B) Effects of the
790 introgression of the inland *DIV1* inversion orientation into the coastal perennial genetic
791 background.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

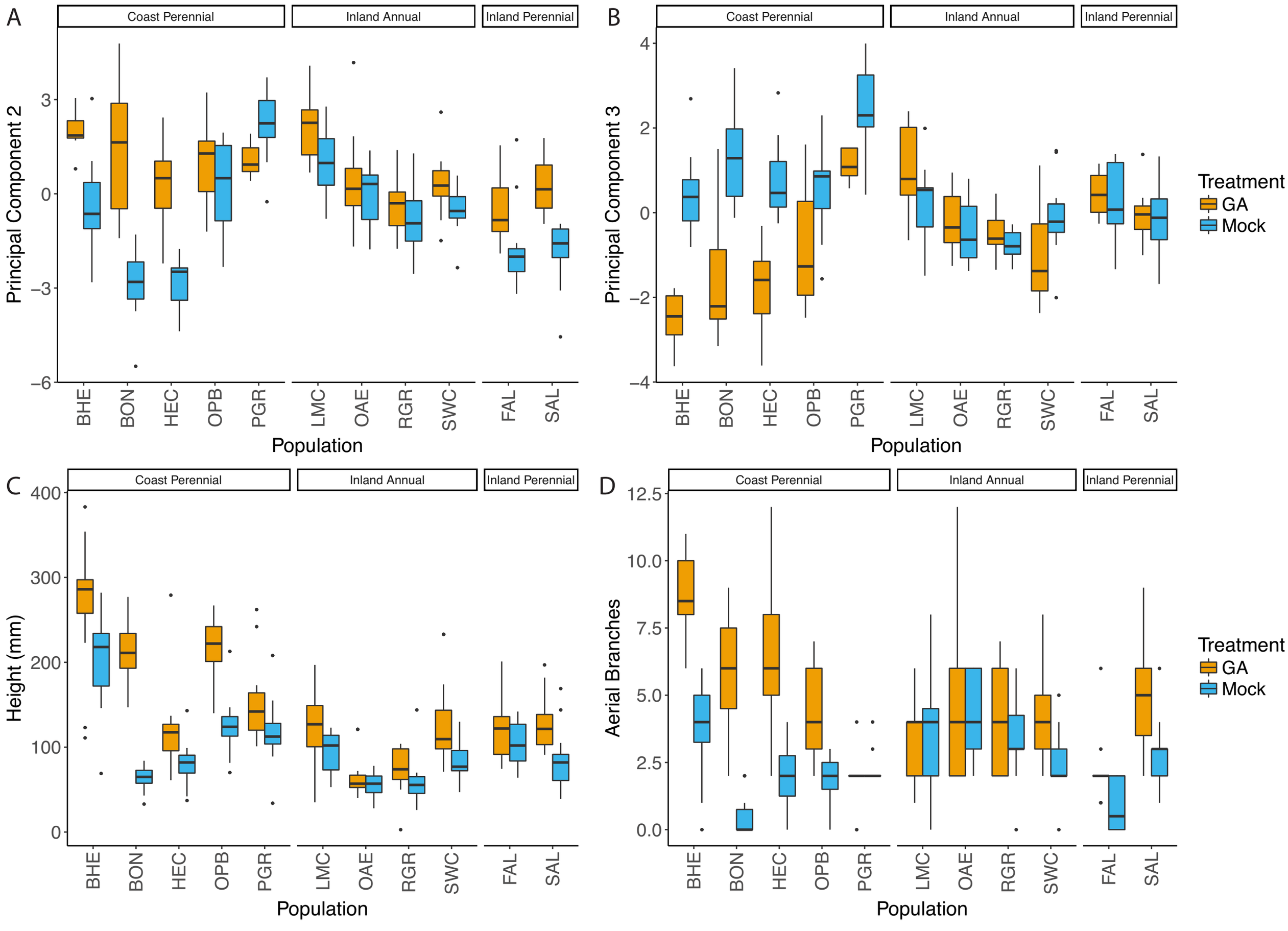
796

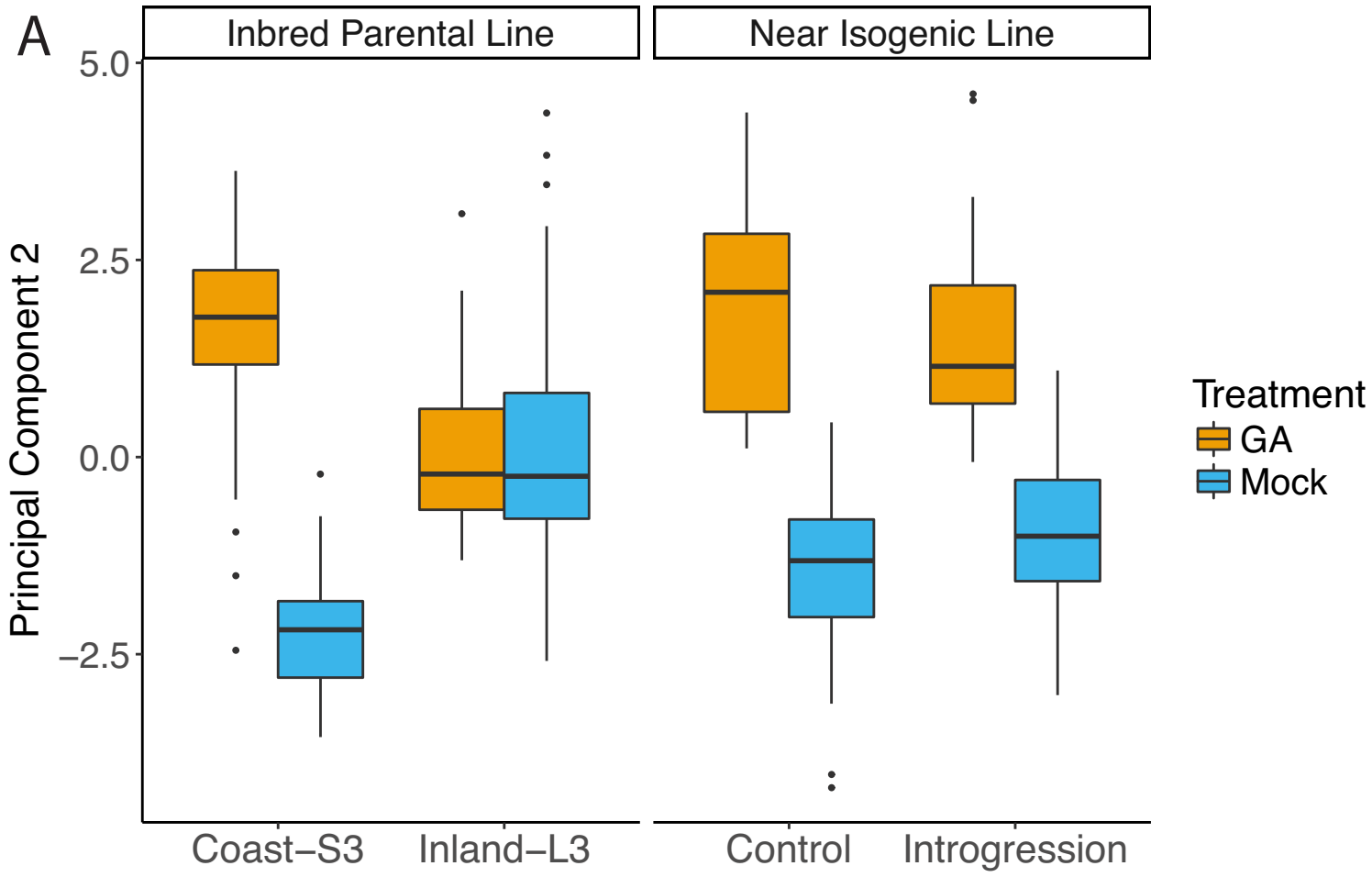
797 **Figure 2.** Box and whisker plots of trait responses by coastal perennial, inland annual, and inland
798 perennial accessions to mock and gibberellin treatments.

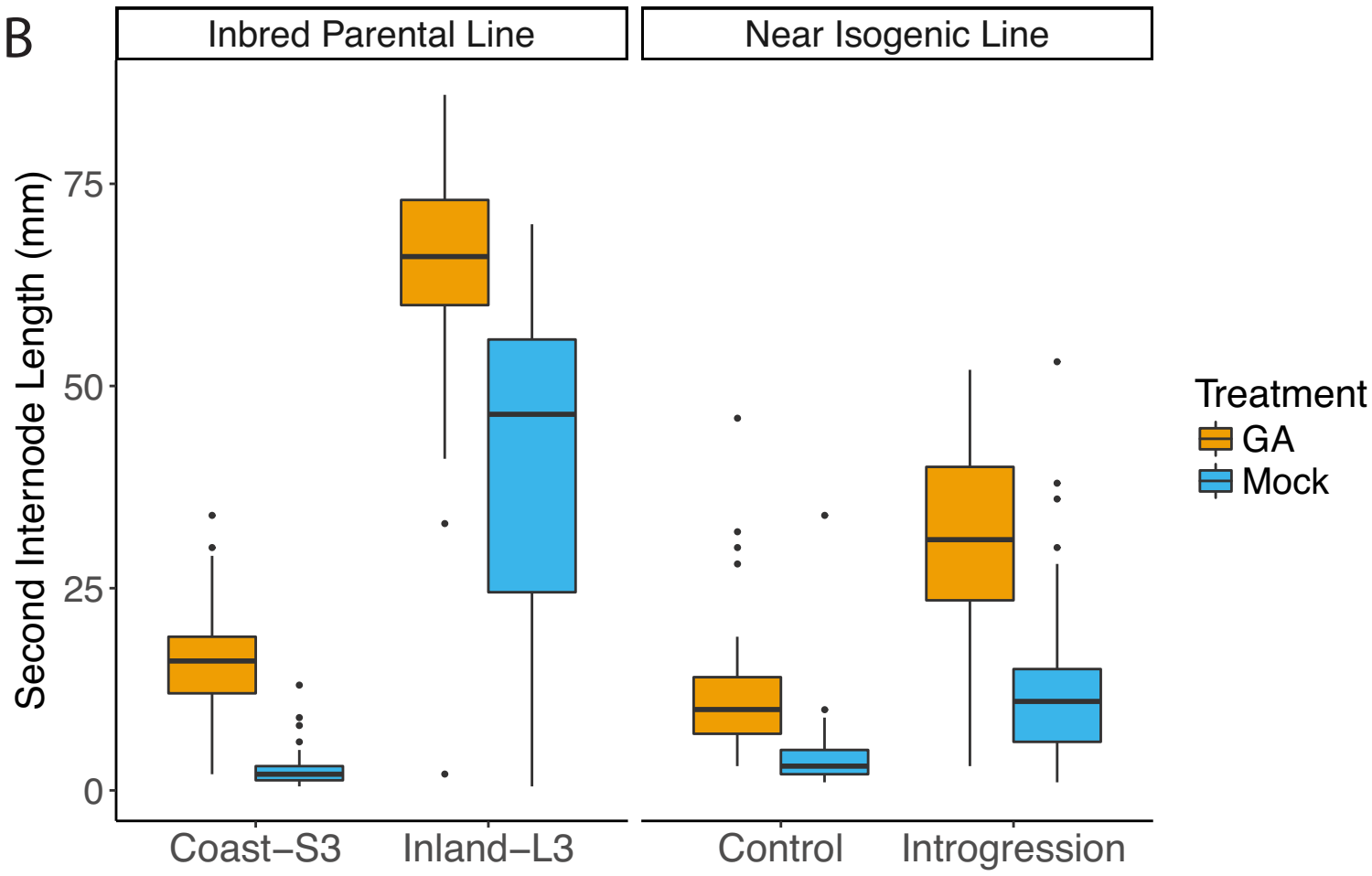
799

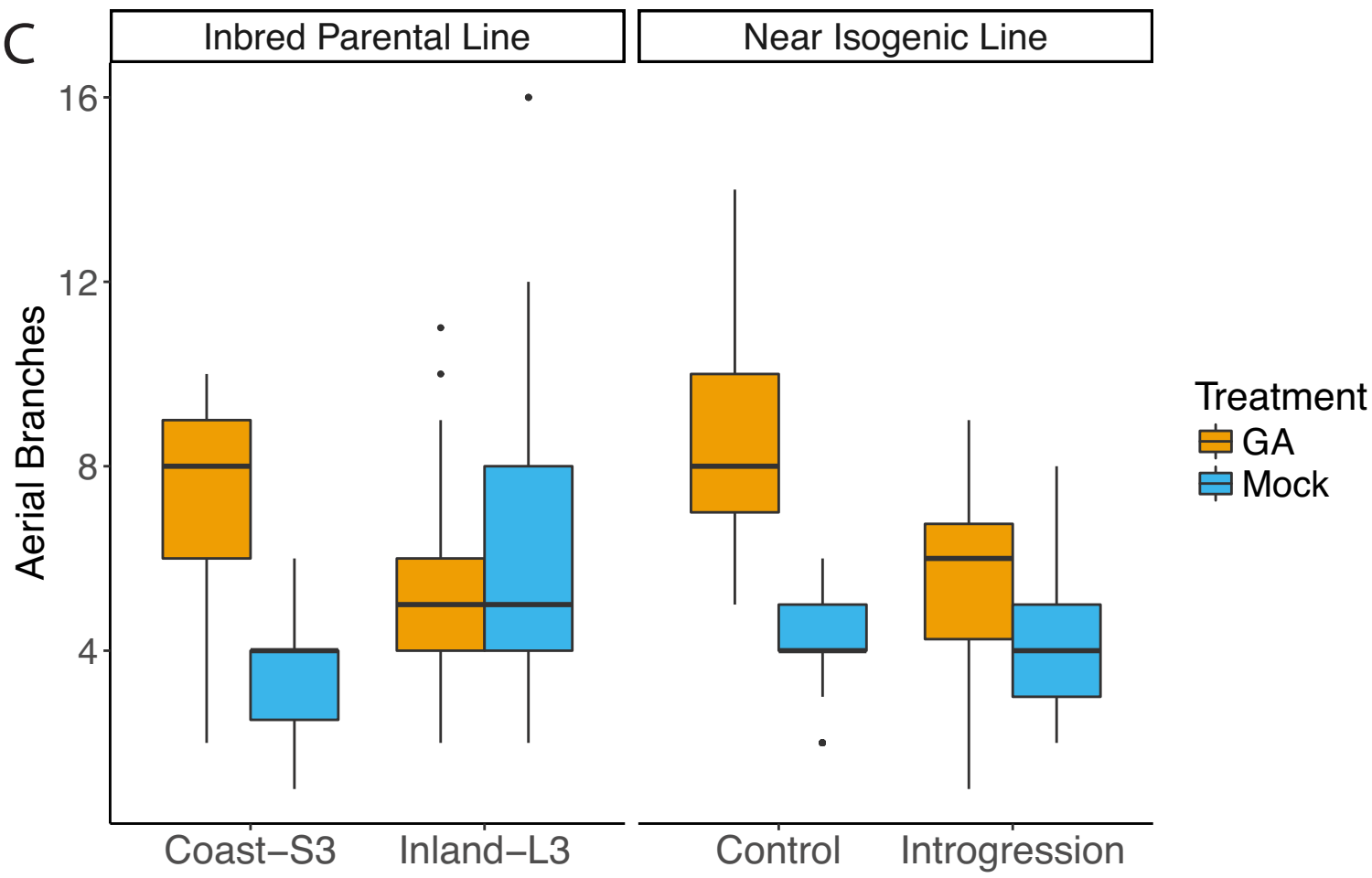
800 **Figure 3.** Box and whisker plots of trait responses by coastal perennial (SWB) and inland annual
801 (LMC) parents as well as coastal perennial genetic background near-isogenic lines (NILs) to
802 mock and gibberellin treatments. The introgression NILs were homozygous for the inland annual
803 orientation of the *DIV1* inversion, while the control NILs were homozygous for the coastal
804 perennial orientation of the *DIV1* inversion.
805
806 **Figure 4.** Concentration of phenylpropanoid glycosides for coastal perennial (SWB) and inland
807 annual (LMC) parents as well as coastal perennial genetic background near-isogenic lines
808 (NILs). The introgression NILs were homozygous for the inland annual orientation of the *DIV1*
809 inversion, while the control NILs were homozygous for the coastal perennial orientation of the
810 *DIV1* inversion.
811

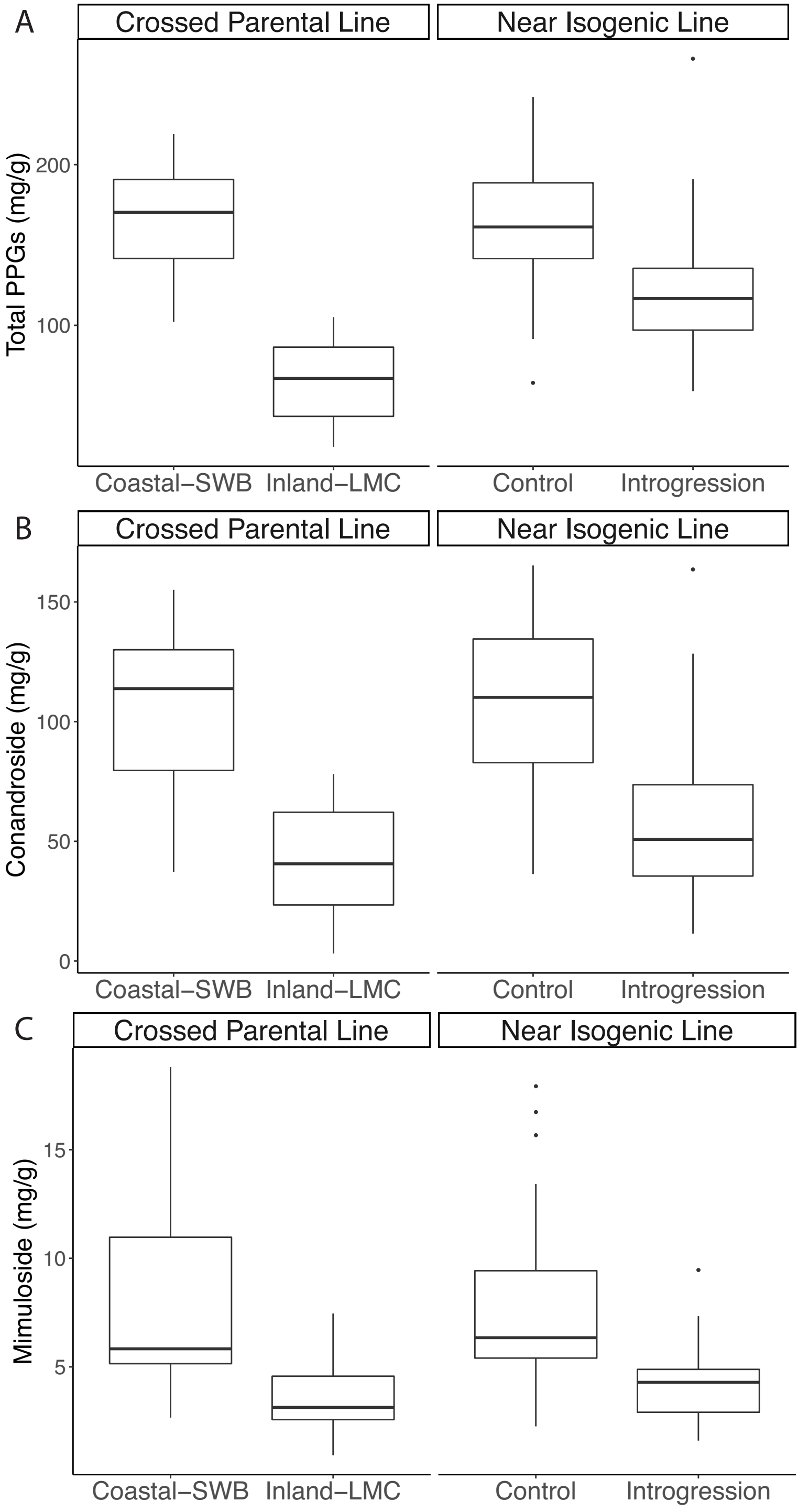

A
HEC
SWC
OPB
RGR
SAL
SWB
LMC
BHE
OAE
FAL
BON
PGR
B
Coastal Parent
Coastal Control
Coastal Intro
C
Inland Mock
Inland GA
Coastal Mock
Coastal GA


A
Coast Perennial
Inland Annual
Inland Perennial
Principal Component 2
3
0
−3
−6
BHE
BON
HEC
OPB
PGR
LMC
OAE
RGR
SWC
FAL
SAL
Population
B
Principal Component 3
4
2
0
−2
−4
Treatment
GA
Mock
C
Height (mm)
400
300
200
100
0
D
Aerial Branches
12.5
10.0
7.5
5.0
2.5
0.0


A
Inbred Parental Line
Near Isogenic Line
Principal Component 2
5.0
2.5
0.0
−2.5
Coast−S3
Inland−L3
Control
Introgression
Treatment
GA
Mock


B
Inbred Parental Line
Near Isogenic Line
Second Internode Length (mm)
75
50
25
0
Coast−S3
Inland−L3
Control
Introgression
Treatment
GA
Mock


C
Inbred Parental Line
Near Isogenic Line
Aerial Branches
16
12
8
4
Coast−S3
Inland−L3
Control
Introgression
Treatment
GA
Mock


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