

1 Running header: Herbivores limit a native plant distribution

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3 Herbivores and edaphic factors constrain the realized niche of a native plant

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23 Abstract

24 Biotic interactions, such as competition and herbivory, can limit plant species ranges to a
25 subset of edaphically suitable habitats, termed the realized niche. Here we explore the role that
26 herbivores play in restricting the niche of the native California annual *Collinsia sparsiflora*. We
27 transplanted seeds from 4 populations, representing 2 distinct ecotypes, into a range of
28 environments both within and outside the observed distribution of *C. sparsiflora* and used path
29 analysis to investigate the direct and herbivore-mediated indirect effects of environmental
30 variables on *C. sparsiflora* survival. We find that *C. sparsiflora* receives more herbivory outside
31 its typical distribution and that increased herbivory is associated with decreased survival,
32 suggesting that herbivores may limit the distribution of *C. sparsiflora*. Additionally, we show
33 that edaphic environmental variables impact *C. sparsiflora* survival both directly and indirectly,
34 by altering interactions with herbivores. While the magnitude of direct effects exceeded the
35 magnitude of indirect effects, many strong herbivore-mediated indirect effects were detected,
36 and several habitat attributes (e.g. slope and aspect) only impacted survival through indirect
37 pathways. Our results also highlight the importance of an evolutionary perspective of the niche,
38 since we detected substantial differences between ecotypes. Both direct and herbivore-mediated
39 indirect effects explained variation in the serpentine ecotype's survival; however, only direct
40 effects contributed to variation in the survival of the non-serpentine ecotype. Thus, we show that
41 herbivores limit the distribution of a common native plant and contribute to differences in niche
42 use between ecotypes.

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44 Keywords: ecological niche modeling; ecotypes; edaphic variation; indirect effect; herbivory;
45 path analysis; plant-insect interaction; realized niche, serpentine soils.

46

47 Introduction

48 Plant populations are patchily distributed across the landscape. No species is equally
49 abundant across the whole spectrum of environmental conditions (Walter 1964), and what
50 factors limit species distributions is a central question of ecology. The niche concept is a useful
51 framework for considering both the local distribution and ecological tolerance of a species. The
52 fundamental niche describes the range of environments an organism can inhabit based on
53 physiological tolerances (Hutchinson 1959, Holt 2003). Biotic interactions with other species
54 may further alter the actual area occupied, termed the realized niche (Hutchinson 1959). Species
55 interactions often vary across environmental gradients (e.g., Bertness and Ellison 1987, Louda
56 and Rodman 1996). As a result, interspecific interactions that decrease species abundances or
57 population growth rates such as competition (Bertness and Ellison 1987, Davis et al. 1998,
58 Choler et al. 2001), predation (Davis et al. 1998), and herbivory (Parker and Root 1981, Smith
59 1987, Louda and Rodman 1996, Harley 2003, DeWalt et al. 2004) can restrict the observed
60 distributions of focal species to a subset of available habitats. Similarly, the presence of
61 mutualists can expand species distributions to otherwise unsuitable habitats (i.e. facilitation,
62 Choler et al. 2001).

63 While herbivory often decreases individual plant fitness and can influence the persistence
64 and growth of plant populations (Parker and Root 1981, Smith 1987, Louda and Rodman 1996,
65 Harley 2003, DeWalt et al. 2004), the general role of insect herbivory in limiting plant
66 population growth rates and restricting plant distributions is debatable (Harper 1969, Crawley
67 1989a, b, Louda 1989). In several systems, herbivores limit the abundance (Smith 1987, Rand
68 2002) or population growth rates of their host plants (Ehrlén 1995, Louda and Potvin 1995,

69 Fagan and Bishop 2000, Bishop 2002). Several other studies provide evidence that the
70 combination of spatial variation in herbivory and strong demographic effects of herbivores
71 restrict plant distributions to a subset of available habitats (Parker and Root 1981, Louda and
72 Rodman 1996, Harley 2003, DeWalt et al. 2004). However, other studies have failed to detect
73 herbivore impacts on abundance or population growth even when plants experience extremely
74 high levels of damage (Parker 2000, Pacheco 2001). Furthermore, most studies demonstrating
75 strong impacts of herbivores on plant distributions and population dynamics come from the
76 biological control literature (reviewed in Crawley 1989a), the effects of herbivores on plants
77 outside their typical distribution (e.g., DeWalt et al. 2004), and other perturbed environments
78 (e.g., Bishop 2002, Fagan et al. 2004). Few studies have convincingly documented strong
79 demographic impacts of herbivores on undisturbed native plant populations (but see Louda and
80 Potvin 1995, Louda and Rodman 1996). In part, this may be due to the “ghost of herbivory”
81 past: plant populations under intense herbivory have already been excluded and/or have evolved
82 anti-herbivore defenses to minimize strong herbivore impacts (Harper 1969, Connell 1980,
83 DeWalt et al. 2004). Thus, the strongest herbivore effects on population dynamics can often
84 only be detected when the plant population has been perturbed from its natural state.
85 Accordingly, transplant experiments into patches that are unoccupied but meet the physiological
86 needs of the plant species may be valuable for investigating the effects of herbivory on plant
87 population dynamics and plant distributions (Crawley 1990).

88 In a previous study, we constructed a niche model describing the distribution of the
89 native California annual *Collinsia sparsiflora* and then experimentally tested the niche model by
90 transplanting *C. sparsiflora* seeds into plots where environmental variables associated with the
91 *C. sparsiflora* distribution had been described (e.g., *C. sparsiflora* presence/absence,

92 calcium:magnesium ratio, phosphorus, slope, aspect, and biomass, see Wright et al., in press).
93 We found that the niche model based on descriptive data successfully predicted *C. sparsiflora*
94 survival, but only for one of two ecotypes tested. Here, we expand upon that work to investigate
95 potential underlying mechanisms responsible for the patterns documented by the niche model. In
96 particular, we investigate how habitat attributes and herbivory interact to influence *C. sparsiflora*
97 survival. We employ path analyses to link variation in habitat characters with variation in
98 herbivory and variation in survival to determine how habitat characteristics both directly and
99 indirectly (by influencing herbivory) impact *C. sparsiflora* population dynamics. Finally,
100 because seeds from 4 source populations representing 2 distinct ecotypes were used in the
101 previous transplant experiment, we can contrast path models for each ecotype to investigate
102 whether similar mechanisms (i.e. direct and indirect effects) influence plant survival across
103 substantial intraspecific variation. We address three questions: (1) Does herbivory differ among
104 transplants within versus outside the observed natural distribution of *C. sparsiflora*? (2) Do
105 habitat variables known to predict *C. sparsiflora* occurrence affect survival directly or indirectly
106 via herbivory? (3) Do the relative roles of direct and herbivore-mediated indirect effects differ
107 between *C. sparsiflora* ecotypes?

108

109 Materials and Methods

110 *Study system*

111 The native, annual plant *Collinsia sparsiflora* Fischer and C. Meyer (Scrophulariaceae
112 *s.l.*) is patchily distributed across the McLaughlin University of California Natural Reserve in the
113 California Coast Range (<http://nrs.ucdavis.edu/mclaughlin.html>). It occurs on both serpentine
114 and non-serpentine soils in grasslands and open oak woodlands. Serpentine soils are

115 characterized by high concentrations of heavy metals and are stressful environments for many
116 plant species (Brady et al. 2005). Previous work has shown that genotypes growing in serpentine
117 versus non-serpentine environments have differentiated into distinct ecotypes (Wright et al.
118 2006). We found that various generalist herbivores attack *C. sparsiflora*, including lepidopteran
119 larvae and flea beetles (Coleoptera). Flea beetles imposed the vast majority of damage observed
120 in this study.

121

122 *Mapping the natural distribution of C. sparsiflora and using environmental data to create the*
123 *realized niche model*

124 In 2001, a 600 x 550 m grid (hereafter referred to as “the grid”) was established across
125 both serpentine and non-serpentine grasslands at the McLaughlin Reserve, with gridpoints every
126 50 m. Within the grid, 6 smaller grids (100 x 50 m, with gridpoints every 10 m), were established
127 to more intensively sample highly variable locations. At each gridpoint (n=528), data were
128 collected on soil chemical composition and texture (22 variables, see Wright et al. 2006 for a full
129 description of these variables), soil depth, slope, aspect, biomass, and *C. sparsiflora* occurrence
130 (sampled in the late spring) (see Wright et al., in press for details on environmental attribute
131 measures). The environmental variables were then used to construct a niche model describing
132 the observed distribution of *C. sparsiflora* (see Wright et al., in press). The final niche model
133 included 5 environmental variables: slope, aspect, Ca:Mg ratio, organic matter, and phosphorus,
134 and a previous study, using the same transplant experiment described here, has shown that the
135 niche model successfully predicted *C. sparsiflora* survival (Wright et al., in press).

136

137 *Experimental design of transplant experiment*

138 We planted *C. sparsiflora* seeds into 100 plots across the grid on the McLaughlin reserve.
139 The 100 plots were chosen from among the 528 gridpoints to maximize variation in the 5
140 variables included in the niche model described above. At each plot, we planted 40 seeds in
141 August 2003; 2 seeds were planted at each cell in the plot in a 5 x 4 array with 5cm between
142 cells. Seedlings were thinned to one individual per cell. Thus, there were a maximum of 20
143 plants in each plot, with 5 plants from each of 4 source populations: NS1, NS2, S1, and S3, see
144 Wright et al. (2006) for a full description of these source populations. Source populations were
145 randomly assigned to locations within each plot. Because of differences in germination, the
146 number of plants per plot ranged from 0 to 19 (mean = 11.52 plants per plot). The planted seeds
147 were the selfed progeny of field-collected seeds grown for one generation in a common
148 greenhouse environment in order to reduce maternal effects resulting from differences in the
149 source population habitat. Plants were scored for germination (weekly observations from
150 December 14, 2003 to January 19, 2004) and survival to flowering (censused weekly from
151 March 4 to April 21, 2004). Herbivory was measured qualitatively (damaged or undamaged) and
152 quantitatively (visual estimates of the proportion of leaf material removed) weekly between
153 December 21 and January 20, 2004. By January 20, a substantial amount of herbivory had been
154 received but little mortality had occurred, thus maximizing sample sizes. At the end of the
155 experiment all experimental plants were harvested and then all biomass (excluding the
156 experimental plants) was dried and weighed to determine plot biomass.

157

158 *Data analysis*

159 *Herbivory across the C. sparsiflora distribution*—We used a simple one-way ANOVA to
160 test whether transplants outside the natural distribution receive more herbivory than transplants

161 within the natural distribution of *C. sparsiflora*. The proportion of plants at each plot that
162 received herbivory was our response variable, and the presence or absence of naturally-occurring
163 *C. sparsiflora* was included as a predictor variable. We also performed similar analyses using
164 mean proportion of leaf area damaged (arcsine-transformed) as our response variable. To more
165 thoroughly investigate this pattern, we also regressed predicted survival (from the niche model
166 described in Wright et al., in press) on herbivory.

167 *Fitness effects of herbivory across the C. sparsiflora distribution*--To determine if the
168 fitness effects of herbivory (i.e., tolerance) varies across the distribution of *C. sparsiflora*, we
169 performed a logistic regression with survival to flowering as a categorical response variable, and
170 *C. sparsiflora* natural presence/absence, herbivory (proportion of leaf area damaged), and the
171 interaction as predictor variables. If herbivory interacts with environmental variables to limit the
172 distribution of *C. sparsiflora*, we expect to detect an interaction between *C. sparsiflora* presence
173 and herbivory, where herbivory decreases fitness more where *C. sparsiflora* is naturally absent.

174 *Direct and herbivore-mediated indirect effects of edaphic variables on C. sparsiflora*
175 *survival*--We used path analysis to examine the causal effects of the edaphic variables found to
176 be important in the initial niche model and herbivory on *C. sparsiflora* survival. These path
177 analyses included the 5 variables in the initial niche model (slope, aspect, Ca:Mg ratio, organic
178 matter, and phosphorus: see Wright et al., in press) as exogenous predictor variables.
179 Aboveground biomass of all surrounding competitors was also included as an exogenous
180 variable because of the strong impacts vegetation heterogeneity has on herbivory in other
181 systems (Karban 1997). Herbivory (proportion of plants damaged per plot) and survival
182 (proportion of seeds surviving to flower) were included as endogenous response variables. Each

183 plot was 1 replicate (n=100); however, at 4 plots, all plants died before herbivory was censused,
184 yielding a final sample size of n=96.

185 We initially tested the full path model which included both direct and herbivore-mediated
186 indirect effects of all exogenous variables on survival using maximum likelihood techniques as
187 implemented by PROC CALIS (SAS Institute, 2001). Because early analyses revealed that
188 phosphorus had minimal effects on herbivory and survival (regression of phosphorus on
189 herbivory $t_{1,98} = -1.41$, $P = 0.16$; survival $t_{1,98} = -1.54$, $P = 0.13$), it was dropped from the model
190 to ensure that the path model was not overidentified. We performed all analyses on the variance-
191 covariance matrix, and all predictor variables were standardized by their variance, so that each
192 variable had a mean of 0 and a variance of 1.

193 Path analysis assumes that the data are normally distributed, that the correlations between
194 predictor variables are not excessively high (low multicollinearity), and that the relationships
195 among variables are linear (Hatcher 1994). Visual inspection of residual plots revealed that all
196 predictor variables used in the analyses were normally distributed. We tested for collinearity
197 among predictor variables by examining variation inflation factors (VIFs) for each independent
198 variable, using the VIF option in PROC REG (SAS Institute 2001). All VIFs were $\ll 10$,
199 indicating that collinearity was negligible. Furthermore, all correlations between predictor
200 variables were < 0.60 . We examined the data for nonlinearities by including quadratic terms in
201 simple regressions between each predictor variable and each response variable. While we
202 detected significant quadratic terms in 3 of 11 cases (in regressions of survival on aspect,
203 survival on Ca:Mg, and herbivory on slope), the quadratic terms were small relative to the linear
204 coefficients, suggesting that the relationship was linear over most of the distribution.
205 Furthermore, in the most extreme case (survival on aspect), linearity was obtained when aspect

206 was log-transformed. Model fits were not qualitatively different when transformed versus non-
207 transformed data were used; therefore, we only present the output from the non-transformed
208 data.

209 The results of the CALIS procedure can be evaluated with several goodness-of-fit
210 indices. These include X^2 statistics that test the null hypothesis that the reproduced covariance
211 matrix has the model structure (i.e., tests that the path diagram fits the data), as well as, the
212 normed fit index (NFI, Bentler and Bonett 1980), the non-normed fit index (NNFI), and the
213 comparative fit index (CFI, Bentler 1989). NFI, NNFI, and CFI values that exceed 0.9 indicate
214 an acceptable fit between the proposed model and the data (Hatcher 1994). The initial analysis
215 revealed that the full model fit the data well (Table 1); however, examination of the path
216 coefficients and the normalized residual matrix of the full model revealed that model fit could be
217 improved by deleting several paths. Thus, we deleted any paths where the path coefficients were
218 not significantly different than zero ($P > 0.1$) and where deletion would not influence the chi-
219 square statistic (Wald test obtained from PROC CALIS output). We also examined the
220 normalized residuals to delete (or add) any paths with large residuals. To determine whether the
221 deletion of these paths significantly reduced model fit, we performed a X^2 difference test by
222 comparing the X^2 goodness-of-fit indices for the revised model versus the full model. If
223 removing paths does not significantly affect model fit, the reduced model is preferred because it
224 contains fewer paths and is more parsimonious (Hatcher 1994). We calculated the relative
225 magnitude of direct versus herbivore-mediated indirect effects of the edaphic soil variables on *C.*
226 *sparsiflora* survival by using the path coefficients to calculate the relative strengths of all
227 potential pathways (Mitchell 1992).

228 *Ecotypic differentiation in the mechanisms underlying the effects of edaphic variables on*
229 *survival*-- To determine whether the relative roles of direct versus herbivore-mediated indirect
230 effects vary between ecotypes, we created and modified 2 path diagrams, one for each ecotype
231 (data from the 2 populations per ecotype were pooled). We then fit the data from each ecotype
232 to each path diagram. Thus we could tell if the serpentine data fit the non-serpentine model and
233 vice-versa. If the X^2 statistics indicate lack of fit, this suggests that different path models are
234 appropriate for the 2 distinct ecotypes. As above, we also calculated the magnitude of direct and
235 herbivore-mediated indirect effects of soil variables on survival for each ecotype.

236 Because plant populations often differ in tolerance to herbivory (i.e., the ability to sustain
237 herbivore damage with minimal reductions in fitness) and because tolerance will influence the
238 magnitude of herbivore-mediated indirect effects, we used logistic regression to calculate
239 tolerance estimates for each source population (2 source populations of each of 2 ecotypes).
240 Survival was included as a dichotomous response variable; herbivory (proportion of leaf area
241 removed) was included as a continuous predictor variable, and source population was included as
242 a fixed factor.

243

244 Results

245 *Does herbivory differ among transplants within versus outside the observed natural distribution*
246 *of C. sparsiflora?*

247 We predicted that if herbivores limit the local distribution of *C. sparsiflora*, individuals
248 transplanted outside the natural distribution would receive more herbivory than individuals
249 transplanted within the natural distribution of *C. sparsiflora*. As predicted, a greater proportion
250 of *C. sparsiflora* plants in plots outside the natural distribution received herbivore damage

251 compared to transplants within the local distribution ($F_{1,93} = 6.77, P = 0.01$, Fig. 1a). The
252 percentage of leaf area removed also tended to be higher outside the natural distribution
253 (Mean \pm SE proportion of leaf material damaged: outside distribution 0.46 ± 0.04 ; within
254 distribution 0.36 ± 0.06), though this difference was not significant ($F_{1,93} = 2.12, P = 0.15$).
255 Furthermore, predicted *C. sparsiflora* survival (based on the niche model described in Wright et
256 al., in press) was negatively correlated with the proportion of transplants that experienced
257 herbivory ($\rho = -0.35, P = 0.0005$, Fig. 1b). This relationship occurred in spite of the fact that
258 herbivory was not included in the construction of the initial niche model and suggests that many
259 of the edaphic variables included in the niche model may have strong indirect effects by altering
260 herbivory. Herbivory was negatively associated with *C. sparsiflora* survival ($X^2 = 51.49, P <$
261 0.0001), and these negative effects tended to be greater at plots outside the natural distribution
262 than at plots within the distribution (i.e., logistic ANCOVAs indicate a marginally-significant
263 interaction between *C. sparsiflora* presence and herbivory on survival, $X^2 = 3.69, P = 0.055$).
264 These results indicate that herbivory is both more intense and has greater impacts on fitness
265 outside the natural distribution, suggesting that herbivory may be an important biotic factor
266 limiting the realized niche of *C. sparsiflora*.

267

268 *Do habitat variables known to predict C. sparsiflora occurrence affect survival directly or*
269 *indirectly via herbivory?*

270 Path analysis revealed that habitat variables influenced the survival of experimental *C.*
271 *sparsiflora* plants directly, as well as indirectly, via effects on herbivory (Fig. 2, Table 2). The
272 final model, which included a subset of direct and indirect effects of the habitat variables,
273 explained 49% of the variation in *C. sparsiflora* survival and 24% of variation in herbivory. As

274 expected, herbivory decreased the proportion of *C. sparsiflora* seeds that survived to flower
275 (standardized path coefficient = -0.22) and resulted in several indirect effects on survival.
276 Including these herbivore-mediated indirect effects in the path model predicting *C. sparsiflora*
277 survival dramatically improved model fit ($X^2 = 31.39$, $df = 5$, $P < 0.0001$).

278 Slope and aspect influenced *C. sparsiflora* survival indirectly by altering herbivory. For
279 example, *C. sparsiflora* growing in plots with steeper slopes experienced greater herbivory,
280 resulting in decreased survival. In contrast, Ca:Mg influenced *C. sparsiflora* survival directly,
281 but had no significant effect on herbivory. Biomass and organic matter influenced *C. sparsiflora*
282 survival through both direct and indirect pathways, but, in some cases indirect and direct effects
283 were in opposing directions. For example, organic matter was negatively associated with
284 herbivory, resulting in a positive indirect effect. This opposed the negative direct effect of
285 organic matter on *C. sparsiflora* survival. In general, the direct effects of the habitat variables on
286 *C. sparsiflora* survival were much greater than the herbivore-mediated indirect effects (Table 2).

287

288 *Do the relative roles of direct and herbivore-mediated indirect effects differ between C.*
289 *sparsiflora ecotypes?*

290 The niche model tested in Wright et al. (in press) described the niche of serpentine
291 ecotypes of *C. sparsiflora*, but did not accurately predict survival of non-serpentine ecotypes.
292 The lack of fit to the non-serpentine data likely resulted because the environmental variables
293 included in the model had very different direct effects on the survival of non-serpentine
294 ecotypes. However, the relationships between herbivory and survival or between the
295 environmental variables and herbivory may have also differed for this ecotype. To investigate
296 this possibility, we fit separate path models for each ecotype (Fig. 3A, B, Table 3). The non-

297 serpentine path model explained less variation in both herbivory and survival than the serpentine
 298 path model (non-serpentine ecotype $R^2_{\text{survival}} = 0.13$, $R^2_{\text{herbivory}} = 0.19$; serpentine ecotype R^2_{survival}
 299 $= 0.50$, $R^2_{\text{herbivory}} = 0.24$). All path coefficients, with the exception of biomass, were much
 300 weaker in the non-serpentine model (Table 3). As a result, the net direct and net indirect effects
 301 were weaker in the non-serpentine model (non-serpentine net direct effects = 0.60, net indirect
 302 effects = 0.04; serpentine direct effects = 1.02, indirect effects = 0.22). In fact, the path
 303 coefficient between herbivory and survival was small in magnitude and not significant for the
 304 non-serpentine ecotype ($t = -0.68$, $P = 0.25$), thus herbivore-mediated indirect effects were
 305 minimal or non-existent for the non-serpentine ecotype.

306 The lack of an herbivore effect on survival potentially occurred because the non-
 307 serpentine populations tended to be more tolerant of herbivory than the serpentine populations
 308 (i.e., herbivore damage reduced fitness of the non-serpentine genotypes less than the serpentine
 309 genotypes). Logistic regression revealed a significant interaction between source population and
 310 herbivory on survival probability ($X^2 = 11.05$, $df = 3$, $P = 0.01$), and analysis of each source
 311 population independently indicated that herbivory tended to reduce the survival probability of the
 312 2 serpentine populations (S1 and S3) more than the 2 non-serpentine populations (NS1 and NS2)
 313 (logistic regression coefficients: S1: -0.035 , $X^2 = 54.86$, $P < 0.0001$; S3: -0.020 , $X^2 = 16.94$, $P <$
 314 0.0001 ; NS1: -0.009 , $X^2 = 2.13$, $P = 0.14$; NS2: -0.019 , $X^2 = 11.58$, $P = 0.0007$). Direct effects
 315 were also weak for non-serpentine ecotypes, with only biomass significantly reducing survival.

316 The optimal path model for the serpentine populations was very different from the non-
 317 serpentine populations. While the direction of effects remained consistent between the 2
 318 ecotypes, both direct and indirect effects were much stronger for the serpentine ecotype.
 319 Calcium:magnesium ratio and aspect both directly impacted survival of the serpentine ecotypes

320 (higher Ca:Mg decreased survival, and northerly aspects increased survival). Slope decreased
321 survival indirectly, because transplants growing on steeper slopes experienced greater rates of
322 herbivory. Biomass and organic matter influenced survival through both direct and indirect
323 pathways. For biomass, the negative indirect effect exacerbated the negative relationship
324 between biomass and survival; while for organic matter, the indirect effect was in the opposite
325 direction of the direct effect (organic matter had a negative direct effect on survival, but a
326 positive indirect effect because plots with more organic matter received less herbivory). In
327 contrast, only the direct effect of biomass significantly impacted the survival of the non-
328 serpentine ecotype.

329

330 Discussion

331 While the role of herbivory in limiting plant distributions and restricting niches has been
332 debated, several studies have convincingly demonstrated that herbivores restrict plant
333 distributions to a narrower region than that allowed by their physiological tolerances (Parker and
334 Root 1981, Louda and Rodman 1996, Harley 2003, DeWalt et al. 2004). For this to occur, the
335 effects of herbivory must be spatially variable (either because herbivory intensities vary or
336 because the fitness effects of herbivory vary), and herbivory must impact plant population
337 growth rates (Louda and Rodman 1996). Spatial variation in edaphic factors is one mechanism
338 that could produce the necessary variation in herbivory. A prior study documented that fine
339 scale variation in edaphic variables produced the observed patchy distribution of the native
340 annual plant *Collinsia sparsiflora* (Wright et al., in press). These habitat variables influence
341 plant population persistence directly, but can also alter herbivory, and therefore, may influence
342 *C. sparsiflora* survival through indirect pathways. Here we employed path analysis to

343 investigate the mechanisms underlying the model described in Wright et al. (in press) and to
344 disentangle the direct effects of habitat attributes from indirect effects that occur when habitat
345 properties alter interactions with other species. We find that many habitat attributes affect *C.*
346 *sparsiflora* survival indirectly, because the abiotic environment influences herbivory.

347 We used transplant experiments, combined with path analysis to investigate the
348 mechanisms underlying the impact of a *multitude* of habitat variables known to restrict the
349 distribution of *C. sparsiflora*. While experimental manipulations of environmental variables
350 (such as employed by Louda and Rodman 1996) are particularly convincing, this manipulative
351 approach becomes unfeasible when several environmental variables are known to influence plant
352 distributions, i.e, the “n-dimensional niche” (Hutchinson 1959, Pulliam 2000). The path analysis
353 approach was especially useful in this study because it allowed us to link variation in several
354 continuous habitat characteristics with variation in herbivory rates and in turn, to explore how
355 these factors both directly and indirectly impact *C. sparsiflora* survival. While path analysis was
356 extremely useful for obtaining a mechanistic understanding of how multiple abiotic
357 environmental characters interact with biotic selective agents to determine the realized niche,
358 additional experiments are needed to determine whether the observed impact of herbivores
359 actually restricts the local distribution of *C. sparsiflora*. In particular, experiments
360 demonstrating that *C. sparsiflora* population growth rates increase outside the natural
361 distribution when plants are protected from herbivores would provide especially convincing
362 evidence that herbivores restrict the distribution of this species.

363 Herbivores may also contribute to the ecotypic differentiation in niche use documented in
364 Wright et al. (in press). Given that the original niche model successfully predicts the survival of
365 serpentine genotypes, but does not predict the survival of non-serpentine genotypes (Wright et

366 al., in press), differences in the path diagrams between the two ecotypes are not unexpected.
367 Indeed, the optimal model constructed from the serpentine dataset failed to fit the non-serpentine
368 data ($X^2 = 11.25$, $df = 5$, $P = 0.047$), and similarly, the optimal path model constructed from the
369 non-serpentine dataset failed to fit the serpentine data ($X^2 = 25.61$, $df = 8$, $P = 0.0012$). Because
370 the serpentine path model includes several strong herbivore-mediated indirect effects, while the
371 non-serpentine genotypes are not influenced by herbivory, variation in the ecotypes' responses to
372 herbivory (i.e., tolerance) appear to be responsible for the differences in the path models.
373 Furthermore, the higher tolerance of the non-serpentine ecotypes may be responsible for the lack
374 of fit of the original niche model to this ecotype, described by Wright et al. (in press). Several
375 soil variables (e.g., slope and biomass) had strong impacts on herbivory on the non-serpentine
376 ecotypes; however, because non-serpentine genotypes were tolerant of herbivory, the herbivore-
377 mediated indirect effects were negligible, thus limiting the impacts of these habitat variables on
378 *C. sparsiflora* survival.

379

380 In conclusion, our data suggest that herbivores play an important role in restricting the
381 realized niche of *C. sparsiflora*. Several of the habitat attributes associated with the natural
382 distribution of *C. sparsiflora* alter herbivory, resulting in strong indirect effects on *C. sparsiflora*
383 survival. However, the importance of herbivore-mediated indirect effects varied between
384 serpentine and non-serpentine ecotypes, likely because of intraspecific variation in tolerance to
385 herbivore damage. While herbivory decreased the survival of the serpentine ecotype, it had no
386 impact on the survival of the non-serpentine ecotype. Because indirect effects were negligible
387 for the non-serpentine ecotype, several soil attributes did not impact survival of this ecotype.
388 Thus, herbivory has minimal impacts on the distribution of non-serpentine ecotypes of *C.*

389 *sparsiflora*. However, spatial variation in herbivory influences the distribution of the serpentine
390 ecotype, and may also promote divergence in niche use between the serpentine and non-
391 serpentine ecotypes.

392

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402

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479 serpentine soils in *Collinsia sparsiflora*. *Evolutionary Ecology Research* **8**:1-21.
480
481

481 Table 1: Goodness of fit indices for the full model (all direct and indirect effects included) versus
 482 the reduced model shown in Fig. 1. The two models do not differ significantly ($X^2 = 1.64$, $df = 3$,
 483 $P = 0.65$). NFI = Normed fit index; NNFI = non-normed fit index; CFI = comparative fit index.

Model	Chi-square	<i>df</i>	<i>P</i>	NFI	NNFI	CFI
Full	0.092	2	0.955	0.9995	1.15	1.00
Reduced	1.732	5	0.885	0.9914	1.10	1.00
Null	202.10	28				

484

485

485 Table 2: Magnitude of direct versus herbivore-mediated indirect effects for each exogenous
 486 variable. Values shown are standardized path coefficients. Pathways and effects shown in bold
 487 are statistically significant ($P < 0.05$). Data from both serpentine and non-serpentine ecotypes
 488 were pooled in this analysis.

Effect	Pathway	Magnitude
Slope		
Direct	$\rho_{\text{survival,slope}}$	0.1052
Indirect	$\Gamma_{\text{herbivory,slope}} \times \Gamma_{\text{survival,herbivory}}$	-0.0815
Aspect		
Direct	$\rho_{\text{survival, aspect}}$	-0.0483
Indirect	$\Gamma_{\text{herbivory,aspect}} \times \Gamma_{\text{survival,herbivory}}$	0.0552
Ca:Mg		
Direct	$\Gamma_{\text{survival, Ca:Mg}}$	-0.2229
Indirect	$\rho_{\text{herbivory,Ca:Mg}} \times \rho_{\text{survival,herbivory}}$	-0.0060
Biomass		
Direct	$\Gamma_{\text{survival, biomass}}$	-0.3757
Indirect	$\Gamma_{\text{herbivory,biomass}} \times \Gamma_{\text{survival,herbivory}}$	-0.0584
Organic matter		
Direct	$\Gamma_{\text{survival, organic matter}}$	-0.1388
Indirect	$\Gamma_{\text{herbivory,organic matter}} \times \Gamma_{\text{survival,herbivory}}$	0.0345

489

490

490 Table 3: Magnitude of direct versus herbivore-mediated indirect effects for each exogenous
 491 variable for the path model fit to the serpentine ecotype data and for the path model fit to the
 492 non-serpentine ecotype data. Values shown are standardized path coefficients. Effects shown in
 493 bold are statistically significant ($P < 0.05$).

Effect	Pathway	Magnitude	Magnitude
		(non-serpentine)	(serpentine)
Slope			
Direct	$\rho_{\text{survival, slope}}$	-0.0682	0.1503
Indirect	$\rho_{\text{herbivory, slope}} \times \rho_{\text{survival, herbivory}}$	0.0050	-0.0320
Aspect			
Direct	$\rho_{\text{survival, aspect}}$	-0.0744	-0.1435
Indirect	$\rho_{\text{herbivory, aspect}} \times \rho_{\text{survival, herbivory}}$	0.0054	-0.0306
Ca:Mg			
Direct	$\rho_{\text{survival, Ca:Mg}}$	-0.1084	-0.2017
Indirect	$\rho_{\text{herbivory, Ca:Mg}} \times \rho_{\text{survival, herbivory}}$	0.0079	0.0430
Biomass			
Direct	$\rho_{\text{survival, biomass}}$	-0.3374	-0.3104
Indirect	$\rho_{\text{herbivory, biomass}} \times \rho_{\text{survival, herbivory}}$	0.0245	0.0662
Organic matter			
Direct	$\rho_{\text{survival, organic matter}}$	-0.012	-0.2098
Indirect	$\rho_{\text{herbivory, organic matter}} \times \rho_{\text{survival, herbivory}}$	0.0009	0.0447

494

495

495 Figure legends:

496 Figure 1: Means \pm SE of the proportion of *C. sparsiflora* transplants damaged by herbivores,
497 within the natural distribution versus outside the natural *C. sparsiflora* distribution (A) and linear
498 regression of herbivory as a function of predicted habitat quality (B). Predicted habitat quality
499 (i.e., probability of *C. sparsiflora* survival) was estimated from the niche model described in
500 Wright et al., in press.

501

502 Figure 2: Reduced path diagram for the full dataset. Dashed lines indicate negative path
503 coefficients; solid lines indicate positive path coefficients. Line thickness indicates the
504 magnitude of effect (i.e., magnitude of the standardized path coefficient). Only significant
505 covariances between exogenous predictor variables are shown. “U” denotes the variation
506 explained by all other causes, not included in the path model.

507

508 Figure 3: Revised path diagrams for the non-serpentine ecotype dataset (A) and for the
509 serpentine ecotype dataset (B). Dashed lines indicate negative path coefficients; solid lines
510 indicate positive path coefficients. Line thickness indicates the magnitude of effect (i.e.,
511 magnitude of the standardized path coefficient). “U” denotes the variation explained by all other
512 causes. Significant covariances are not shown for figure clarity.

513

513 Figure 1

514

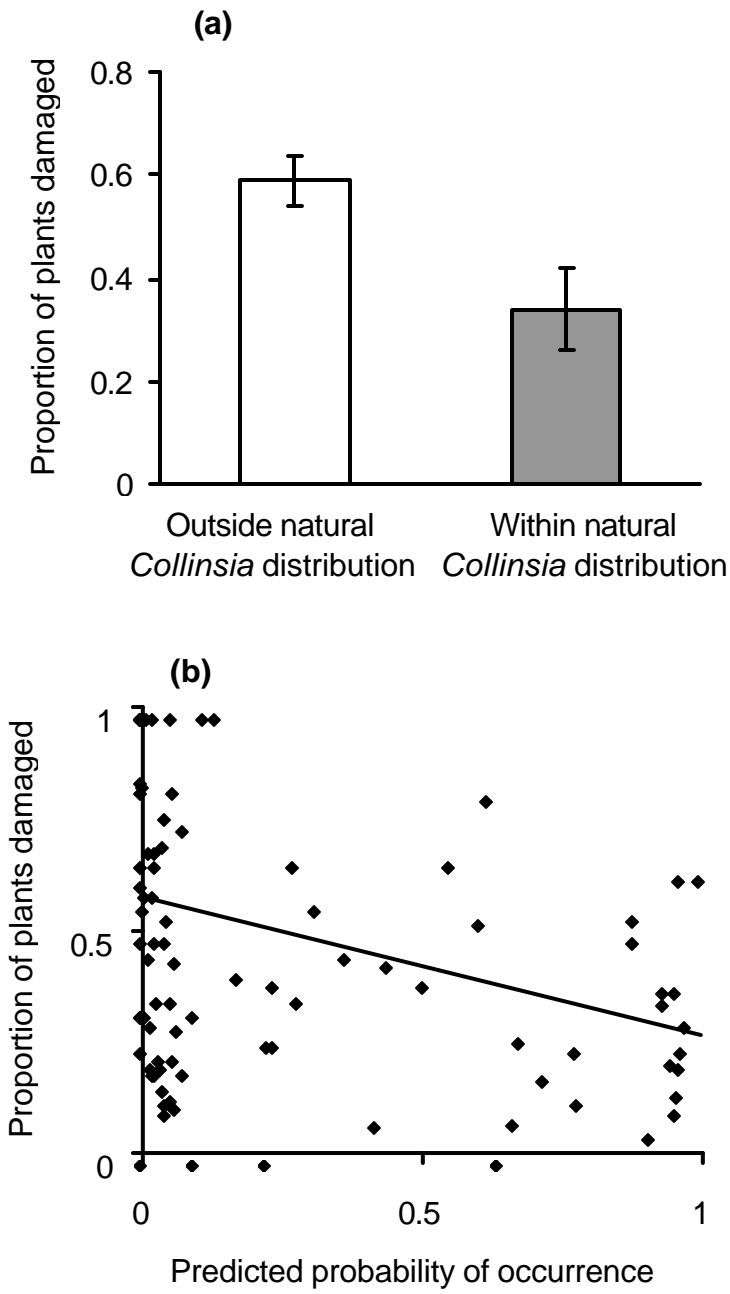
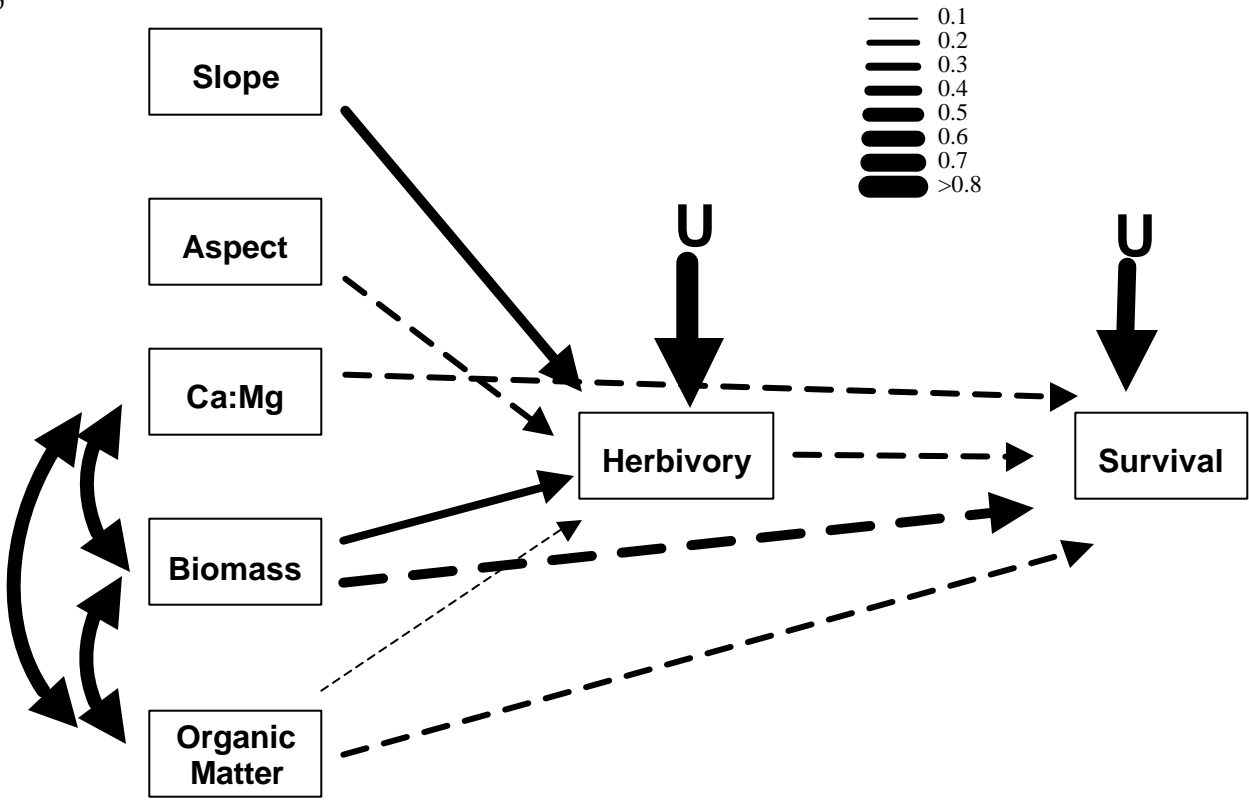


Figure 2

514

515



515 Figure 3

516

(a) Non-serpentine ecotype

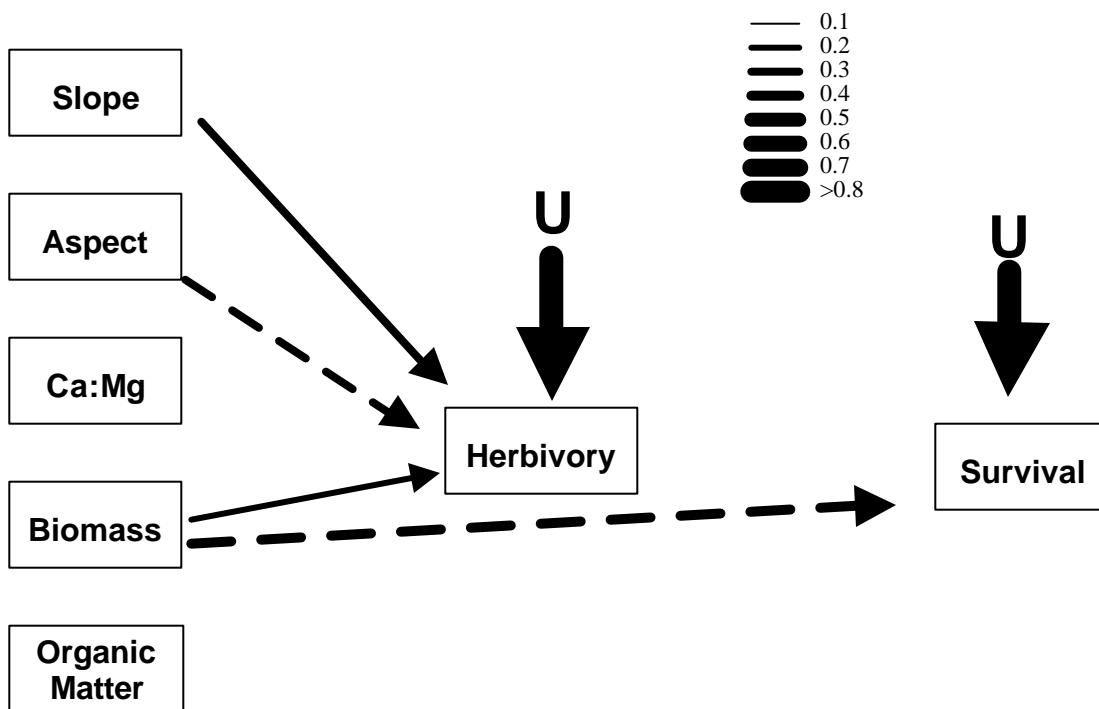
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(b) Serpentine ecotype

