

1 **A test of the evolution of increased competitive ability in two invaded regions.**

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21 **Abstract**

22 Non-native plant species invasions can have significant ecological and economic impacts.  
23 Finding patterns that predict and explain the success of non-native species has thus been an  
24 important focus in invasion ecology. The evolution of increased competitive ability (EICA)  
25 hypothesis has been a frequently used framework to understand invasion success. Evolution of  
26 increased competitive ability predicts that (1) Non-native populations will escape from  
27 coevolved specialist herbivores that were present within the native range and this release from  
28 specialist herbivores should result in relaxed selection pressure on specialist-related defense  
29 traits, (2) There will be a trade-off between allocation of resources for resistance against  
30 specialist herbivores and allocation to traits related to competitive ability, and (3) This shift will  
31 allow more allocation to competitive ability traits. We tested the predictions of EICA in the  
32 model plant *Mimulus guttatus*, a native of western North America (WNA). We compared how  
33 well the predictions of EICA fit patterns in two non-native regions, the United Kingdom (UK),  
34 an older more successful invasion, and eastern North America (ENA), a younger less successful  
35 invasion. We completed extensive herbivore surveys and grew plants derived from multiple  
36 populations in each region in a common greenhouse environment to test adherence to the  
37 predictions of EICA. We found evidence of specialist herbivore escape in the UK, but not the  
38 ENA plants. Compared to native plants the UK plants had lower levels of resistance traits, were  
39 taller, and produced larger and more flowers, while the ENA plants had mostly equivalent traits  
40 to the WNA plants. Plants from the UK conformed to the predictions of EICA more closely than  
41 those from ENA. The UK invasion is an older, more successful invasion, suggesting that support  
42 for EICA predictions may be highest in more successful invasions.

## 44 **Introduction**

45 The translocation of non-native species into areas outside of their native range provides unique  
46 opportunities for the study of evolution (Cox 2004), including how selection pressures from  
47 herbivores can shape plant defense evolution (Callaway and Maron 2006). Comparisons between  
48 divergent biotic and abiotic factors in the native and non-native habitats can aid understanding of  
49 how these variables shape evolution in non-native plants (Whitney and Gabler 2008). The testing  
50 of theories blending ecological and evolutionary explanations can provide important insight into  
51 how non-native plants are successful and how defense traits evolve; these tests often involve  
52 comparison of genotypes from the native and non-native ranges (Orians and Ward 2010). Better  
53 understanding of the mechanisms of non-native plant success may allow improved control and/or  
54 more accurate predictions of the impacts that non-native species can have on native ecosystems.

55 Many hypotheses have been proposed to find a common reason for why plants successfully  
56 invade non-native regions (Catford et al 2009), dating back to Darwin's naturalization hypothesis  
57 (Diez et al 2008). Hypotheses have speculated on the potential for non-native plants to more  
58 efficiently use resources than native plants (Coley et al 1985), or have proposed that non-native  
59 plants are able to exploit an empty or less-crowded niche in the invaded habitat (Mack et al  
60 2000; Hierro et al 2005). Many of these hypotheses also incorporate the idea that a competitive  
61 advantage is gained through an enemy release in a non-native habitat from co-evolved, specialist  
62 herbivores present in the native species range (Keane and Crawley 2002; Orians and Ward  
63 2009). According to one prominent hypothesis, the evolution of increased competitive ability  
64 (EICA) hypothesis, enemy release results in the allocation of resources to reproductive fitness  
65 and/or competitive ability traits rather than to defenses. Relaxed selection for defense traits  
66 would allow for the evolution of traits or trait values that allow plants to become more

67 competitive and contribute to their invasive success (Blossey and Notzold 1995). Across a  
68 gradient of invasion success native populations will express higher resistance and lower  
69 allocation to competitive ability traits while the reverse, lower resistance and higher allocation to  
70 competitive ability traits, is expected on the most successful non-native invaded site. For  
71 instance, Blossey and Notzold (1995) found that plants in non-native populations of *Lythrum*  
72 *salicaria* (Lythraceae) in eastern North America produced more seeds and had greater biomass  
73 than those in native, European populations. Increases in seed production and biomass were  
74 correlated with a decline in defenses against two specialist herbivores that, at the time, were not  
75 present in eastern North America. By generating testable predictions of the role that ecology  
76 plays in shaping the evolution of non-native plants, EICA hypothesis has become one of the most  
77 widespread frameworks to explore the ability of non-native plants to succeed (Bossdorf et al  
78 2005; Orians and Ward 2010).

79 Two specific, testable predictions of EICA to explain the success of non-native plant populations  
80 include: Firstly, non-native populations will escape from coevolved specialist herbivores that  
81 were present within the native range. This release from specialist herbivores should result in  
82 relaxed selection pressure on specialist-related defense traits (e.g., Vila et al 2005). Secondly,  
83 EICA predicts that there will be a trade-off between allocation of resources for resistance against  
84 specialist herbivores and allocation to traits related to competitive ability. This shift to allow  
85 more allocation to competitive ability traits (e.g. increased reproduction, large plants, etc.)  
86 permits the non-native populations to compete successfully in their new habitat (Blossey and  
87 Notzold 1995; Rotter and Holeski 2018).

88 Experimental tests of EICA can be complicated by a number of factors, including the difficulty  
89 in knowing the most relevant defense traits against specialists, and inferring which competitive

90 ability traits are most important in a particular non-native environment. Further, to test  
91 evolutionary trade-offs, traits must be studied in a common garden environment, as the  
92 measurement of phenotypes in the field yields trait values influenced by both genetic and  
93 environmental variation. Perhaps in part because of these complications, there has been mixed  
94 support for EICA (Bossdorf et al 2005; Felker-Quinn et al 2013; Rotter and Holeski 2018). For  
95 example, in a recent meta-analysis that found some validation for EICA, evidence was strongest  
96 when looking at actual herbivory (e.g. field damage or feeding trials), while there was very little  
97 support when studies looked directly at resistance traits (Rotter and Holeski 2018).

98 While a number of studies have tested independent premises of EICA, fewer have conducted  
99 simultaneous assessment of both resistance and competitive ability-related traits in a common  
100 garden setting, which is necessary to detect evolutionary trade-offs particularly in a comparative  
101 context between two invasions of relative age and success (Rotter and Holeski 2018). In  
102 addition, very few studies have directly compared the accuracy of EICA predictions across  
103 multiple invasions of the same species that differ in age and success. Here we test the predictions  
104 of EICA in *Mimulus guttatus*, using populations in the native range of western North America, as  
105 well as non-native populations in two areas of introduction, eastern North America and the  
106 United Kingdom. Specifically we tested for:

- 107 1. An escape in non-native populations from co-evolved specialist herbivore species present  
108 in the native western North America range. This would be supported by the lack of  
109 specialist herbivores feeding on *M. guttatus* in the non-native populations in eastern  
110 North America and/or the United Kingdom.
- 111 2. A decrease in herbivore resistance traits within the non-native populations, relative to  
112 native. This would be demonstrated by reduced levels of genetic-based herbivore

- 113 resistance traits, or in increased performance of herbivores feeding on non-native, vs.  
114 native plants.
- 115 3. A genetic-based increase in trait values related to fitness/competitive ability within the  
116 non-native populations, relative to native. This would be demonstrated by increased trait  
117 values for fitness/ competitive ability traits in the eastern North America and/or UK  
118 populations, relative to native, when plants are grown in a common garden environment.
- 119 4. Genetic-based tradeoffs between herbivore resistance traits and competitive ability traits.  
120 For EICA, evidence of this would include negative correlations between resistance and  
121 competitive ability traits in non-native plants, although negative correlations between  
122 resistance and fitness/competitive ability may also exist in native plants.
- 123 5. These predictions should be most closely followed by plants from a seemingly successful  
124 invasion (the United Kingdom plants that have filled available niches) than those of the  
125 less successful (eastern North America invasion), which consists of relatively few small  
126 populations that have not expanded and have mostly been locally extirpated.

## 127 **Methods**

### 128 *Study system*

129 *Mimulus guttatus* Fisch. ex DC. (*Erythranthe guttata* G.L. Nesom) is a species complex native to  
130 moist habitats throughout western North America (WNA). In the past few decades *Mimulus spp.*  
131 and in particular *M. guttatus*, have become important model organisms for the study of  
132 evolutionary ecology and genetics (Wu et al 2008; Yuan 2018). *Mimulus guttatus* has been  
133 introduced throughout the globe where it has escaped numerous times from cultivation. Non-  
134 native *M. guttatus* populations are located in the United Kingdom (UK), western Europe, New  
135 Zealand, and eastern North America (ENA) (Hall and Willis 2006, Vallejo-Marin and Lye 2013).

136 Historical records and genetic evidence suggest that the first *M. guttatus* introduced in the United  
137 Kingdom originated from Alaska (referred to as cordilleran) (Puzey and Vallejo-Marin 2014).  
138 The first records of naturalized *M. guttatus* in the UK are from the first half of the 1800s and this  
139 taxon is currently widespread and locally abundant in the UK (Preston et al 2002; Vallejo-  
140 Marinand and Lye 2013; Puzey and Vallejo-Marin 2014). In contrast, it is unknown when *M.*  
141 *guttatus* was first introduced into ENA, but we found no collections before the early 1900's and  
142 most extant populations were observed since the 1960's. The source populations of the ENA  
143 populations is currently uncertain, but they likely represent a mix of multiple accidental  
144 introductions (e.g. through introduction of debris on military or construction equipment) and/or  
145 cultivated escapes (Gleason and Cronquist 1991).

146 The degree of invasiveness differs between the UK populations and the ENA populations. For  
147 instance, in particular areas of Europe there is concern over its spread into natural areas (Truscott  
148 et al 2006) and new locations (Tokarska-Guzikand and Dajdok 2010). Within the United  
149 Kingdom, the presence of *M. guttatus* is associated with local declines in native species richness  
150 (Truscott et al 2008). In contrast, many of the reported populations in ENA appear to not be  
151 spreading or have disappeared entirely (Timothy Block, personal communication, Gleason and  
152 Cronquist 1991).

### 153 *Plant material*

154 We collected seed from wild populations in the summer of 2015, 2016, and 2017 in both the  
155 native (WNA) and the non-native (ENA and the UK) regions (Figure 1, Table S1). Populations  
156 were chosen to maximize geographic spread in all regions as well as to capture life history  
157 variation across the *M. guttatus* range (e.g., annual and perennial populations). We also grouped  
158 native populations into geographic clades (sub-regions in this paper) based on the genetic

159 population structure results from Twyford and Friedman (2015) who found 5 broad genetic  
160 clusters that were geographically separated. In each population, we collected seeds from >20  
161 plants separated by at least a meter to avoid clones and from multiple flowers on each plant.  
162 Populations were then grown in the greenhouse (Flagstaff AZ, USA) for at least one generation,  
163 originating from multiple wild-collected, maternal sib families from each natural population.

#### 164 *Herbivore communities*

165 The first prediction of EICA is that there is a release from specialist herbivore pressure in non-  
166 native populations. To test for this prediction in *M. guttatus*, we collected herbivores at each seed  
167 collection site and made herbivore collections from additional populations in each region. Most  
168 sites were surveyed over at least two seasons. Plant damage was estimated at each field site as  
169 the proportion of plants in the population with visible damage measured in a discrete scale (none:  
170 no damage on any plant; low: 1-10% of plants damaged by herbivores; moderate: 10-60% of  
171 plants damaged; high: 60-90% of plants damaged; extreme: >90% of plants damaged). For  
172 invertebrate herbivores, surveys consisted of timed visual searches and timed sweep netting (the  
173 latter only when *M. guttatus* density was high enough to preclude herbivores on other plant  
174 species). All invertebrates were collected and identified to the lowest taxonomic level possible.  
175 Herbivores were considered as those animals seen actively feeding on a plant, or those on the  
176 plant and likely able to feed on *M. guttatus* (such as a hemipteran resting on a plant but not  
177 actively feeding). We also noted if the damage was caused by a mammal and any signs of what  
178 mammal species may have been responsible. In addition to these field-based surveys in both  
179 ranges, we conducted a literature review on reports of herbivores and noted their geographic  
180 range. We also looked at feeding records in the literature for herbivores of plant species closely



181 related to *M. guttatus* (i.e., Scrophulariaceae *sensu lato*) to see if there were any specialist  
182 herbivores that may be able to shift hosts onto *M. guttatus* in the non-native regions.

### 183 *Resistance traits*

184 Following a release from specialist herbivores, EICA predicts the evolution of lower levels of  
185 some herbivore resistance traits. To test this part of EICA we used plants derived from native  
186 and non-native populations to assess patterns of genetic- based trait variation from a common  
187 greenhouse environment. We assessed specific leaf area (SLA), leaf water content, leaf dry  
188 matter content (LDMC), trichome density, and foliar phytochemistry. After growing the plants in  
189 a common greenhouse environment for one month, we harvested one leaf from the fourth true-  
190 leaf pair. We weighed the leaf to get wet mass and then scanned the leaf (Epson Perfection V19)  
191 to find leaf area using Image J (Rueden et al 2017). Freeze dried leaves (see below) were used to  
192 estimate dry weight and calculate specific leaf area (SLA), leaf water content, and leaf dry matter  
193 content (LDMC). Leaf water content and LDMC are associated with performance of some  
194 generalist herbivores consuming native *M. guttatus* (Rotter unpublished data) and have been  
195 included as resistance traits in other EICA studies (Bossdorf et al. 2005). Trichome density was  
196 measured by counting all the trichomes at the basal section of the adaxial side of each leaf within  
197 the field of view of a dissecting microscope at 25x magnification. This density was converted to  
198 trichome density per cm<sup>2</sup> (Holeski 2007).

199 For phytochemical analysis, we quantified phenylpropanoid glycosides (PPGs), the predominant  
200 foliar bioactive secondary compound in the species (Holeski et al 2013; Keefover-Ring et al  
201 2014). The leaf opposite the leaf in feeding trials (detailed below) was cut at the base of the  
202 petiole with scissors and flash frozen in liquid nitrogen before being transferred to a -20 degree C  
203 freezer. Tissue was then lyophilized using a pre-chilled FreeZone triad freeze dry system

204 (Labconco; Kansas City, USA). We finely ground the freeze-dried tissue in a small capacity ball  
205 mill (dental amalgamator with steel bearings). Samples were stored and extracted as described in  
206 Holeski et al. 2013. We quantified the PPG content of each sample via high-performance liquid  
207 chromatography [HPLC; Agilent 1260 HPLC with a diode array detector and Poroshell 120 EC-  
208 C18 analytical column (4.6 · 250 mm, 2.7 µm particle size); Agilent Technologies, Santa Clara,  
209 CA] maintained at 30°C, as described in Kooyers et al. (2017). The seven PPGs analyzed in this  
210 study represent the PPGs present in detectable levels in the populations used in this study.

### 211 *Herbivore feeding trials*

212 Herbivore response to plant resistance traits are often diffuse and vary depending on many  
213 different factors. In addition to quantifying resistance traits, we also measured resistance through  
214 two performance trials. For these trials, we used a subset of plant populations that represent the  
215 range of native and non-native populations (Table S1). We conducted no-choice performance  
216 trials with neonate Lepidopteran larvae of the specialist herbivore *Junonia coenia* and the  
217 generalist herbivore *Trichoplusia ni* (Rotter and Holeski 2017; Rotter et al 2018). One leaf from  
218 a leaf pair was placed in an envelope and treated as described above for analysis of PPGs. We  
219 assessed trichome density on the second leaf of the leaf pair, as described in Holeski (2007). The  
220 leaf scored for trichomes was then placed into a water pic and placed in a plastic container. In  
221 each container, we placed a single recently emerged first instar caterpillar. Leaves were  
222 immediately replaced with leaves from the same plant (with the opposite leaf harvested for  
223 phytochemical analysis) if/when the caterpillar consumed the entire leaf or if the leaf wilted.  
224 After larvae had fed for 10 days, we ended each trial, froze the caterpillars, and then dried and  
225 weighed them to determine caterpillar final dry mass. Larval initial (wet) weights were all within  
226 0.001 µg of each other for a particular species, so we assumed that initial dry mass was identical

227 across larvae within each species. Higher caterpillar mass and growth rates are important  
228 indicators of greater pupal survival rates as well as increased adult fitness (Haukioja and  
229 Neuvonen 1985; Awmack and Leather 2002). Additionally, a more rapid growth rate allows  
230 greater survival when faced with pressure from predators and parasitoids (Feeny 1976; Benrey  
231 and Denno 1997).

### 232 *Plant fitness traits*

233 Finally, EICA predicts an increase in fitness/competitive ability traits with a release from  
234 specialist herbivores and the decline of herbivore resistance traits. To test plant fitness traits we  
235 used the plants from the resistance traits measurements. We grew all plants for a total of six  
236 months prior to harvest with the exception of several populations of annual plants that were  
237 harvested after they stopped producing flowers. We assessed traits related to reproductive  
238 development, reproductive fitness, and vegetative fitness. We assessed reproductive development  
239 by counting the number of days until a plant first flowered. We also measured the corolla width  
240 (bigger flowers have been associated with pollinator preference; Martin 2004) of this first flower  
241 on the day after it was fully emerged. We collected pollen from the first two flowers. Pollen was  
242 then stained, counted, and evaluated for viability (decreased viability is a sign of inbreeding  
243 depression within *M. guttatus*; Carr and Dudash 1995) with a hemocytometer following the  
244 procedure in Kearns and Inouye (1993). We self-pollinated each plant with the next three  
245 flowers, saturating each stigma with as much pollen as possible. Seeds were collected from these  
246 flowers and total seed was counted. Finally, the total number of flowers produced by a plant  
247 were counted at the time of plant harvest. Plants that did not flower by the end of the six-month  
248 trial (n=32 plants) were excluded from these analyses. Vegetative traits quantified included  
249 specific leaf area and leaf water content, which were measured as described above during our

250 quantification of resistance traits. At harvest, we measured the total height (length) of the plant,  
251 from the root crown to the end of the largest shoot. We then dried all plants in a drying oven and  
252 measured aboveground biomass, belowground biomass, and total (aboveground + belowground)  
253 biomass.

#### 254 *Statistical analysis*

255 To compare herbivore communities, we used non-parametric multidimensional scaling (NMDS)  
256 to look at herbivore family and functional feeding guild differences between native and non-  
257 native populations of *M. guttatus*. The NMDS was performed using PC ORD v. 6 (McCune and  
258 Mefford, 2016). We used Jaccard distance as the similarity measure, and the program was run on  
259 “Autopilot” mode under the “slow and thorough” method, with principal axes rotation.  
260 Significance of the ordination was based on a Monte Carlo test with 250 iterations. To validate  
261 the NMDS we looked for differences between the non-native populations and the native sub-  
262 regions in the above herbivore communities using multi-response permutation procedures  
263 (MRPP). We also used ANOVA (transformed with either a log or root transformation as  
264 assessed by Q-Q plots; we used Kruskal-Wallis tests if we could not obtain a normal  
265 distribution) to look at the differences of field measured herbivory and herbivore richness  
266 between regions and sub-regions. Trait values, fitness and resistance traits, were analyzed using a  
267 nested ANOVA (plant family nested within population and population as a factor) to look for  
268 differences between the two non-native ranges and the native geographical clades. We further  
269 used Tukey post-hoc tests for pairwise comparisons between the non-native UK and ENA  
270 populations and between the native subregions. Lastly, we wanted to test for the predicted  
271 tradeoffs between herbivore resistance traits and competitive ability traits in the non-native  
272 populations compared to native populations. To narrow down important traits as well as suits of

273 traits we used PCA to find the two most important contributors to variation (components) for  
274 resistance traits and then for fitness/competitive ability traits for the two introduced regions. We  
275 took these components and used a linear regression (with population means of the components to  
276 account for population structure) to look for the relationship between the PCA components for  
277 resistance traits and the fitness/competitive ability PCA components. In addition to using the  
278 PCA components, we used correlation matrices to look at all pairwise trait tradeoffs (using  
279 population means) for each region. All ordinations and MRPPs were run in PC ORD v. 6  
280 (McCune and Mefford 2016) with all other analysis conducted in R (ver. 3.1.1; R Core team  
281 2013).

## 282 **Results**

### 283 Herbivores and herbivore communities

284 We found no evidence of specialist herbivores of *M. guttatus* in the non-native populations of the  
285 UK or in ENA. Within both non-native regions, all herbivores found have not been reported to  
286 consume plants from Scrophulariaceae *sensu lato*. However, the pool of potential specialist  
287 species is greater in eastern North America than in the UK. For example, at several of the ENA  
288 sites we observed adults of the specialists *Euphydryas phaeton* and *Junonia coenia* in the  
289 proximity of the *M. guttatus* populations, although no caterpillars of these species were found  
290 feeding on *M. guttatus* in ENA. Both species feed on plants related to *M. guttatus* and those that  
291 share similar phytochemistry (i.e., PPGs) making it possible that they could select these plants  
292 for oviposition with their offspring consuming the plants. In contrast we did not find any similar  
293 occurrence in the UK populations.

294 In the field, the percent of damaged plants differed between the regions and sub-regions ( $H=$   
295  $8.89$ ,  $DF = 2$ ,  $p = 0.012$ , Figure 2A). We found three times fewer plants damaged in the invaded  
296 UK than in the native WNA region (Dunns non-parametric comparison  $p < 0.001$ ), while the  
297 ENA populations had equivalent levels of field damage to the native WNA region (Dunns non-  
298 parametric comparison  $p = 0.154$ ). The comparisons between the introduced populations and  
299 individual native sub-regions showed that the UK populations had significantly less field  
300 herbivory than all the native sub-regions except the northern sub-region (Dunns non-parametric  
301 comparison  $p < 0.001$  for all except northern), while the ENA populations had equivalent  
302 herbivore damage (Dunns non-parametric comparison  $p < 0.087$ ) compared to all the native sub-  
303 regions ( $H = 18.21$ ,  $DF = 5$ ,  $p = 0.003$ ). Herbivore richness did not differ significantly between  
304 the UK, ENA, and WNA ( $F_{2,37} = 0.83$ ,  $p = 0.444$ , Figure 2B). This was also true when comparing  
305 the non-native regions to the native sub-regions ( $F_{5,34} = 2.33$ ,  $p = 0.063$ ).

306 Herbivore communities, at the family level, differed between the native subregions and the non-  
307 native populations (MRPP  $A = 0.085$ ,  $p < 0.001$ , Table 1) with the two non-native regions (ENA  
308 and the UK) having similar herbivore families to one another ( $A = -0.017$ ,  $p = 0.86$ ; Figure 3).  
309 The similarity in herbivore communities in ENA and the UK was generally driven by families  
310 dominated by generalist herbivores such as terrestrial gastropods and mammals. Differences  
311 between the UK populations and the native Cordilleran populations (which includes Alaska and  
312 is thus from which the UK populations are thought to be derived;  $A = 0.092$ ,  $p < 0.001$ ), were  
313 driven in part by the lack of leaf mining Agromyzidae in the UK. Results of the MRPP supported  
314 the results of the NMDS.

315 We also found substantial geographic variation in herbivore community composition within the  
316 native subregions. Native subregions were generally separated because of specialist insects that

317 dominated in particular subregions. For instance, leaf mining Agromyzidae flies were common  
318 in the cordilleran subregion as a dominant herbivore while the more southern subregions were  
319 dominated by specialist caterpillar species. Herbivore functional feeding guild differences across  
320 regions were similar to these herbivore community patterns (Table 1), and were driven by  
321 generalist chewers being more common in the non-native regions.

### 322 Herbivore resistance traits

323 In comparing traits between non-native and native regions, we focus on trait comparisons  
324 between populations from the non-native ENA and the native WNA regions and between the  
325 non-native UK populations and their likely ancestral WNA Cordilleran subregion (see study  
326 system for details). See the eastern North America supplement for a greater breakdown within  
327 these and the native subregions.

328 We found mixed evidence of an overall relaxation of selection on resistance traits predicted by  
329 EICA in the non-native *M. guttatus* populations. Physical resistance traits varied between native  
330 and non-native regions. Trichome density was significantly different between all regions ( $F_{2, 518}$   
331 = 86.63,  $p < 0.001$ , Figure 4). In support of EICA, native WNA populations had, on average,  
332 three and a half times higher trichome density than the non-native ENA plants, which was  
333 similar when using the native sub-regions ( $F_{5, 516} = 56.62$ ,  $p < 0.001$ , Figure 4). In contrast to the  
334 predictions of EICA, the UK population had one and half times higher trichome density than the  
335 native Cordilleran sub-region (Tukey post hoc:  $p = 0.002$ ). Specific leaf area was not  
336 significantly different between any of the native and non-native regions ( $F_{2,518} = 1.82$ ,  $p = 0.121$ ,  
337 Figure 4). Leaf water content in the UK populations was slightly higher than the Cordilleran  
338 populations and the non-native ENA populations was slightly higher than the native WNA  
339 populations ( $F_{2,517} = 4.53$ ,  $p = 0.011$ , Figure 4), suggesting a relaxation in herbivore defense. Leaf

340 dry matter content did not differ significantly across any of the native and non-native regions  
341 ( $F_{2,517} = 0.93$ ,  $p = 0.392$ , Figure 4).

342 Concentrations of chemical resistance compounds (PPGs) varied across the native and non-  
343 native regions ( $F_{2,454} = 56.62$ ,  $p = 0.004$ , Figure 5). Potentially in contrast to EICA, the eastern  
344 North American populations had higher levels of total PPGs than the native WNA plants (Tukey  
345 post hoc:  $p=0.004$ ). However, in line with the predictions of EICA, the non-native UK plants had  
346 lower amounts of total PPG concentration than the native Cordilleran subregion. When  
347 considering individual PPG compounds, there was no consistent overall pattern. For instance, the  
348 non-native ENA plants had high concentrations of calceolarioside B relative to the native WNA  
349 plants but a significantly lower concentration of verbascoside. Similarly, in the UK versus  
350 Cordilleran comparison, the non-native UK plants had higher concentrations of calceolarioside B  
351 than the cordilleran plants but lower concentrations of other PPGs such as conandroside (Figure  
352 5).

353 We found no evidence that specialists herbivores performed better on plants from non-native  
354 regions than from native, as predicted by EICA in lab trials. We found no difference in  
355 performance of a generalist or a specialist herbivore feeding on tissue from native vs. non-native  
356 regions. The generalist caterpillar *Trichoplusia ni* performed similarly on tissue from all regions  
357 ( $F_{2,115} = 0.06$ ,  $p = 0.940$ , Figure 6), as well as between the non-native regions and native  
358 subregions ( $F_{5,112} = 1.73$ ,  $p = 0.131$ ). Performance of the specialist caterpillar *Junonia coenia*  
359 also did not differ significantly across native and non-native regions ( $F_{2,41} = 1.87$ ,  $p = 0.168$ ).  
360 However, there were differences in *J. coenia* performance between the non-native regions and  
361 the native subregions ( $F_{5,38} = 2.77$ ,  $p = 0.032$ ) for instance caterpillars performed worse on the  
362 ENA plants compared to the southern, northern, and cordilleran subregions. Both of the



363 caterpillar species performed equally well on the native Cordilleran subregion plants and the  
364 non-native UK plants. Interestingly, the generalist herbivore performed worst on the WNA  
365 subregion in which the specialist herbivore species had the highest performance.

#### 366 Fitness/competitive ability traits

367 Reproductive traits varied across plants from the native and non-native regions. The non-native  
368 ENA populations tended to have relatively equivalent trait values for most traits when compared  
369 to the native WNA populations. In contrast, the non-native UK populations deviated from the  
370 Cordilleran subregion of WNA for many, but not all traits (Figure 7).

371 In the greenhouse, days till flower differed among regions ( $F_{2,481} = 27.28$ ,  $p < 0.001$ , Figure 7).

372 However, the two non-native regions did not significantly differ from their native regions of  
373 origin; the ENA plants flowered around the same time as the WNA plants (Tukey post hoc:  $p =$   
374  $0.761$ ) and the UK plants flowered at the same time as the Cordilleran subregion plants (Tukey  
375 post hoc:  $p = 0.998$ ). In support of EICA, both non-native regions had on average larger corolla  
376 widths than the native WNA region (Tukey post hoc: both  $p < 0.001$  compared to WNA plants).

377 This same trend held when comparing the non-native regions to the native subregions ( $F_{5,481} =$   
378  $35.83$ ,  $p < 0.001$ , Figure 7) with the UK plants having larger flowers than Cordilleran plants  
379 (Tukey post hoc:  $p < 0.001$ ). While pollen viability was variable across regions ( $F_{2,381} = 8.38$ ,  $p =$   
380  $0.003$ , Figure 7), trends between regions were opposite those predicted by EICA. Pollen

381 viability was lower in the ENA populations than in the WNA as well as in the UK plants versus  
382 the Cordilleran plants (Tukey post hoc:  $p > 0.001$  for both comparison). Total flower production  
383 was variable across regions ( $F_{2,518} = 6.41$ ,  $p = 0.001$ , Figure 7). Conforming to EICA predictions,  
384 the ENA plants produced slightly more flowers on average than WNA plants (Tukey post hoc:  $p$   
385  $= 0.007$ ), and the UK populations produced on average one and half times more flowers than the

386 native Cordilleran subregion (Tukey post hoc:  $p > 0.001$ ). Finally, seed production varied across  
387 regions ( $F_{2,518} = 5.83$ ,  $p = 0.008$ , Figure 7). The ENA plants produced an equivalent amount of  
388 seeds to the WNA plants (Tukey post hoc:  $p = 0.064$ ). The UK populations produced twice as  
389 many seeds on average compared to the Cordilleran subregion (Tukey post hoc:  $p < 0.001$ ).

390 For vegetative traits, plants from ENA tended to not conform to the predictions of EICA, while  
391 the non-native UK populations did, for most but not all traits. Plant height varied across regions  
392 ( $F_{2,502} = 50.92$ ,  $p < 0.001$ , Figure 7). Patterns in both non-native regions were compatible with  
393 the predictions of EICA. Plants from both the non-native ENA population and the UK  
394 population were larger than their native counterparts (Tukey post hoc:  $p < 0.001$ ), with the UK  
395 plants being over twice as tall on average than the native Cordilleran subregion.

396 There was variation across the regions for total plant biomass (aboveground + belowground;  
397  $F_{2,503} = 47.92$ ,  $p < 0.001$ , Figure 7), as well as aboveground biomass and belowground biomass  
398 considered independently ( $F_{2,497} = 55.36$ ,  $p < 0.001$ ;  $F_{2,501} = 12.03$ ,  $p < 0.001$ , respectively). The  
399 non-native ENA populations had equivalent total, aboveground, and belowground biomass to the  
400 native WNA populations (Tukey post hoc:  $p = 0.899$ ;  $p = 0.924$ ;  $p = 0.941$ , respectively). As  
401 predicted by EICA, the UK populations had almost twice as much total biomass and  
402 aboveground biomass, and also higher root biomass than the Cordilleran subregion (Tukey post  
403 hoc:  $p < 0.001$  for all biomass comparisons).

404 Shoot to root ratios varied across regions ( $F_{2,497} = 14.36$ ,  $p < 0.001$  Figure 7), with the non-native  
405 ENA populations having the largest ratio, which was significantly larger than that for WNA  
406 (Tukey post hoc:  $p = 0.002$ ). Shoot:root ratios for the UK populations were equivalent to those of  
407 the Cordilleran populations (Tukey post hoc:  $p = 0.625$ ).

408 Tradeoffs between herbivore resistance traits and fitness/competitive ability

409 We found some evidence of resistance-fitness/competitive ability trade-offs in the non-native  
410 UK region. For herbivore resistance traits, PCA one (24.8%) was associated with chemical traits  
411 such as conandroside, calceolarioside A and B, and unknown PPG 16. The second component  
412 (15.8%) was associated with trichome density, SLA, and unknown PPG 10. For  
413 fitness/competitive traits, PCA one (28.5%) was associated with plant height, number of flowers,  
414 and pollen viability while component two was associated with days till first flower, corolla  
415 width, and root mass. We found negative associations (suspected tradeoffs) between  
416 fitness/competitive ability PCA component one and resistance traits PCA component two ( $R^2 =$   
417  $0.29$ ,  $p = 0.012$ ; Figure 8). Additionally, we found a positive relationship between  
418 fitness/competitive ability component two and resistance component one ( $R^2 = 0.17$ ,  $p = 0.045$ ).  
419 The other regressions had non-significant relationships (Table 2).

420 We found no signs of resistance-fitness/competitive ability trade-offs in the ENA plants. The  
421 PCA for resistance traits in the ENA plants had the first component (25%) associated with  
422 conandroside, calceolarioside A, and unknown PPG 16 with the second component (20.9%)  
423 associated primarily with verbascoside, mimuloside, and unknown PPG 10. The fitness/  
424 competitive traits PCA had a first component (40%) associated primarily with number of  
425 flowers, shoot mass, and corolla width. The second component (18.8%) was associated with root  
426 mass, pollen viability and seed production. All the components had non-significant relationships  
427 to one another (Table 2).

428 The native region (WNA) also showed evidence of resistance vs. fitness/competitive ability  
429 tradeoffs. Herbivore resistance PCA first component (21.2%) was associated with unknown PPG  
430 16, calceolarioside B, and conandroside while the second component (14%) was associated with

431 calceolarioside A, unknown PPG 10, and mimuloside. The first component (33.3%) for fitness/  
432 competitive ability traits was composed primarily of corolla width, plant height, and shoot mass.  
433 The second component (16.5%) was associated with days till first flower, number of flowers, and  
434 pollen viability. The only significant relationship we found for WNA plants was a negative  
435 relationship between fitness/competitive ability component two and resistance component one  
436 ( $R^2 = 0.16$ ,  $p = 0.041$ , Figure 8). All other comparisons were non-significant (Table 2).

437 Finally, Cordilleran plants showed no signs of tradeoffs. The herbivore resistance PCA  
438 component one (25.6%) was associated with unknown PPG 16, calceolarioside A, and  
439 conandroside and the second component (16.9%) was associated with calceolarioside B,  
440 verbascoside, and unknown PPG 10. The first component of the fitness/ competitive ability traits  
441 (41.9%) was associated with corolla width, plant height, and number of flowers produces, the  
442 second fitness/ competitive ability component (23.4%) was associated with root mass, seed  
443 count, and percent pollen viability. We found no evidence of tradeoffs between these  
444 components (Figure 8, Table 2). In addition to regression results we also detected weak signs of  
445 tradeoffs between specific resistance traits and competitive/ fitness traits through correlation  
446 analysis. There results are similar to the regression results (Figures S1-S3).

## 447 **Discussion**

448 By comparing two different plant invasions of differing ages to their native counterparts we  
449 found some, but not comprehensive, support for EICA. Support was strongest in the non-native  
450 UK, the older and more successful of the two invasions. Both the non-native UK and the ENA  
451 plants had different herbivore communities than the native WNA plants. However, there was  
452 adherence to the EICA prediction of a reduction in herbivore damage as well as clear evidence of  
453 specialist herbivore escape in only the UK range. We found relatively minor support for the

454 prediction that there would be a decline of herbivore resistance traits in the non-native plants,  
455 with some changes in trait values in the non-native vs. native regions, but no differences in  
456 herbivore performance in no-choice trials. The UK plants were larger, taller, and produced more  
457 seeds and flowers than their native counterparts, in accordance with EICA predictions, while the  
458 non-native ENA plants were generally smaller and had poorer pollen production than the native  
459 WNA plants. Lastly the UK plants exhibited some tradeoffs between resistance traits and fitness/  
460 competitive ability while the ENA plants did not, confirming the predictions that release from  
461 specialist herbivores can result in allocational tradeoffs that allow for increases in fitness/  
462 competitive ability.

#### 463 Enemy release and resistance traits in the non-native populations

464 We found some evidence of escape from coevolved specialist herbivores in both of the non-  
465 native regions. However, this did not translate to the same pattern of relaxed defenses in the two  
466 non-native regions. Each non-native region had several resistance traits present at lower levels  
467 than in their native ancestral regions. The non-native ENA populations had lower trichome  
468 density and higher leaf water content than did the native WNA populations, while the non-native  
469 UK populations had higher leaf water content and lower levels of total PPGs than the native  
470 Cordilleran region. However, levels of some defenses were also higher in the non-native regions  
471 than the native, and we found no difference in performance of generalist and specialist  
472 herbivores feeding on native vs. non-native plants. Within a non-native range, even if they are  
473 escaping co-evolved specialist herbivores, introduced plants often encounter generalist  
474 herbivores that may prefer to attack these non-native plants (Maron and Vilà 2001; Parker and  
475 Hay 2005; Liu et al 2007). One of the few other studies that have compared two invaded regions  
476 within the context of EICA found that populations of the invasive plant *Senecio jacobaea* in a

477 region with a biological control agent (i.e. re-association with a specialist herbivore, see also  
478 Sieman and Rogers 2003 and Valverde et al. 2015) did not conform to EICA predictions as well  
479 as an invaded region without this control agent present (Rapo et al 2010). Both non-native  
480 ranges in our study had different herbivores communities attacking them than the native region,  
481 although herbivory pressures were not necessarily lessened in the non-native environments.  
482 Plants in ENA still suffered equivalent damage to WNA plants while, although they suffered less  
483 damage, UK plants still had equivalent herbivore richness (per population) as the native plants  
484 did. Another explanation of these results may stem from the longevity of the UK invasion versus  
485 the relatively new ENA invasion. The UK plants may have initially experienced a herbivore  
486 release that was reduced or eliminated over time, and thus may have evolved a new defense  
487 strategy most effective in the current habitat.

488 Although other studies have generally detected EICA-predicted relaxation of resistance to  
489 specialist herbivores in non-native regions in feeding trials (Rotter and Holeski 2018), these  
490 changes in herbivore resistance traits were not detected in herbivore performance trials in our  
491 study. An alternative hypothesis, the novel weapons hypotheses (Callaway and Ridenour 2004;  
492 Inderjit et al 2006), which predicts that enemy release and non-native success is the result of the  
493 non-native plants unique chemical unpalatability to herbivores in the non-native range, may  
494 explain the lack of differences between caterpillar performance. This would suggest that the non-  
495 native populations may have retained resistance traits because they are beneficial in the non-  
496 native region. The presence of overlap in resistance traits, as some traits likely deter both  
497 generalists and specialists, resulting in the overall maintenance of traits that defend against  
498 generalist herbivores. This would lead to the maintenance of certain resistance traits that may  
499 deter specialist herbivores despite their absence. For instance, the PPG conandroside has a

500 negative impact on the performance of the generalist herbivores *Grammia incorrupta* and  
501 *Spodoptera exigua* as well as a negative impact on the specialist herbivore *Junonia coenia*  
502 (Rotter et al 2018).

### 503 Changes to competitive ability in non-native plants

504 The EICA prediction that trait values related to fitness and/or competitive ability will be higher  
505 in non-native regions was partially supported by our data. Like resistance traits, we did not see  
506 similar patterns in fitness and/or competitive ability traits between the two non-native regions.  
507 Fitness/competitive ability traits tended not to conform to the predictions of EICA for the non-  
508 native ENA region; these trait values were generally very similar to those for the native WNA  
509 region. In contrast, fitness/competitive ability trait values were greater in the non-native UK than  
510 the native Cordilleran region for many traits, in accordance to EICA predictions. Several other  
511 studies have looked at genetic-based phenotypic differences, particularly physiological and floral  
512 traits, between native and non-native *M. guttatus* (van Kleunen and Fischer 2008; Murren et al  
513 2009; Martinez 2018). In these studies there was an observed adaptation of the non-native plants  
514 to local abiotic conditions as well as producing more flower-bearing stems (van Kleunen and  
515 Fischer 2008) with non-native plants exhibiting increased flower sizes (Murren et al 2009),  
516 which is similar to our findings in the UK plants. For competitive traits, relative growth rate was  
517 not found to be different between native and non-native *M. guttatus* populations (Martinez  
518 2018). In the UK, *M. guttatus* has been shown to readily spread through both vegetative and seed  
519 propagules during high flow events allowing for successful spread (Truscott et al 2006), although  
520 this study focused on non-native populations and did not include a native population comparison.

### 521 Tradeoffs

522 We found equivocal support for EICA-predicted trade-offs between defense and  
523 fitness/competitive ability. In the native WNA and non-native ENA comparison, there were  
524 actually fewer detected trade-offs in the non-native region (0) than in the native (1). The native  
525 Cordilleran vs. non-native UK comparison was compatible with EICA, with no trade-offs  
526 detected in the native region, and one detected in the non-native UK. EICA's predictions for the  
527 success of non-native plants are based on the assumption of allocational tradeoffs existing  
528 between herbivore resistance and traits associated with competitive ability (Bloosey and Notzold  
529 1995; Orians and Ward 2008). Here, we did find an increase in trait values for traits associated  
530 with fitness/competitive ability in one non-native range (the UK), but these increases were not  
531 overwhelmingly associated with decreases in resistance traits. A recent meta-analysis found that  
532 non-native plants may in fact not have to make these trade-offs and instead are able to increase  
533 resistance traits and fitness/competitive ability (Rotter and Holeski 2018). This may present  
534 some support for hypotheses predicting that non-native plants are able to exploit resources more  
535 efficiently or take advantage of unoccupied niche space (Burke and Grime 1996; Davis et al  
536 2001). In fact, there may be a synergy between enemy release and the use of resources as species  
537 that are limited by defending themselves may gain a significant advantage when these resources  
538 are in abundance (Blumenthal 2006).

539 This lack of clear tradeoffs, as predicted by EICA, has also been found in other reviews focused  
540 on EICA (Bossdorf et al 2005; Felker-Quinn et al 2013). Both of these studies found overall that  
541 non-native plant populations changed in their herbivore resistance traits as well as their fitness/  
542 competitive ability traits but these changes did not reflect EICA predictions of a tradeoff (a direct  
543 relationship between an increase in fitness/ competitive ability and a decrease in herbivore  
544 resistance traits). These studies suggested that more specific looks at relevant traits was needed



545 in testing EICA predictions. Although it is possible that we missed some of the key traits that are  
546 involved in tradeoffs, our study was relatively comprehensive in our trait selection particularly  
547 for traits important to the ecology of *M. guttatus*.

#### 548 Can EICA predict the success of *M. guttatus* invasions?

549 Finally, our prediction that the more successful and older invasion (the UK) would display more  
550 evidence of adherence to EICA than the less successful and recent invasion (ENA), was  
551 supported. The non-native UK populations showed greater adherence to multiple predictions of  
552 EICA (Table 3) than the non-native ENA region. Within the EICA framework, species that have  
553 become extremely successful invaders such as *Triadica sebifera* (Huang et al 2010; Carillo et al  
554 2014) might conform more closely to EICA than relatively non-invasive non-natives such as  
555 *Lepidium draba* (Cripps et al 2009). In the UK, *M. guttatus* has successfully spread throughout  
556 the country filling many of the available niches. In ENA the invasion is thought to be more  
557 recent, *M. guttatus* has become extirpated from several of the locales where it has previously  
558 been reported, and no new populations have been reported since at least the early 2000's. Our  
559 results correspond with those of other studies that compared different non-native plants within  
560 the same region that had differing level of invasiveness (ability to spread and dominate  
561 communities). Plants that were ranked as more invasive had lower rates of herbivory than those  
562 non-natives that were not considered as invasive (Cappuccino and Carpenter 2005). This  
563 supports the idea that the strongest evidence for EICA may be found in more successful  
564 invasions.

565 The relative success of the UK invasion versus the ENA invasions and the differences in  
566 adherence to EICA across invasions may be in part explained by the nature of their introductions.  
567 The UK plants were introduced as a garden plant; these plants are typically pre-selected in the

568 field for traits correlated with increased fitness/ competitive ability (Reichard and White 2001;  
569 Dehnen-Schmutz et al 2007). In contrast, the ENA plants most likely came from multiple  
570 haphazard introductions. In addition to these pre-selected traits UK plants have had more time to  
571 be influenced from the new herbivore communities and respond to evolutionary tradeoffs. This  
572 lag time is somewhat common in other non-native plant invasions (Crooks 1999) and could  
573 explain the greater conformity of the UK populations to the EICA predictions. It is possible that  
574 given more time the ENA plants will become more successful and their relative adherence to  
575 EICA predictions may also change.

576 There are many different frameworks for understanding the success of non-native organisms  
577 (Catford et al 2009) and it is likely that there is not a single one that can consistently and fully  
578 explain why a non-native species becomes successful across systems (Gurevitch et al 2011; Lau  
579 and Schultheis 2015). This is the case with our results; although we found some evidence to  
580 support EICA, particularly in the non-native UK region, there were several patterns that were not  
581 necessarily compatible with EICA (e.g., caterpillar performance was not different between the  
582 native and non-native plants and the sometimes positive relationship between resistance traits  
583 and fitness/ competitive ability in the UK plants). However, we do present evidence that the  
584 release from (or at least a shift in herbivore suites) can lead to evolutionary changes in plant  
585 resistance traits that result in an increase in competitive ability particularly in invasions that have  
586 persisted longer and have achieved higher success.

587

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596

#### 597 **Compliance with ethical standards**

598 The authors declare no conflicts of interests.

599

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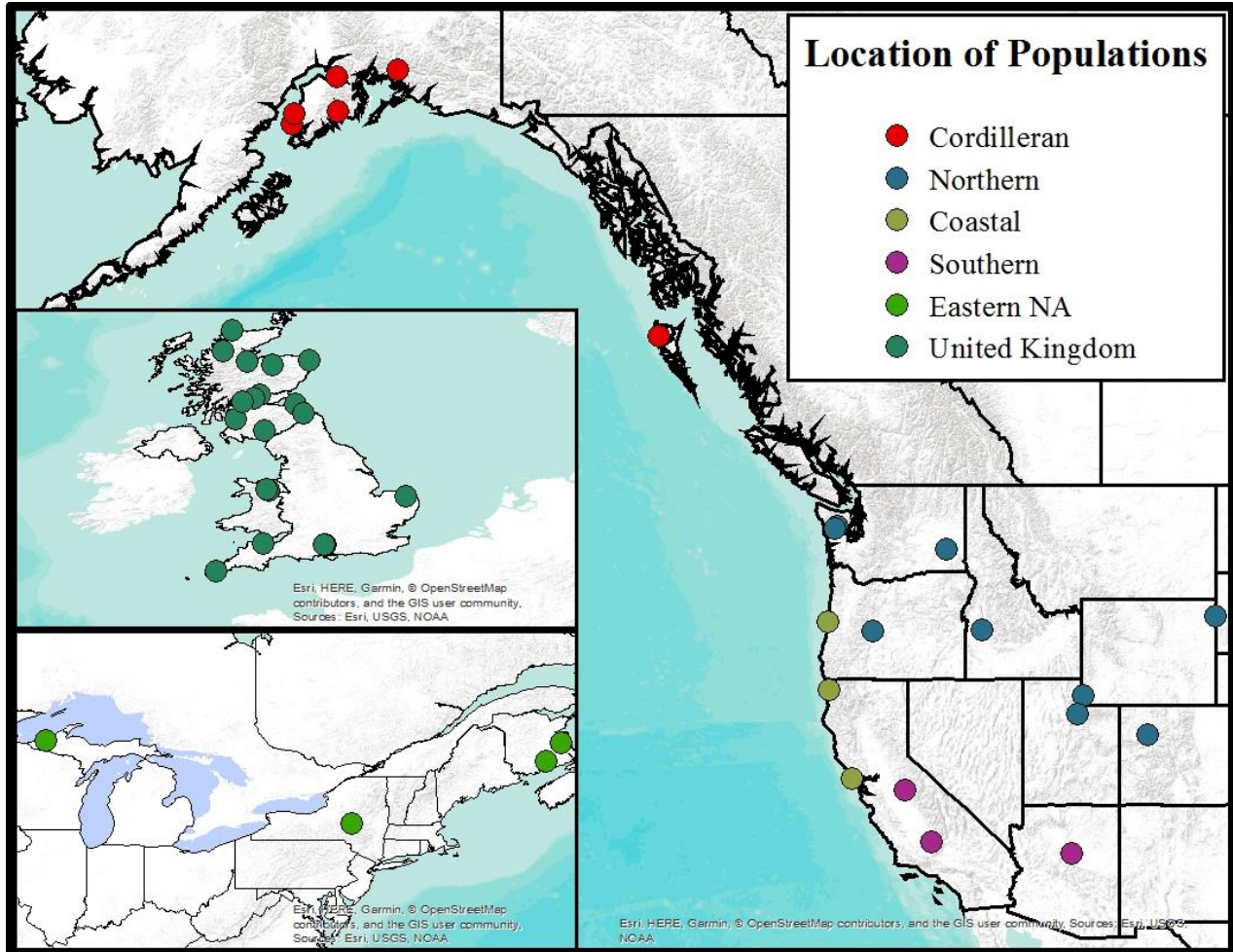
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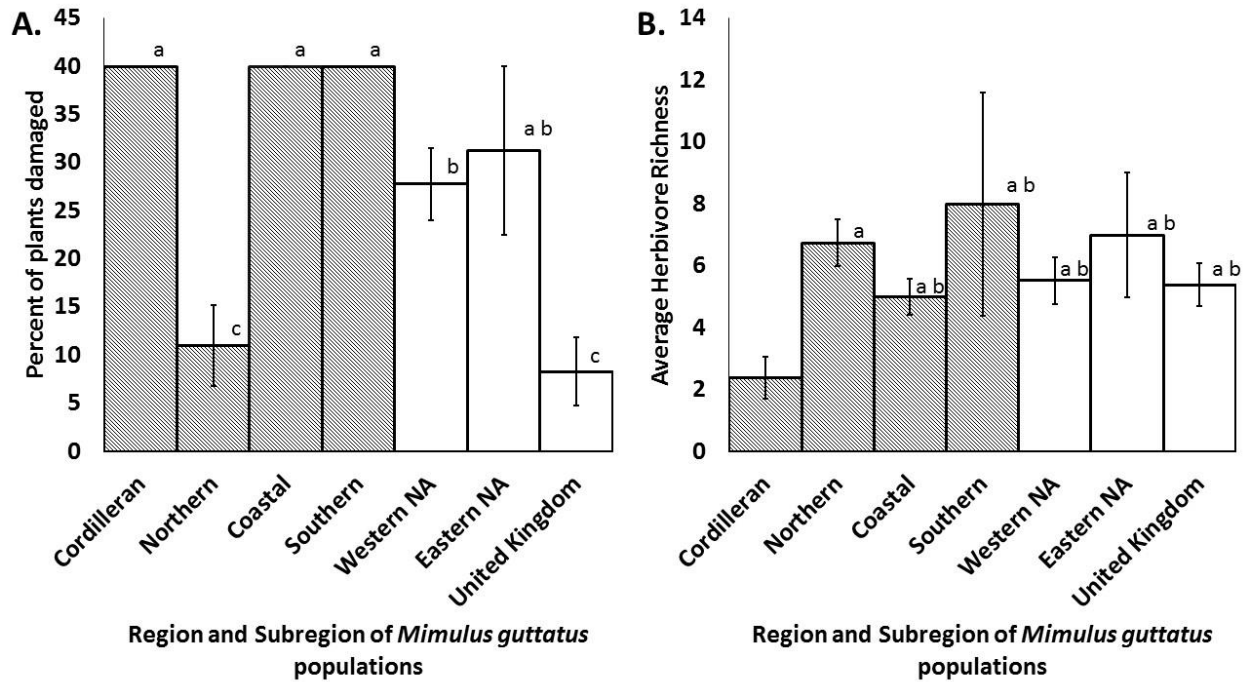
767 **Figures**



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769 **Figure 1.** Locations of populations used in this study. Sub-regions within the native range (W. North  
 770 America) are based on molecular evidence from Twyford and Friedman (2015).

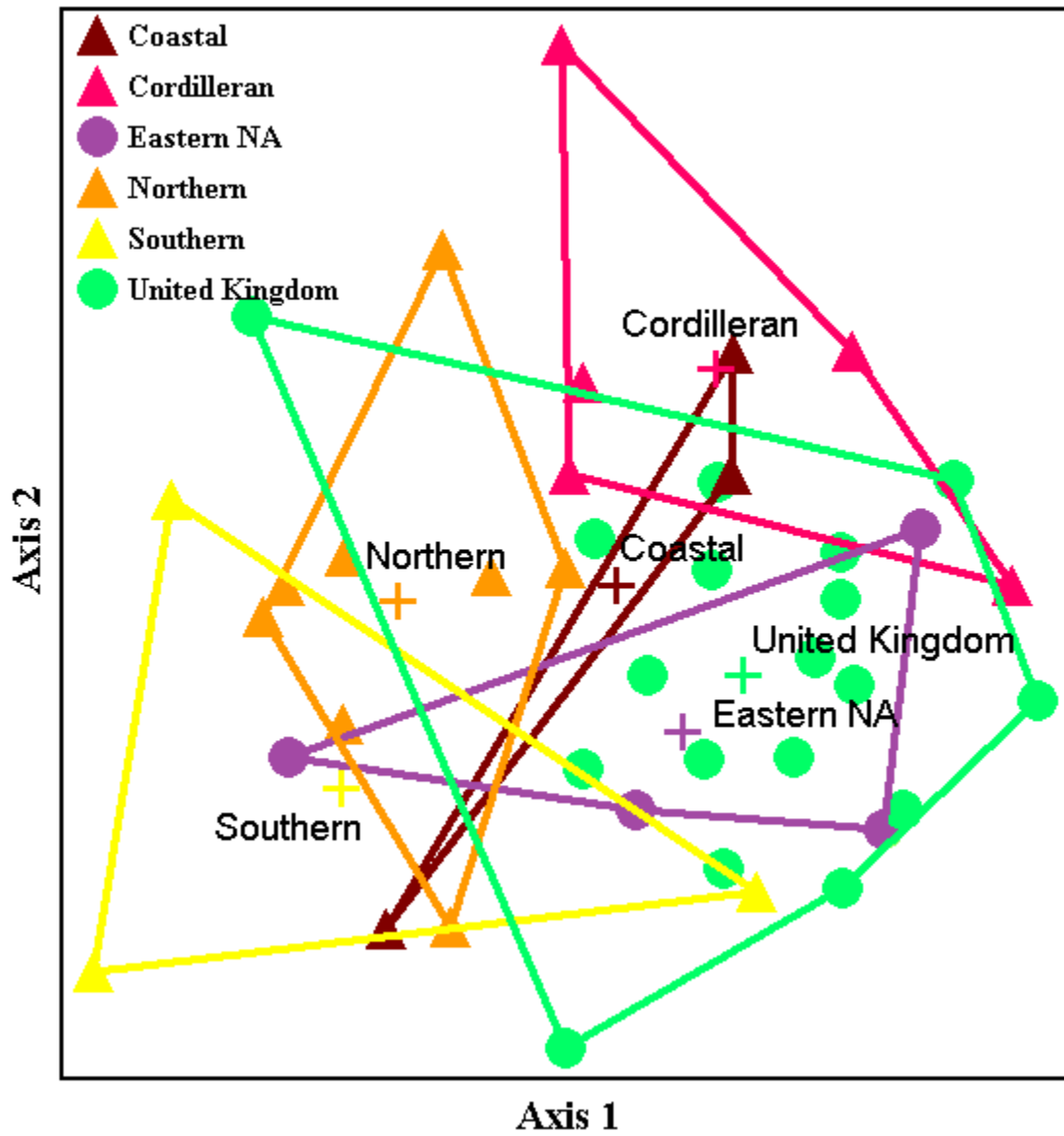




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772 **Figure 2.** A. Percent of plants with herbivore damage in wild growing *Mimulus guttatus* populations  
 773 between regions (in white) and native subregions (patterned). B. Average herbivore species richness  
 774 found in the field feeding on *Mimulus guttatus* populations between regions (in white) and native  
 775 subregions (patterned). Error bars represent  $\pm 1$  standard error. Letters indicate equivalent values based on  
 776 a Tukey HSD post-hoc test. Non-transformed data displayed.

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779 **Figure 3.** NMDS of herbivore communities based on family for the two non-native regions and the four  
 780 native subregions. A 2D solution was the best solution with final stress being 25.71. Stress of axis 1 was  
 781 51.74 and axis 2 was 27.891. Although there is high stress, these results resembles MRPP results and are  
 782 ecologically relevant (See Minchin 2018).

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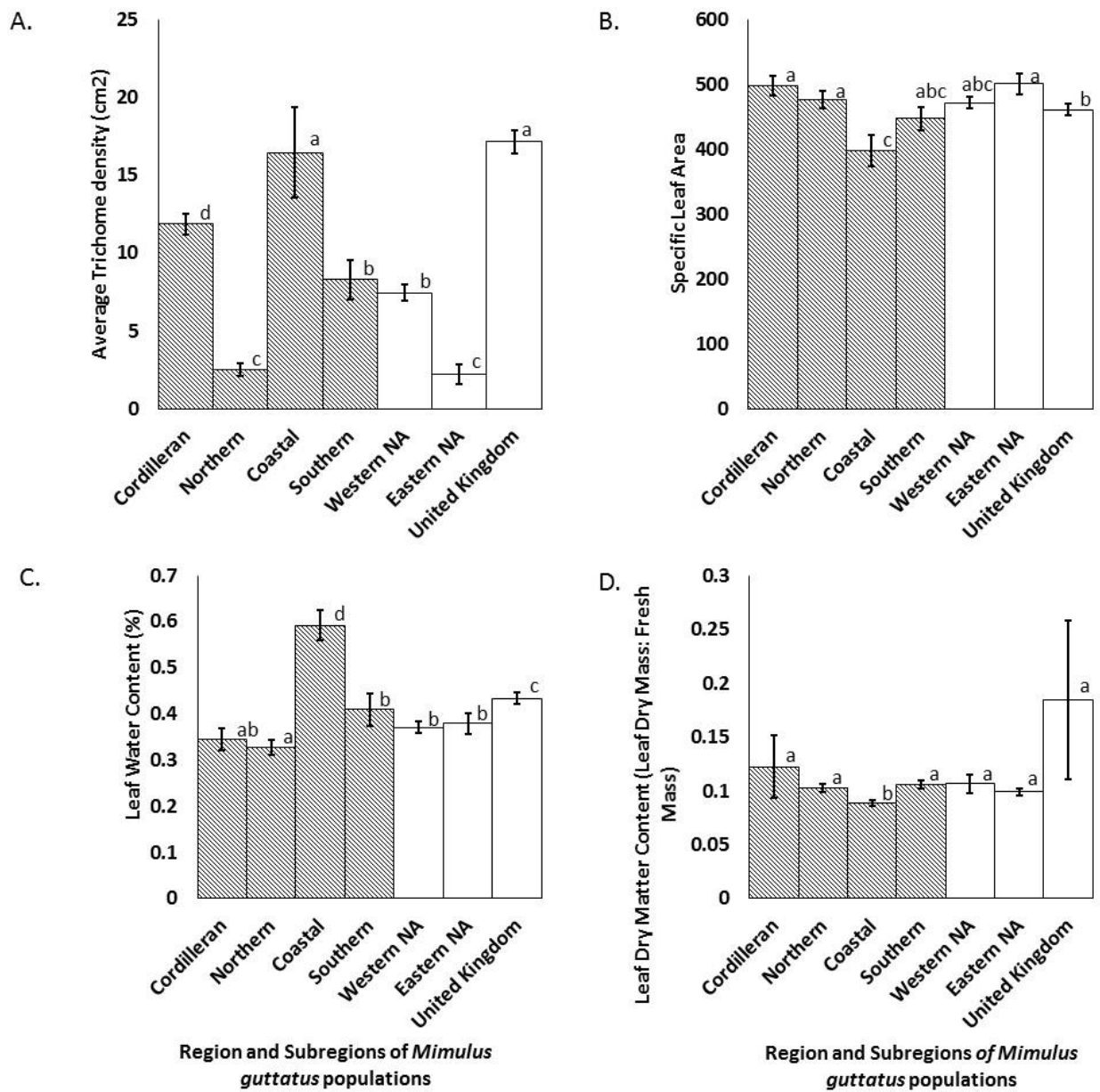
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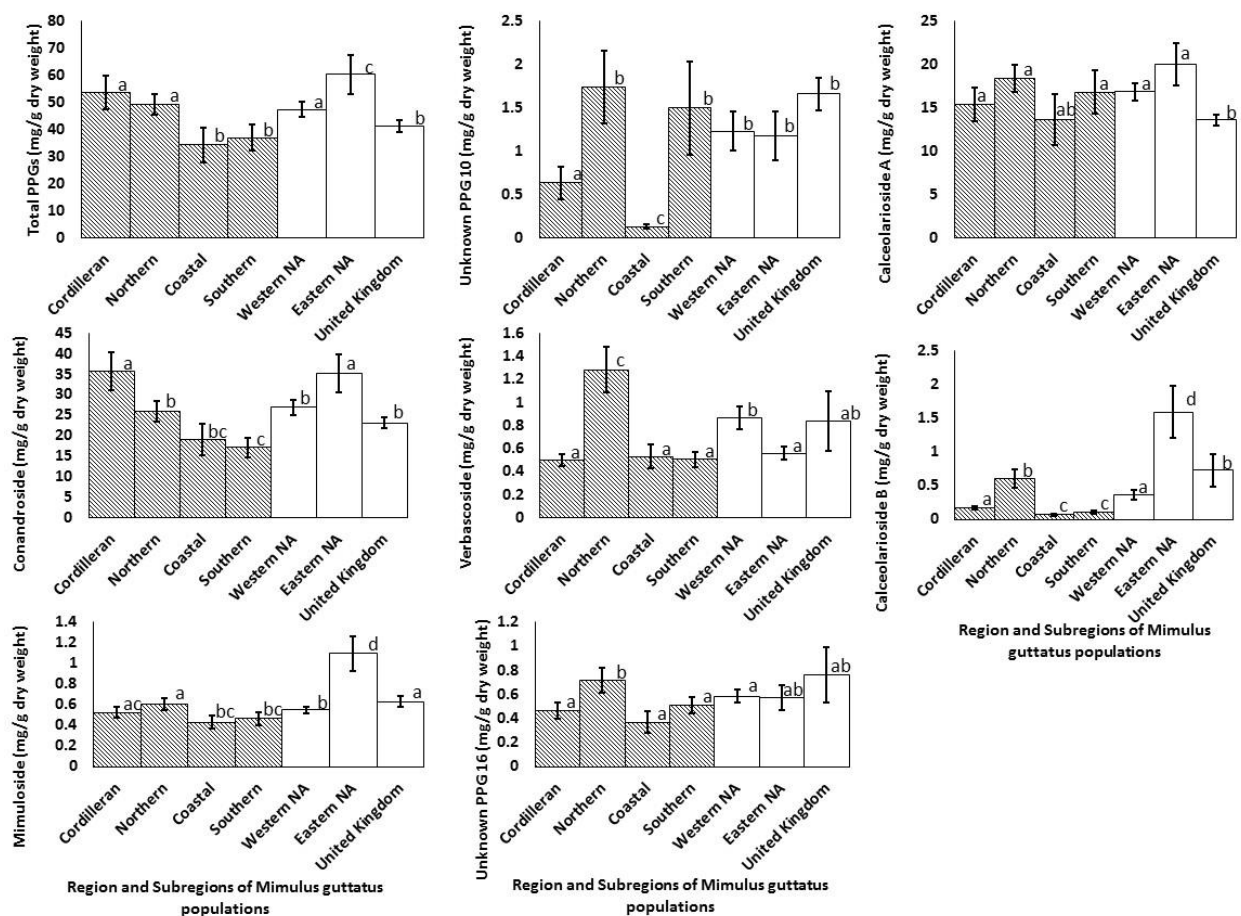
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793 **Figure 4.** Average physical resistance traits (A. Trichomes, B. Specific Leaf Area, C. Water Content, D.  
794 Dry Leaf Matter) in *Mimulus guttatus* populations between regions (in white) and native subregions  
795 (patterned). Error bars represent  $\pm 1$  standard error. Letters indicate equivalent values based on a Tukey  
796 HSD post-hoc test. Non-transformed data displayed.



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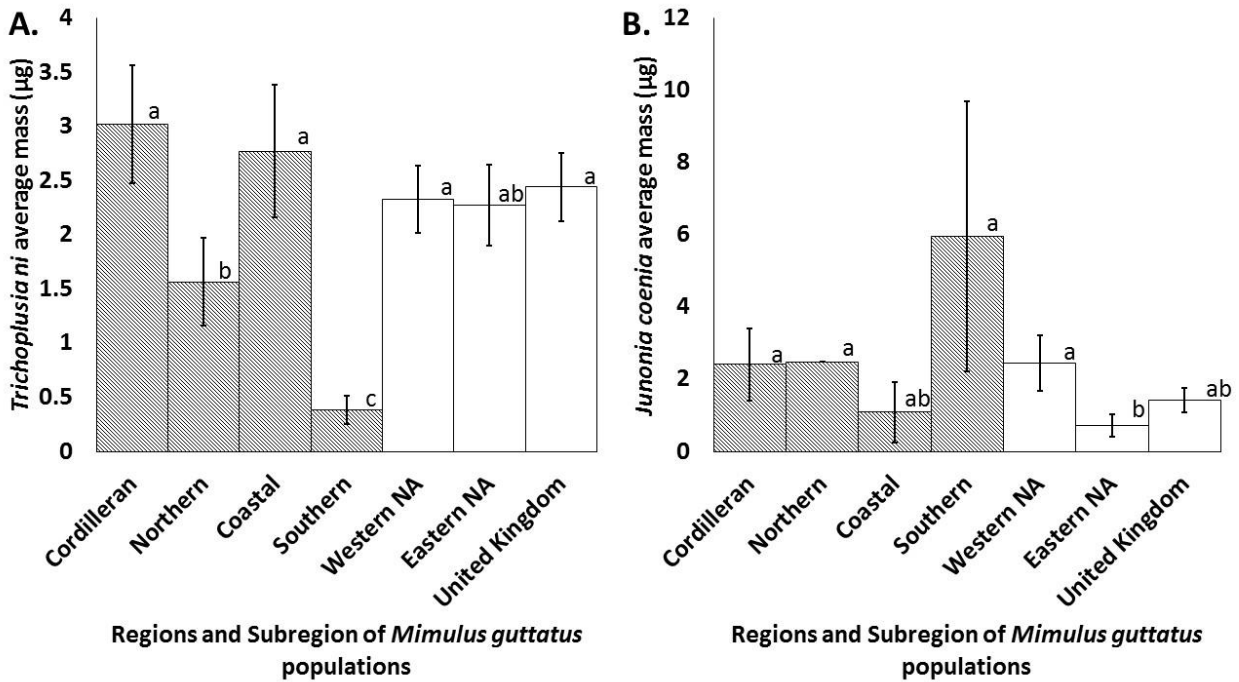
800 **Figure 5.** Average concentration (mg/ dry weight) of foliar phenylpropanoid glycosides within regions  
 801 (white) and subregions (patterned) of *M. guttatus* populations. Error bars represent one standard error.  
 802 Error bars represent  $\pm 1$  standard error. Letters indicate equivalent values based on a Tukey HSD post-hoc  
 803 test. Non-transformed data displayed.

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810 **Figure 6.** Average performance (mass µg) of (A.) the generalist caterpillar *Trichoplusia ni* and (B.) the  
 811 specialist caterpillar *Junonia coenia* within regions (white) and subregions (patterned) of *M. guttatus*  
 812 populations. Error bars represent  $\pm 1$  standard error. Letters indicate equivalent values based on a Tukey  
 813 HSD post-hoc test. Non-transformed data displayed.

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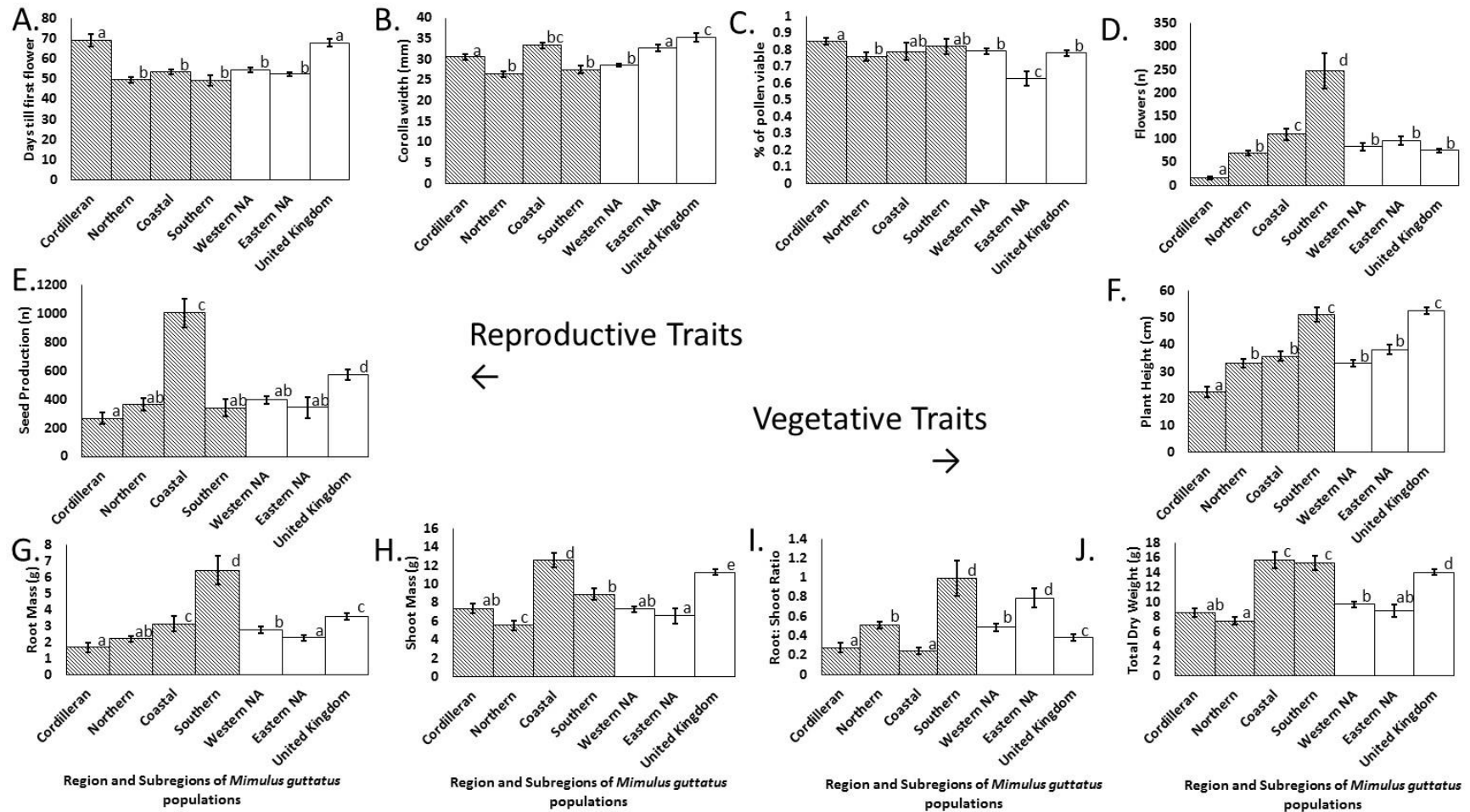
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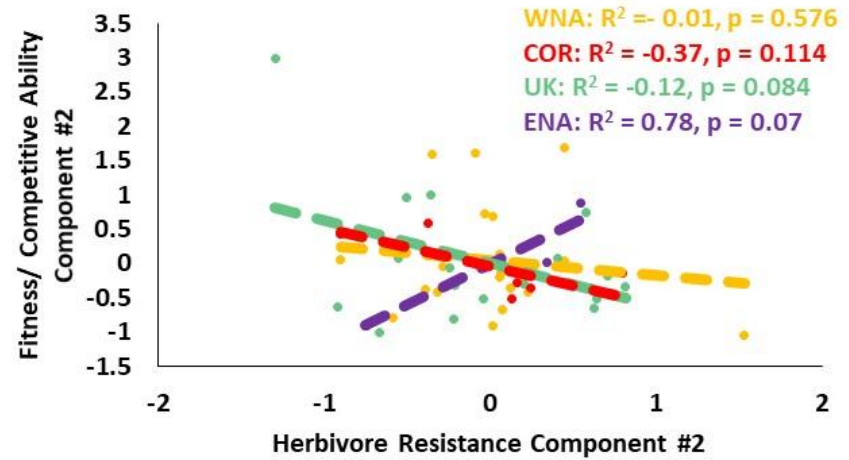
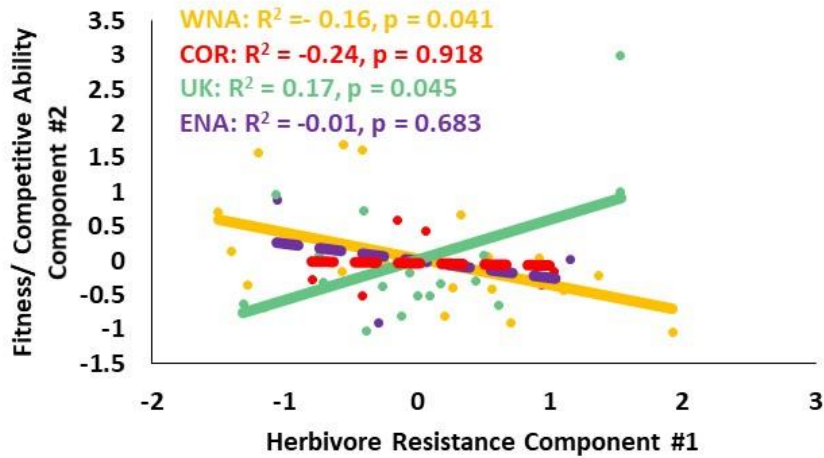
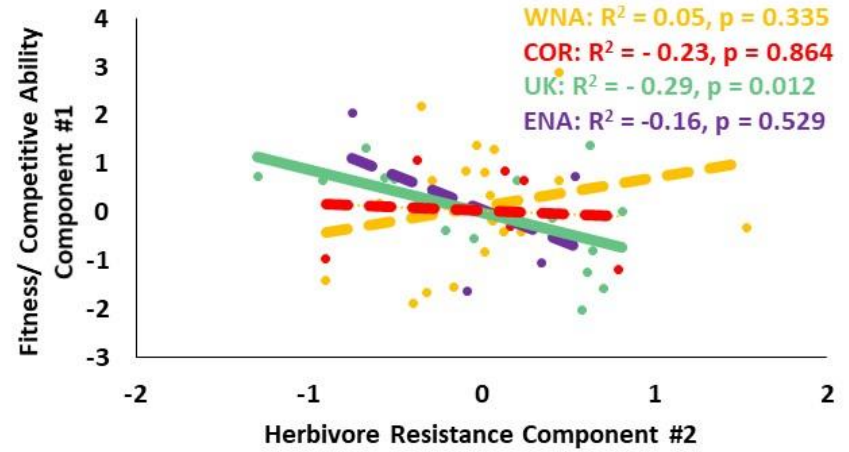
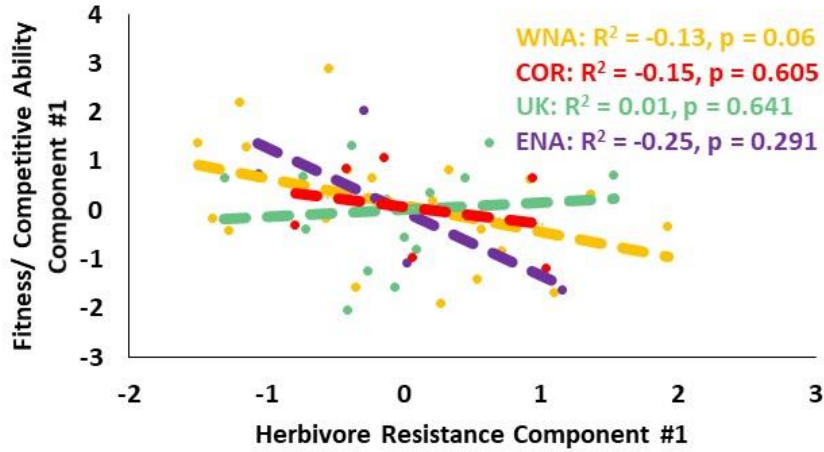
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**Figure 7.** Averages of measures of fitness / competitive ability traits. Reproductive traits: A. Number of days till first flower, B. Width of first corolla, C. Percent of pollen that is viable, D. Total number of flowers produced, E. Number of seeds from first three flowers. Vegetative traits: F. Plant height, G. Root dry mass, H. Shoot dry mass, I. Root:shoot ratio, J. Total dry biomass for *Mimulus guttatus* populations between regions (in white) and native subregions (patterned). Error bars represent  $\pm 1$  standard error. Letters indicate equivalent values based on a Tukey HSD post-hoc test. Non-transformed data displayed.



**Figure 8.** Regressions between fitness/ competitive traits PCA components and herbivore resistance trait PCA components of population means. WNA plants in Orange, Cordilleran plants (COR) in red, UK plants in teal and Eastern North American plants in green. Significant trend lines shown as solid with insignificant trend lines dotted.

**Tables.**

**Table 1.** MRPP results for differences between the non-native regions and the native sub-regions herbivore community at the family level (on bottom and in grey) and functional feeding group (on top in white). The full model was significant for herbivore communities at the family level ( $A = 0.085$ ,  $p < 0.001$ ) and for functional feeding groups ( $A = 0.131$ ,  $p < 0.001$ ). Bolded results are significantly different pair wise comparisons.

	Coastal	Cordilleran	Eastern NA	Northern	Southern	United Kingdom
Coastal		$A = 0.083$ $p = 0.082$	$A = -0.063$ $p = 0.817$	$A = -0.062$ $p = 0.894$	$A = -0.069$ $p = 0.081$	$A = -0.029$ $p = 0.884$
Cordilleran	$A = 0.020$ $p = 0.332$		<b><math>A = 0.154</math></b> <b><math>p = 0.019</math></b>	<b><math>A = 0.292</math></b> <b><math>p &lt; 0.001</math></b>	<b><math>A = 0.177</math></b> <b><math>p = 0.008</math></b>	<b><math>A = 0.131</math></b> <b><math>p &lt; 0.001</math></b>
Eastern NA	$A = -0.078$ $p = 0.908$	<b><math>A = 0.128</math></b> <b><math>p = 0.0149</math></b>		$A = 0.066$ $p = 0.066$	$A = -0.031$ $p = 0.625$	$A = 0.014$ $p = 0.228$
Northern	$A = -0.042$ $p = 0.890$	<b><math>A = 0.181</math></b> <b><math>p &lt; 0.001</math></b>	<b><math>A = 0.067</math></b> <b><math>p = 0.028</math></b>		$A = -0.026$ $p = 0.680$	<b><math>A = 0.126</math></b> <b><math>p &lt; 0.001</math></b>
Southern	$A = -0.053$ $p = 0.812$	<b><math>A = 0.126</math></b> <b><math>p = 0.019</math></b>	$A = 0.005$ $p = 0.433$	$A = 0.025$ $p = 0.215$		$A = 0.039$ $p = 0.072$
United Kingdom	$A = -0.038$ $p = 0.998$	<b><math>A = 0.092</math></b> <b><math>p &lt; 0.001</math></b>	$A = -0.017$ $p = 0.866$	<b><math>A = 0.099</math></b> <b><math>p &lt; 0.001</math></b>	$A = 0.028$ $p = 0.062$	



**Table 2.** Regression tradeoff results of fitness/ competitive ability PCA components vs herbivore resistance traits PCA components of population means. Significant results are in bold. PCA components are different for each of the regions, and are listed in the text.

	Herbivore Resistance Component #1	Herbivore Resistance Component #2
Fitness/ Competitive Ability Component #1	WNA: $R^2 = 0.13$ , $p = 0.06$ , $\beta = -0.42$ COR: $R^2 = 0.15$ , $p = 0.605$ , $\beta = -0.26$ UK: $R^2 = 0.01$ , $p = 0.641$ , $\beta = 0.11$ ENA: $R^2 = 0.25$ , $p = 0.291$ , $\beta = -0.71$	WNA: $R^2 = 0.05$ , $p = 0.335$ , $\beta = 0.22$ COR: $R^2 = 0.23$ , $p = 0.864$ , $\beta = -0.09$ <b>UK: <math>R^2 = 0.29</math>, <math>p = 0.012</math>, <math>\beta = -0.57</math></b> ENA: $R^2 = 0.16$ , $p = 0.529$ , $\beta = -0.47$
Fitness/ Competitive Ability Component #2	<b>WNA: <math>R^2 = 0.16</math>, <math>p = 0.041</math>, <math>\beta = -0.45</math></b> COR: $R^2 = 0.24$ , $p = 0.918$ , $\beta = -0.05$ <b>UK: <math>R^2 = 0.17</math>, <math>p = 0.045</math>, <math>\beta = -0.57</math></b> ENA: $R^2 = 0.01$ , $p = 0.683$ , $\beta = -0.47$	WNA: $R^2 = 0.01$ , $p = 0.576$ , $\beta = -0.13$ COR: $R^2 = 0.37$ , $p = 0.114$ , $\beta = -0.71$ UK: $R^2 = 0.12$ , $p = 0.084$ , $\beta = -0.42$ ENA: $R^2 = 0.78$ , $p = 0.07$ , $\beta = 0.92$

**Table 3.** Comparisons between the two non-native ranges (United Kingdom, and eastern North America) and how they aligned with the predictions of evolution of increased competitive ability (EICA). United

Kingdom plants are compared against the native cordilleran subregion and the eastern North America plants are compared against the overall native western North American range.

<b>Non-native Region</b>	<b>Support of EICA Predictions</b>	<b>Neutral to EICA predictions</b>	<b>Contrary to EICA Predictions</b>
United Kingdom	<ul style="list-style-type: none"> <li>• Specialist herbivore escape</li> <li>• Reduced herbivore damage in field plants</li> <li>• Lower total PPGs, Unknown PPG 16, calceolarioside A, and conandroside concentrations</li> <li>• Larger corolla width, more flowers, greater seed production, taller, more root, shoot and biomass.</li> <li>• Evidence of tradeoffs</li> </ul>	<ul style="list-style-type: none"> <li>• Equivalent Traits: <ul style="list-style-type: none"> <li>○ Specific leaf area</li> <li>○ Leaf dry matter content</li> <li>○ Calceolarioside B and mimuloside</li> <li>○ Specialist and generalist herbivore performance</li> <li>○ Days till flower</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Higher average trichome density</li> <li>• Greater leaf water content</li> <li>• Poorer pollen viability</li> </ul>
Eastern North America	<ul style="list-style-type: none"> <li>• Lower average trichome density</li> <li>• Lower verbascoside concentration</li> <li>• Larger corolla width, taller</li> </ul>	<ul style="list-style-type: none"> <li>• Potential escape from specialist herbivores</li> <li>• Equivalent Traits: <ul style="list-style-type: none"> <li>○ herbivore damage in field plants</li> <li>○ specific leaf area</li> <li>○ leaf water content</li> <li>○ leaf dry matter content</li> <li>○ Unknown PPG 10</li> <li>○ Calceolarioside A</li> <li>○ Unknown PPG 16</li> <li>○ Specialist and generalist herbivore performance</li> <li>○ Days till flower</li> <li>○ Lower production</li> <li>○ Seed production</li> <li>○ Root, shoot and total biomass</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Higher concentration of Total PPGs, conandroside, calceolarioside B, and mimuloside</li> <li>• Poorer pollen viability</li> <li>• No evidence of tradeoffs</li> </ul>

**Supplemental Information**

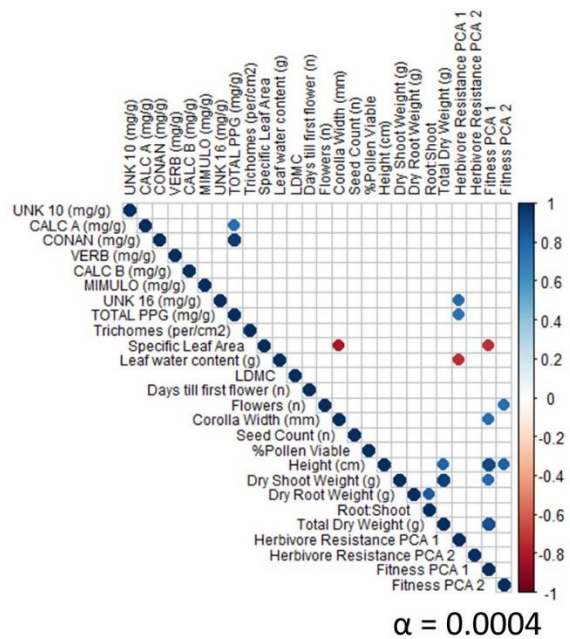
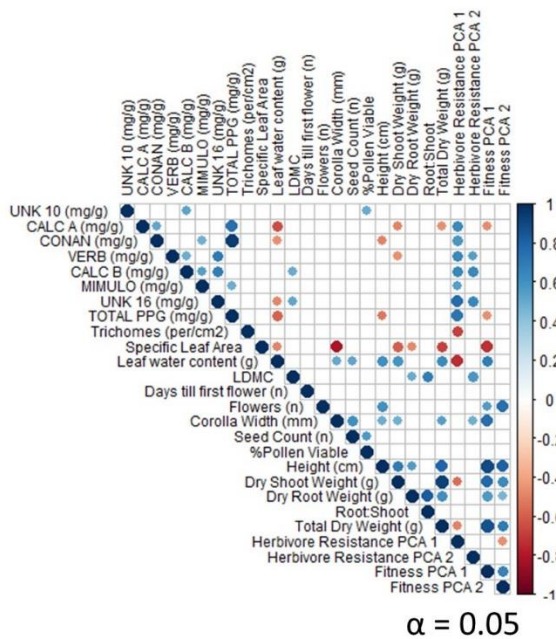
**Table S1.** Locations of all populations used in this study. Name of population is population name in the monkeyflower seed library and the number of individuals used in the common garden for trait measurements. Plant life history, region and subregion based on field observation and literature (Stace 2010, Twyford and Freidman 2015).

<b>Population Name (n)</b>	<b>Annual/ Perennial/ Facultative</b>	<b>Region</b>	<b>Subregion</b>	<b>State/Province/ Nation, Country</b>	<b>Coordinates</b>
Anchor River (13)	Perennial	Western North America	Cordilleran	Alaska, USA	N 59° 44.468', W 151° 44.850'
Bird Point Creek (12)	Perennial	Western North America	Cordilleran	Alaska, USA	N 60° 57.147', W 149° 24.673'
Crooked Creek (9)	Perennial	Western North America	Cordilleran	Alaska, USA	N 61° 08.295', W 146° 19.479'
South Deep Creek (13)	Perennial	Western North America	Cordilleran	Alaska, USA	N 60° 01.744', W 151° 40.988'
Lowell Creek (13)	Perennial	Western North America	Cordilleran	Alaska, USA	N 60° 06.078', W 149° 27.704'
TSG (13)	Perennial	Western North America	Cordilleran	British Columbia, Canada	N 53° 41.888', W 131° 91.573'
Harris Creek (12)	Facultative	Western North America	Northern	Idaho, USA	N 43° 51.966', W 116° 08.882'
Nowhere Ditch (12)	Perennial	Western North America	Northern	Washington, USA	N 46° 77.261', W 117° 57.776'
Cultus River (13)	Facultative	Western North America	Northern	Oregon, USA	N 43° 49.337', W 121° 47.845'
North Fork Quinault River (14)	Facultative	Western North America	Northern	Washington, USA	N 47° 34.201', W 123° 39.033'
K. Moon Seep (13)	Annual	Western North America	Northern	Wyoming, USA	N 41° 20.517', W 110° 54.714'
Thanks Amanda Ditch (12)	Annual	Western North America	Northern	Colorado, USA	N 39° 48.404', W 107° 35.370'
Lone Grave Spring (15)	Annual	Western North America	Northern	South Dakota, USA	N 44° 21.034', W 104° 03.536'
Dispersed Camp Spring (12)	Annual	Western North America	Northern	Utah, USA	N 40° 37.684', W 111° 10.719'

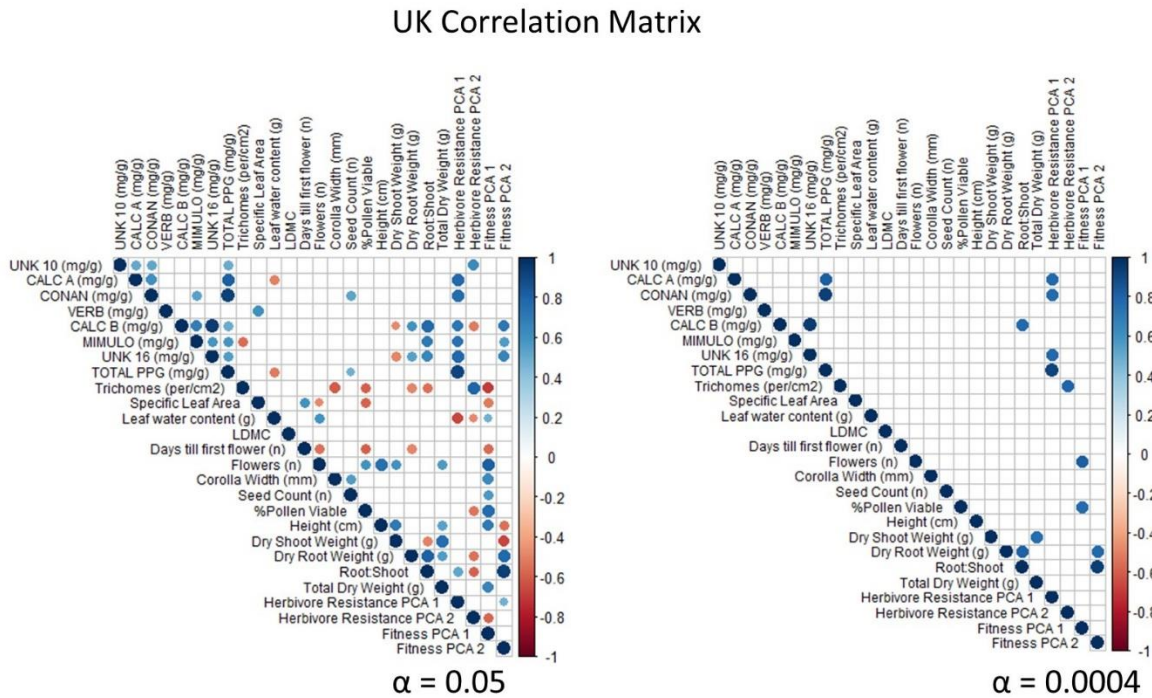
Heceta Head Lighthouse (12)	Perennial	Western North America	Coastal	Oregon, USA	N 44° 08.100', W 124° 07.358'
Population E (12)	Perennial	Western North America	Coastal	California, USA	N 38° 04.875', W 122° 08.696'
Klamath Bog (12)	Perennial	Western North America	Coastal	California, USA	N 41° 39.144', W 124° 04.221'
Bagby Boat Launch (9)	Annual	Western North America	Southern	California, USA	N 37° 36.369', W 120° 08.061'
Kern Canyon (12)	Perennial	Western North America	Southern	California, USA	N 39° 25.380', W 115° 03.845'
Dairy Farm Spring (12)	Perennial	Western North America	Southern	Arizona, USA	N 34° 09.458', W 111° 48.192'
Bass River (12)	Perennial	Eastern North America	Eastern North America	New Brunswick, Canada	N 46° 32.904', W 65° 06.607'
Springfield Ditch (12)	Perennial	Eastern North America	Eastern North America	New Brunswick, Canada	N 46° 41.476', W 65° 49.201'
Ontonagon Spring (13)	Perennial	Eastern North America	Eastern North America	Michigan, USA	Protected Plant Species
Fly Creek (12)	Perennial	Eastern North America	Eastern North America	New York, USA	On Private Property
John Muir Footpath (13)	Perennial	United Kingdom	United Kingdom	Scotland, United Kingdom	N 55° 59.698', W 002° 33.400'
Balfron Mud Flat (13)	Perennial	United Kingdom	United Kingdom	Scotland, United Kingdom	N 56° 03.918', W 004° 23.453'
Durness Stream (12)	Perennial	United Kingdom	United Kingdom	Scotland, United Kingdom	N 58° 34.031', W 004° 44.291'
Loch Broom Hill (12)	Perennial	United Kingdom	United Kingdom	Scotland, United Kingdom	N 57° 49.738', W 005° 03.975'
River Ness (12)	Perennial	United Kingdom	United Kingdom	Scotland, United Kingdom	N 57° 28.816', W 004° 13.999'
Packhorse Bridge (13)	Perennial	United Kingdom	United Kingdom	Scotland, United Kingdom	N 57° 21.159', W 003° 20.246'
Dunblane River (9)	Perennial	United Kingdom	United Kingdom	Scotland, United Kingdom	N 56° 11.199', W 003° 57.872'
Deer Abby Creek (14)	Perennial	United Kingdom	United Kingdom	Scotland, United Kingdom	N 57° 31.394', W 002° 03.482'
West Park Farm (12)	Perennial	United Kingdom	United Kingdom	Scotland, United Kingdom	N 56° 18.097', W 003° 47.038'
River Ayre Seep (13)	Perennial	United Kingdom	United Kingdom	Scotland, United Kingdom	N 55° 27.690', W 004° 37.542'

River Nith Bridge (12)	Perennial	United Kingdom	United Kingdom	Scotland, United Kingdom	N 55° 03.810', W 003° 36.533'
St. Catherine Pasture (12)	Perennial	United Kingdom	United Kingdom	Wales, United Kingdom	N 52° 59.361', W 003° 27.960'
Cerria Condrudion (12)	Perennial	United Kingdom	United Kingdom	Wales, United Kingdom	N 53° 00.345', W 003° 32.949'
Coldstream Bridge (12)	Perennial	United Kingdom	United Kingdom	England, United Kingdom	N 55° 39.288', W 002° 14.363'
Exford Bridge (12)	Perennial	United Kingdom	United Kingdom	England, United Kingdom	N 51° 07.980', W 003° 38.506'
Crowan Field (14)	Perennial	United Kingdom	United Kingdom	England, United Kingdom	N 50° 09.795', W 005° 17.578'
Houghton Lodge Stream (12)	Perennial	United Kingdom	United Kingdom	England, United Kingdom	N 51° 04.806', W 001° 31.009'
Brampton Stream and Field (12)	Perennial	United Kingdom	United Kingdom	England, United Kingdom	N 52° 46.087', E 001° 17.870'

### WNA Correlation Matrix

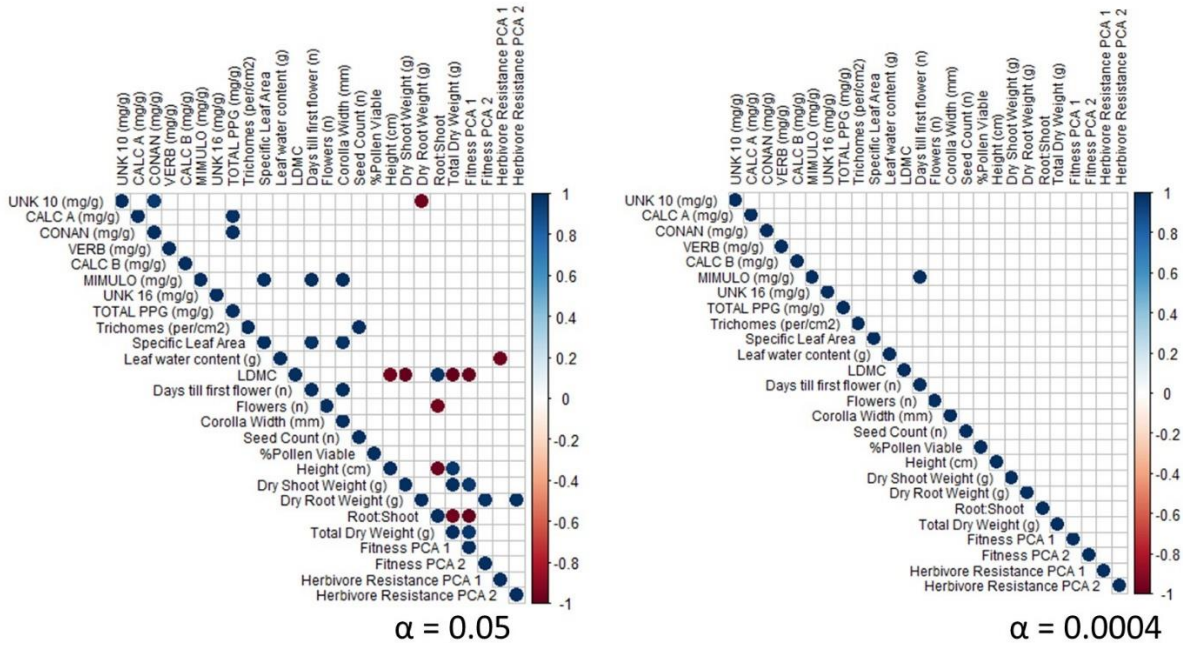


**Figure S1.** Correlation matrix for population means of all continuous pairwise traits measured for native western North America populations (WNA). Blue indicates a positive  $r$  value and red being a negative  $r$  value. Only significant  $r$  values are displayed. Left figure is with  $\alpha$  set at 0.05 and right figure is adjusted  $\alpha$  of 0.0004 for multiple tests.



**Figure S2.** Correlation matrix for population means of all continuous pairwise traits measured for United Kingdom populations (UK). Blue indicates a positive  $r$  value and red being a negative  $r$  value. Only significant  $r$  values are displayed. Left figure is with  $\alpha$  set at 0.05 and right figure is adjusted  $\alpha$  of 0.0004 for multiple tests.

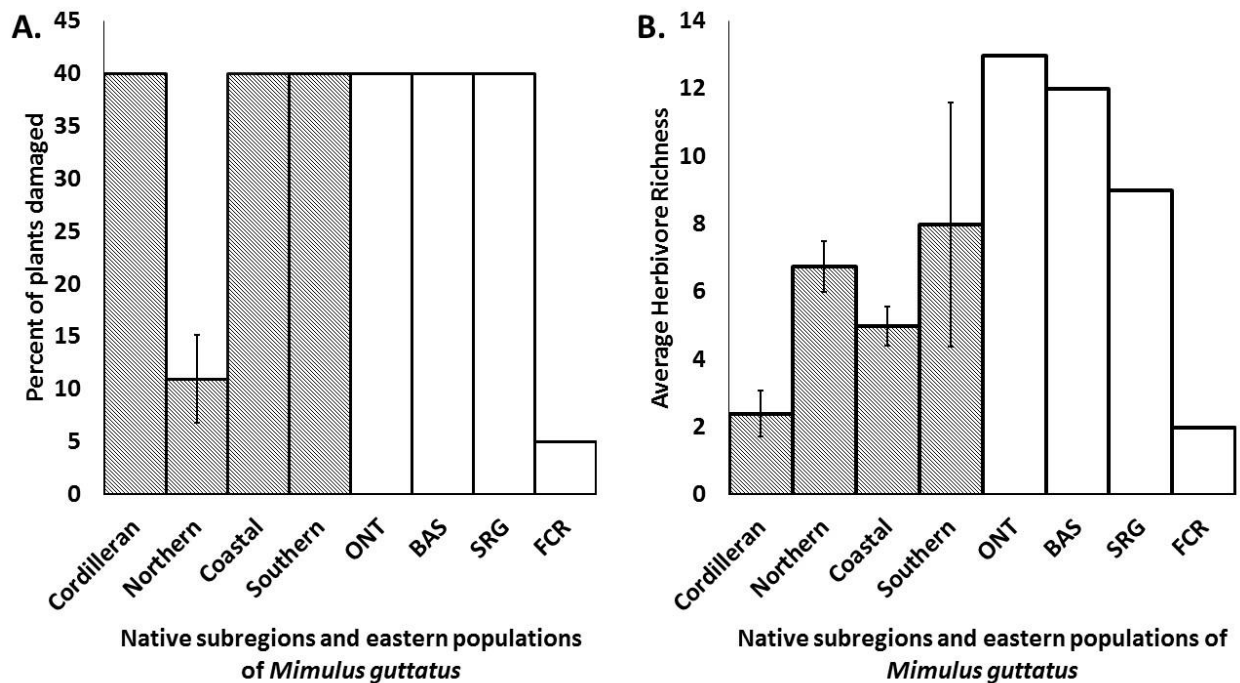
### ENA Correlation Matrix



**Figure S3.** Correlation matrix for population means of all continuous pairwise traits measured for eastern North America populations (ENA). Blue indicates a positive  $r$  value and red being a negative  $r$  value. Only significant  $r$  values are displayed. Left figure is with  $\alpha$  set at 0.05 and right figure is adjusted  $\alpha$  of 0.0004 for multiple tests.

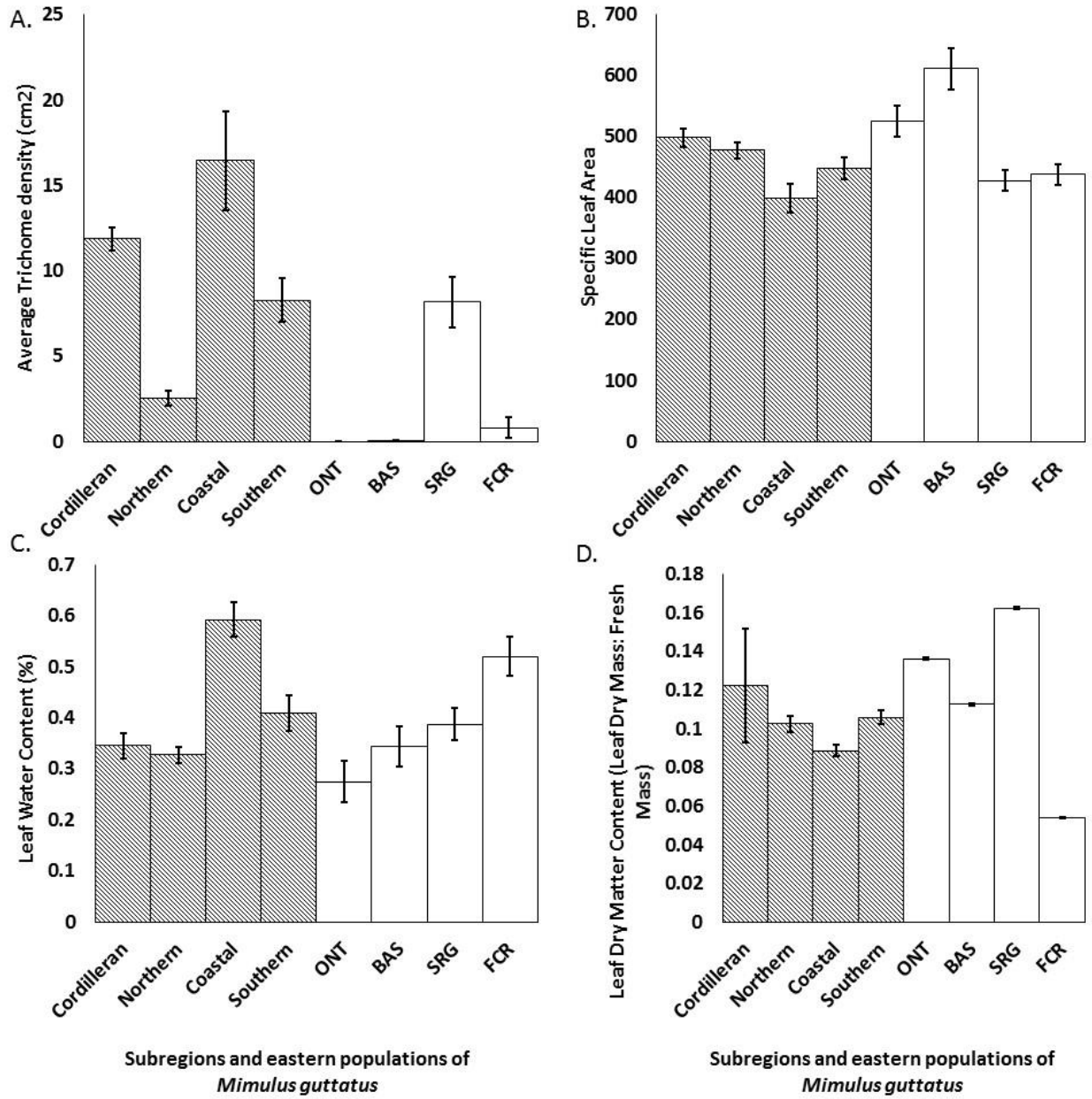
## Eastern North America Supplemental

There is a lack of information on the origins in the eastern North American populations. These populations likely represent multiple introductions of varying different invasion routes with unknown origins. The following figures are presented with comparisons of averages from the individual eastern North American populations to native subregions. See Table S1 for population names and locations.

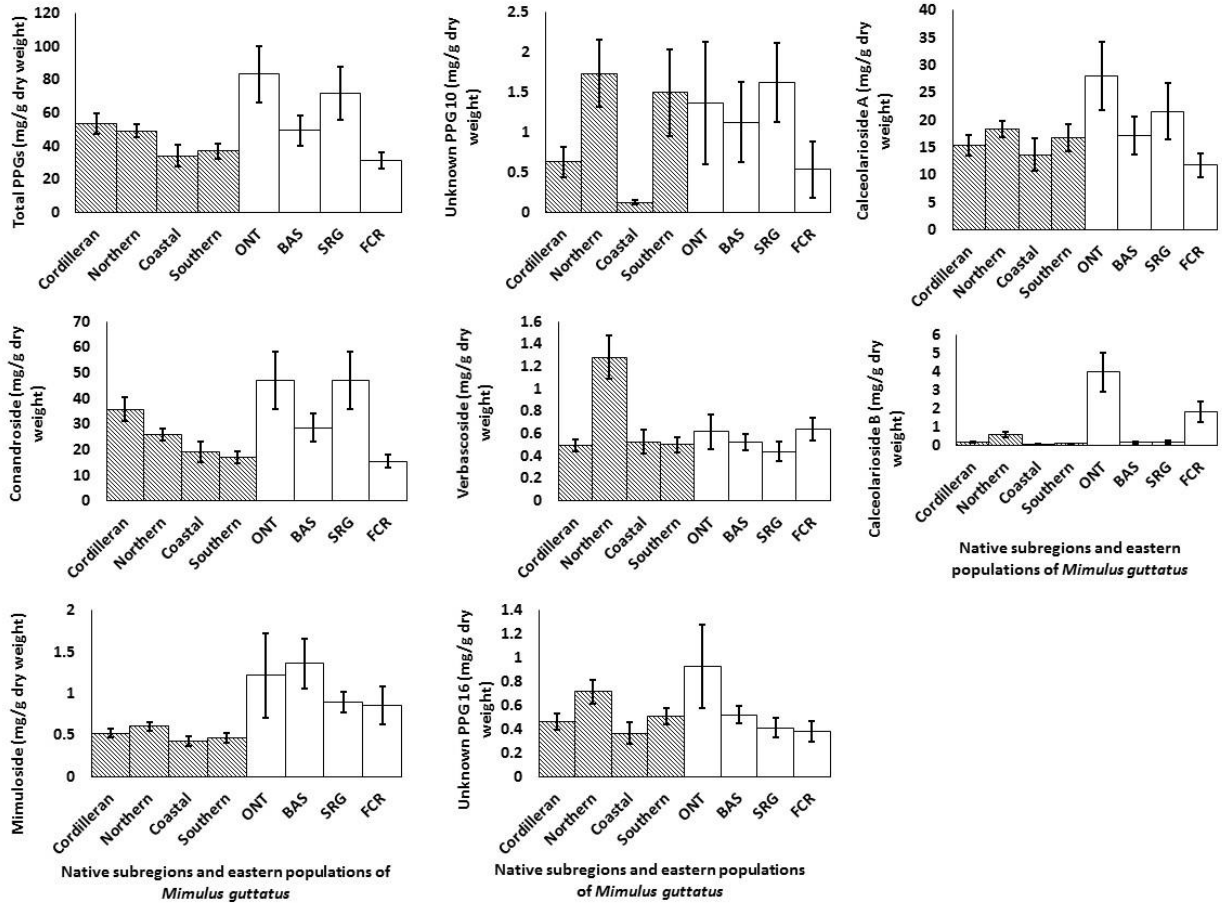


**ENA S1.** A. Percent of plants with herbivore damage in wild growing *Mimulus guttatus* populations between eastern North American populations (in white) and native subregions (patterned). B. Average herbivore species richness found in the field feeding on *Mimulus guttatus* populations between eastern North American populations (in white) and native subregions (patterned). Error bars represent  $\pm 1$  standard error. Non-transformed data displayed.

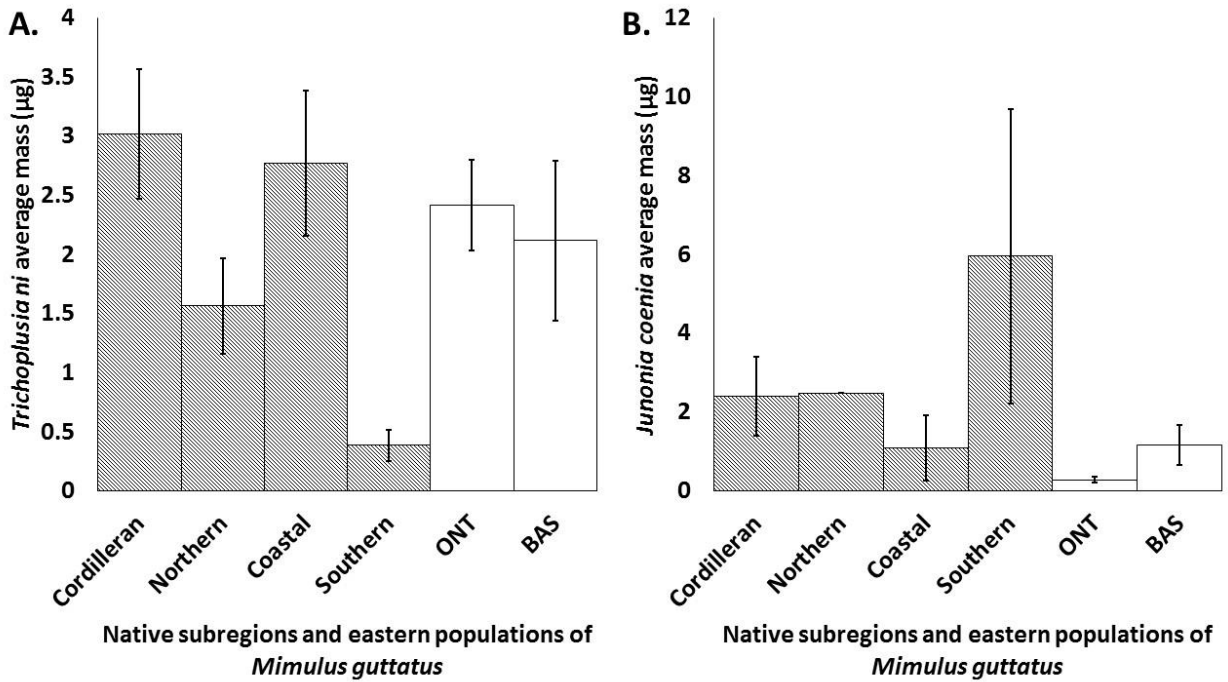




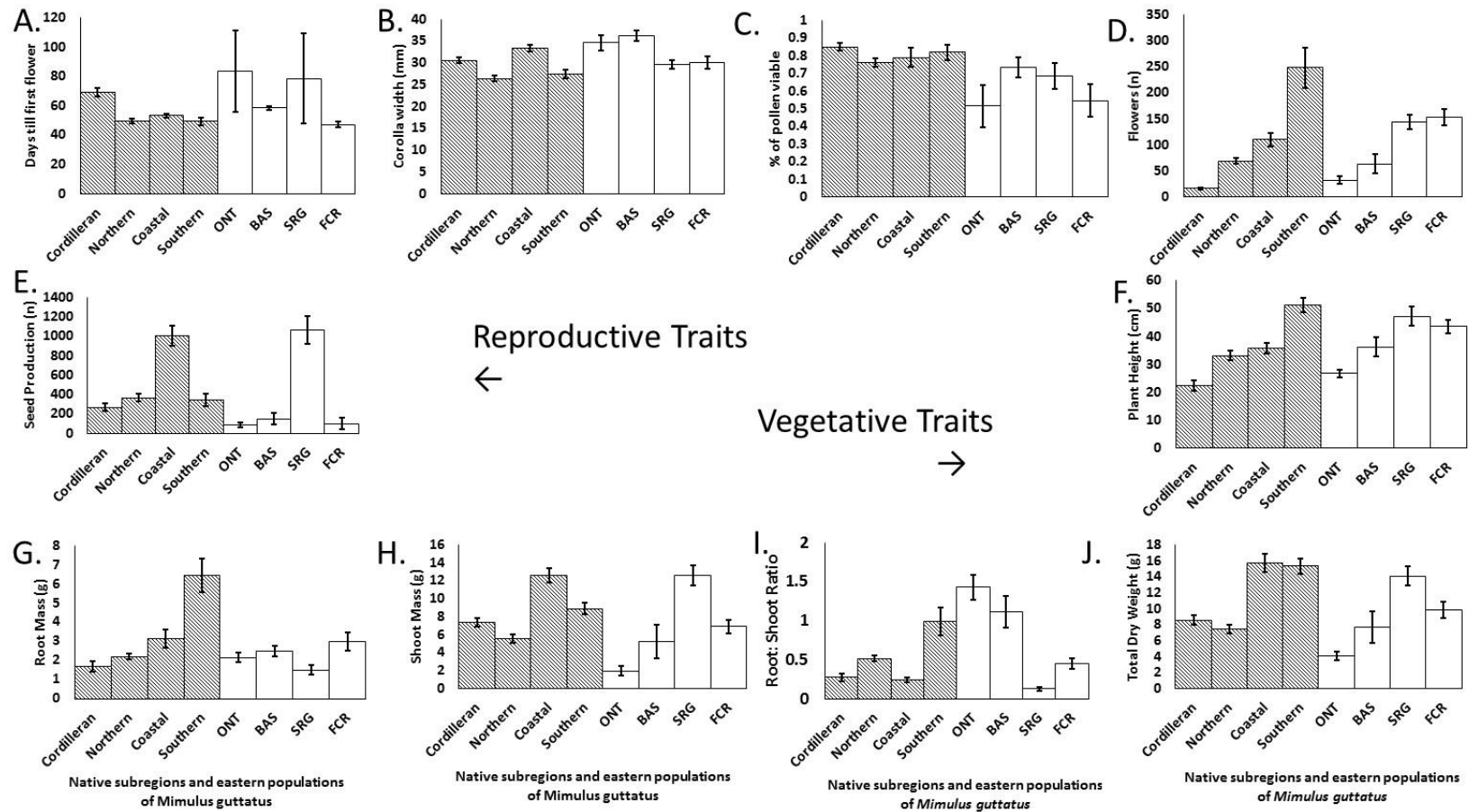
**ENA S2.** Average physical resistance traits (A. Trichomes, B. Specific Leaf Area, C. Water Content, D. Dry Leaf Matter) in *Mimulus guttatus* populations between eastern North American populations (in white) and native subregions (patterned). Error bars represent  $\pm 1$  standard error. Non-transformed data displayed.



**ENA S3.** Average concentration (mg/ dry weight) of foliar phenylpropanoid glycosides within eastern North American populations (white) and subregions (patterned) of *M. guttatus* populations. Error bars represent one standard error. Error bars represent  $\pm 1$  standard error. Non-transformed data displayed.



**ENA S4.** Average performance (mass µg) of (A.) the generalist caterpillar *Trichoplusia ni* and (B.) the specialist caterpillar *Junonia coenia* within eastern North American populations (white) and subregions (patterned) of *M. guttatus* populations. Error bars represent  $\pm 1$  standard error. Non-transformed data displayed



**ENA S5.** Averages of measures of fitness / competitive ability traits. Reproductive traits: A. Number of days till first flower, B. Width of first corolla, C. Percent of pollen that is viable, D. Total number of flowers produced, E. Number of seeds from first three flowers. Vegetative traits: F. Plant height, G. Root dry mass, H. Shoot dry mass, I. Root:shoot ratio, J. Total dry biomass for *Mimulus guttatus* populations between eastern North American populations (in white) and native subregions (patterned). Error bars represent  $\pm 1$  standard error. Non-transformed data displayed.

