

1 **Title: Selection favors adaptive plasticity in a long-term reciprocal transplant experiment**

2 **Authors:** Jill Anderson<sup>1\*</sup>, M. Inam Jameel<sup>1</sup>, Monica A. Geber<sup>2</sup>.

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4 <sup>1</sup> Department of Genetics and Odum School of Ecology, University of Georgia Athens, GA  
5 30602, USA

6 <sup>2</sup> Ecology and Evolutionary Biology Department, Cornell University, Ithaca, NY 14853, USA

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9 \*corresponding author: Jill T. Anderson

10 Email: jta24@uga.edu

11 Phone: 706-542-0853

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13 Running title: Selection favors plasticity

14 **Abstract:** Spatial and temporal environmental variation can favor the evolution of adaptive  
15 phenotypic plasticity, such that genotypes alter their phenotypes in response to local conditions  
16 to maintain fitness across heterogeneous landscapes. When individuals show greater fitness in  
17 one habitat than another, asymmetric migration can restrict adaptive responses to selection in the  
18 lower quality environment. In these cases, selection is predicted to favor traits that enhance  
19 fitness in the higher-quality source habitat at the expense of fitness in the marginal habitat,  
20 resulting in specialization to the high-quality environment. Here, we test whether plasticity is  
21 adaptive in a system regulated by demographic source-sink dynamics. *Vaccinium elliottii*  
22 (Ericaceae) occurs in dry upland and flood-prone bottomland forests throughout the southeastern  
23 United States, and shows patterns consistent with source-sink dynamics. We conducted a multi-  
24 year field experiment to evaluate whether plasticity in foliar morphology is advantageous. Both  
25 across habitats and within the high-quality upland environment, selection favored plasticity in  
26 specific leaf area and stomatal density. Stabilizing selection acted on plasticity in these traits,

27 suggesting that extreme levels of plasticity are disadvantageous. We conclude that even in  
28 systems driven by source-sink dynamics, temporal and spatial variation in conditions can favor  
29 the evolution of plasticity.

30 **keywords:** reciprocal transplant, specific leaf area, stomatal density, phenotypic plasticity,  
31 natural selection, stabilizing selection, woody perennial

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### 33 **Introduction**

34 Species that inhabit spatially or temporally heterogeneous landscapes often exhibit  
35 phenotypic plasticity, such that individuals shift their phenotype in response to environmental  
36 stimuli (e.g., Dudley and Schmitt 1996; Boersma et al. 1998; Galloway and Etterson 2007; Lind  
37 and Johansson 2007; Forsman 2015; Hendry 2015). If individuals can sense and react to reliable  
38 cues, selection can favor plasticity under temporal variation, when individuals experience  
39 multiple conditions across their lifespan (Moran 1992; Stratton and Bennington 1998), and under  
40 spatial variation when progeny establish in non-parental habitats (Alpert and Simms 2002). For  
41 example, populations of the annual plant *Erodium cicutarium* maintain higher plasticity in  
42 spatially heterogeneous serpentine soil patches than in the more homogeneous non-serpentine  
43 areas (Baythavong 2011). Additionally, adaptive plasticity can enable population persistence  
44 during environmental change (Charmantier et al. 2008; Nicotra et al. 2010). However,  
45 phenotypic plasticity could also be neutral or represent a maladaptive or passive response to  
46 stress (Hendry 2015). Evaluating the fitness consequences of plasticity is crucial for predicting  
47 evolutionary responses to environmental heterogeneity (Nicotra et al. 2010). Nevertheless,  
48 testing whether plasticity confers a fitness advantage remains challenging because analyses

49 require fitness and trait data from replicated accessions transplanted into at least two  
50 environments, ideally in natural habitats in the field.

51 Plasticity could be especially advantageous for species with spatially-extensive gene flow  
52 because offspring can disperse broadly into different environments (Alpert and Simms 2002;  
53 Hendry 2015). To that point, the amount of plasticity in island populations of the frog, *Rana*  
54 *temporaria*, increased as a function of the amount of gene flow from populations in disparate  
55 habitats, along with the degree of local environmental variation (Lind et al. 2011). In addition,  
56 plasticity could enhance fitness for long-lived species, which experience multiple years of  
57 fluctuating conditions before reaching reproductive maturity (Bradshaw 1965). For example,  
58 directional selection favored morphological plasticity in response to flooding and competition in  
59 a clonal perennial buttercup (*Ranunculus reptans*) (Van Kleunen et al. 2007). We hypothesize  
60 that stabilizing selection could also operate on trait plasticity. Stabilizing selection often favors  
61 intermediate phenotypes (e.g., Dudley 1996; Brooks et al. 2005; Wadgymar et al. 2017; Taylor et  
62 al. 2018), but few studies have evaluated nonlinear selection on trait plasticity. Canalized  
63 genotypes with limited plasticity could be at a fitness disadvantage under spatial or temporal  
64 variation because they cannot shift their phenotypes. Similarly, highly plastic lines could also  
65 experience reduced fitness if they are too phenotypically labile, either altering phenotypes too  
66 readily in response to environmental variation or expressing exaggerated trait values. Thus, we  
67 might expect fitness to be maximized at an intermediate trait plasticity.

68 Many species inhabit landscapes in which habitat patches vary in quality or some habitat  
69 types occur more frequently (Kawecki 2008). The evolution of adaptive plasticity could be  
70 constrained if habitat quality differs, such that individuals have higher fitness in some habitat  
71 than others, or if habitat types vary in abundance. In the case of demographic source-sink

72 dynamics, migration from source populations sustains sink populations; this asymmetric  
73 migration could potentially counteract selection within sink populations, leading to local  
74 maladaptation there (Pulliam 1988; Sultan and Spencer 2002; Kawecki 2008). In these systems,  
75 traits favorable in the source environment are expected to evolve at the cost of adaptations to the  
76 marginal habitat (Kawecki 2008). Here, we extend this logic to the evolution of plasticity.  
77 Adaptive phenotypic plasticity is a strategy that maximizes fitness across habitat types  
78 (Baythavong and Stanton 2010; Baythavong 2011). In systems regulated by source-sink  
79 dynamics, the evolutionary response to selection is biased toward traits that are adaptive in the  
80 more frequent or higher quality source environment (Holt and Gaines 1992; Stanton and Thiede  
81 2005; Kawecki 2008). Given the potential costs and limitation of plasticity (DeWitt et al. 1998),  
82 we would not expect adaptive plasticity to evolve in response to conditions in the sink  
83 environment under source-sink population dynamics unless selection within the source habitat  
84 favors plasticity. Instead asymmetrical gene flow in a source-sink system could result in the  
85 evolution of specialization to the source environment (Holt and Gaines 1992; Sultan and Spencer  
86 2002).

87         The high bush blueberry, *Vaccinium elliotii* (Ericaceae), is a perennial woody shrub  
88 endemic to the southeastern United States, where it grows across a gradient of water stress from  
89 seasonally flooded bottomland hardwood forests with dense canopies to more arid upland forests  
90 with high light levels in the understory (Radford et al. 1968; Godfrey and Wooten 1981;  
91 Anderson et al. 2010). These contrasting conditions could impose divergent natural selection,  
92 favoring alternate phenotypic optima in each habitat. This species demonstrates demographic  
93 source-sink dynamics (Pulliam 1988), as reciprocal transplant experiments and genotyping via  
94 microsatellite markers suggest that asymmetric gene flow from abundant upland populations into

95 sparse bottomland populations could constrain adaptation to bottomland forests (Anderson and  
96 Geber 2010). Nevertheless, *V. elliottii* expresses extensive plasticity in morphology (specific leaf  
97 area, foliar nitrogen content, root:shoot ratio, allocation to shallow roots) and physiology  
98 (photosynthesis, stomatal conductance and water use efficiency) to flood vs. drought treatments  
99 in the greenhouse, and to bottomland vs. upland forests in the field (Anderson et al. 2010). Thus,  
100 this system presents a disconnect between the expectation that selection should favor adaptations  
101 to the source environment (upland habitats) and the observation of extensive plasticity across  
102 habitat types.

103         Here, we examine selection on plasticity in three foliar traits (Table 1), which are linked  
104 to physiological function and subject to divergent selection across flooding/aridity gradients in  
105 other systems: specific leaf area, leaf lamina area (hereafter: leaf area), and stomatal density  
106 (Steinger et al. 2003; Wright et al. 2004; Carlson et al. 2015; Maire et al. 2015; Ramírez-Valiente  
107 et al. 2018). Stomatal anatomy influences the rate of stomatal conductance (Lawson et al. 1998;  
108 Franks and Beerling 2009). A recent meta-analysis revealed that stomatal density increases with  
109 light intensity across species (Poorter et al. 2019), which leads to the hypothesis that selection  
110 would favor increased stomatal densities in the high-light upland environment. Alternatively,  
111 selection could favor lower stomatal density in arid upland environments to prevent water loss  
112 from transpiration (Woodward et al. 2002; Carlson et al. 2015). Specific leaf area is often  
113 correlated with photosynthetic rate and typically decreases in high-light and arid environments  
114 (Steinger et al. 2003; Wright et al. 2004; Terashima et al. 2011; Maire et al. 2015), leading to our  
115 prediction that selection favors reduced specific leaf area in upland habitats. Finally, arid, high  
116 light environments induce small leaves in other systems (Valladares et al. 2000; Carlson et al.

117 2015; Ramírez-Valiente et al. 2018), and we predict that selection in upland environments will  
118 favor reduced leaf area.

119         We test the hypothesis that phenotypic plasticity is adaptive by (1) examining how trait  
120 values vary with transplant habitat and growing season to quantify spatial and temporal  
121 plasticity; (2) investigating whether divergent selection across habitat types accords with the  
122 direction of plasticity and (3) determining whether plasticity confers a fitness advantage across  
123 the landscape. For example, if the bottomland environment induces higher trait values than  
124 upland forests (as is the case for specific leaf area), we predict that selection should favor a larger  
125 trait optimum in the bottomlands and a smaller trait optimum in the uplands, and that plasticity in  
126 this trait should be associated with greater fitness averaged across habitat types. If temporal or  
127 spatial variation within the source environment favors plasticity within that habitat type, then  
128 adaptive plasticity could evolve across the landscape despite source-sink population dynamics.  
129 For this reason, we also hypothesize that plasticity is beneficial within the source (upland)  
130 habitat. Finally, we assess nonlinear selection to test whether stabilizing selection favors  
131 intermediate levels of plasticity. To evaluate our hypotheses, we leverage data from a multi-year  
132 field experiment exposing individuals of a woody perennial plant to the suite of environmental  
133 factors that differ between discrete habitat types.

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**Table 1:** Predictions of trait variation across habitat types, divergent selection, and selection on plasticity. For each trait, we indicate whether data from this study support the predictions and reference the corresponding figure. *Vaccinium ellioittii* achieves greatest fitness in upland environments.

		<b>Specific Leaf Area (SLA)</b>	<b>Stomatal density</b>	<b>Leaf area</b>
<b>Trait plasticity</b>	<b>Predictions</b>	Higher in bottomlands than uplands	Higher in uplands than bottomlands	Larger in bottomlands than uplands
	<b>Results</b>	Supported for all years (Fig. 1a, 1d)	Supported for some years (Fig. 1c, 1f)	Not supported (Fig. 1b, 1e)
<b>Divergent selection</b>	<b>Predictions</b>	Selection for higher SLA in bottomlands than in uplands	Selection for reduced stomatal density in bottomland than in uplands	Selection for larger leaves in bottomlands than in uplands
	<b>Results</b>	Supported (Fig. 2c)	Not supported (Fig. 2a)	Not supported; instead stabilizing selection favored intermediate leaf area (Fig. 2b)
<b>Selection on plasticity</b>	<b>Predictions</b>	Selection favors plasticity	Selection favors plasticity	Selection favors plasticity
	<b>Global analysis</b>	Supported: Stabilizing selection for intermediate plasticity (Fig. 3a)	Supported: Selection for increased plasticity in specific leaf area (Figs. 3b and c).	Not supported: No pattern
	<b>Within uplands only</b>	Supported for both cohorts (Fig. 4c, d)	Supported: Directional selection for increased plasticity for 2005 cohort (Fig. 4a) and stabilizing selection for 2006 cohort (Fig. 4b)	Not supported: No pattern

163  
164 *Methods*

165 **Focal system**

166 *Vaccinium elliotii* (Ericaceae, Elliott's blueberry) is an outcrossing highbush blueberry,  
167 which produces insect-pollinated flowers in March-April and sets animal-dispersed seeds in  
168 June-July (Martin et al. 1951; Anderson and Geber 2010). This species has low population  
169 genetic differentiation ( $F_{ST}= 0.032$ ) and high rates of gene flow between populations within and  
170 across habitat types (Anderson and Geber 2010). We conducted fieldwork in the Coastal Plain of  
171 South Carolina, where *V. elliotii* inhabits xeric upland and flood-prone bottomland forests. We  
172 established reciprocal field gardens in two upland and two bottomland forest sites at Francis  
173 Beidler Forest, a National Audubon Sanctuary in the diffuse brown-water floodplain of Four  
174 Holes Swamp (33° 13'N 80° 20'W) (Anderson et al. 2010). We sampled natural populations  
175 throughout the Four Holes Swamp watershed and in the Pee Dee and Santee watersheds of S.C.,  
176 all of which share similar climates (Anderson et al. 2010). In these systems, bottomland  
177 hardwood forests flood 3-139 days/year (average  $\pm$  SD: 43.6  $\pm$  26.1 days/year), but floodwaters  
178 are typically no deeper than several centimeters during a flooding event (Anderson et al. 2010).  
179 During the growing season, precipitation ranges from 0-377 mm/month (average  $\pm$  SD: 125.3  $\pm$   
180 79.8 mm/month), which can induce drought stress in upland forests (Anderson et al. 2010). The  
181 clay soils of bottomland forest are nutrient rich relative to sandy upland forest soils, but the  
182 dense canopy restricts light to the understory (Anderson et al. 2010).

183 Historically, upland forests dominated the landscape of the southeastern U.S., and these  
184 forests were dissected by river systems associated with large tracts of wetland forests (Hickman  
185 1990; Phillips 1994, and references therein). Across this region, human activities have caused  
186 extensive loss of forested habitat (Abernethy and Turner 1987; Hickman 1990; Carter and Biagas  
187 2007; Cabbage et al. 2018). Humans disproportionately converted upland forests to agriculture



188 owing to favorable drainage conditions (Phillips 1994). Cubbage et al. (2018) estimated that  
189 bottomland hardwood forests (not permanently flooded swamps) cover ~9.3 million hectares in  
190 13 states of the southeastern USA (~11.4% of all timberland in this region), whereas non-  
191 wetland upland forests cover ~47.8 million hectares (~58% of timberland). Thus, despite large-  
192 scale deforestation, upland forests still occur with greater frequency than bottomland hardwood  
193 forests in the southeastern U.S.

194 Previous work with *V. elliotii* documented plasticity in morphological and  
195 ecophysiological traits, asymmetric gene flow from upland to bottomland, and demographic  
196 source-sink dynamics with transplants from both habitat types expressing higher survival and  
197 growth in upland than bottomland forests and under drought than flooded conditions (Anderson  
198 and Geber 2010; Anderson et al. 2010). Additionally, Anderson and Geber (2010) found that the  
199 abundance of adult *V. elliotii* individuals was five times greater in upland than bottomland  
200 populations, and naturally-occurring upland individuals had >13.5 times greater reproductive  
201 success than their bottomland counterparts. Here, we expand upon this earlier examination of  
202 patterns of plasticity in specific leaf area by quantifying the extent of spatio-temporal plasticity  
203 in three foliar traits (specific leaf area, stomatal density, and leaf area) across a longer timeframe  
204 and examining divergent selection on traits as well as selection on plasticity in these traits.  
205 Specifically, we conducted genotypic selection analyses, including field fitness from planting  
206 (2005-2006) through April 2014 and trait data measured during 2-3 growing seasons to provide  
207 the long-term records necessary for evaluating selection in this perennial species. We find  
208 relatively low levels of correlations across traits in this experiment (Table S1).

209 This study focused on vegetative cuttings taken from adult plants in the field that had  
210 experienced multiple episodes of selection across their lifespans. To propagate adult tissue, we

211 collected 2-5 cuttings (10cm of new growth) from 20-30 adult plants in 17 upland and 15  
212 bottomland populations throughout South Carolina in the summers of 2004 and 2005 (Anderson  
213 et al. 2010). We stored cuttings on ice in the field. In the greenhouse, we applied rooting  
214 hormone to the stem (Rhizopon AA #3, 0.8% IBA, Rhizopon bv, Hazerswoude, Holland), and  
215 positioned cuttings under an automated misting system for 2-3 months until roots established.  
216 We grew rooted cuttings in the greenhouse until May (2005 and 2006) when they were ~20 cm  
217 tall and had woody tissue.

218         Typically, researchers rear field-collected seeds under greenhouse conditions for a  
219 generation to homogenize maternal effects prior to conducting common garden experiments.  
220 However, that procedure is not possible when the focal species is a long-lived woody plant that  
221 takes many years to reproduce. We minimized variation in maternal effects by growing plants in  
222 the same environment under benign greenhouse conditions for 6 months prior to the initiation of  
223 the reciprocal transplant experiments. If maternal effects were prominent in our system, we  
224 would have expected experimental transplants to show patterns that resemble local adaptation  
225 (Galloway and Etterson 2007). Instead, transplants had elevated fitness in upland transplant  
226 gardens and depressed fitness in bottomland gardens (Anderson and Geber 2010), suggesting  
227 that maternal effects are minimal.

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### 229 **Field reciprocal transplant**

230         In spring 2005, we transplanted N=1685 cuttings from 412 genotypes and 22 populations  
231 into two upland and two bottomland common gardens in the Four Holes Swamp. We expanded  
232 this study in spring 2006, when we transplanted N=548 cuttings (106 genotypes from 22  
233 populations) into these same experimental gardens. Some families were represented by only one

234 individual within a transplant habitat, precluding genotypic selection analysis. We restricted the  
235 dataset to families for which at least two individuals were planted into each transplant habitat,  
236 resulting in a sample size of N=1189 cuttings in the 2005 cohort (mean  $\pm$  SD:  $3.23 \pm 1.45$   
237 individuals per family per habitat type; 183 genotypes; 17 source populations), and N=466  
238 cuttings in the 2006 cohort ( $2.94 \pm 0.97$  individuals per family per habitat type; 79 genotypes; 13  
239 source populations).

240 To reduce transplant shock, we watered all experimental individuals two times per week  
241 for two weeks after planting. Flooding stress differed substantially between the two transplant  
242 years, with growing season rainfall exceeding the long-term average by 51mm/month in 2005  
243 (N. Brunswig and M. Dawson, unpub. precipitation records). Additionally, after approximately  
244 half of the bottomland transplants were established in 2005, a 45-day long flood occurred in  
245 bottomland sites, and the water table remained high even after the floods receded (Anderson and  
246 Geber 2010; Anderson et al. 2010). In contrast, monthly precipitation was 16 mm lower than the  
247 average growing season value during the 2006 season. By replicating this field experiment, we  
248 captured temporal environmental variation in conditions during establishment.

249 We monitored experimental individuals from 2008 (the last sampling point included in  
250 Anderson and Geber 2010; Anderson et al. 2010) until 2014. During the first two years of  
251 growth, we visited each individual twice per month to record the time of mortality.  
252 Subsequently, we visited each plant in October 2007, March 2008, March 2009, April 2011,  
253 October 2012, March 2013 and April 2014. In October of 2006, 2007 and 2012, we collected an  
254 average of 5 living sun and 5 living shade leaves per living plant, scanned leaves to extract leaf  
255 areas with ImageJ (Schneider et al. 2012), dried leaves at 50°C for 3-4 days, and weighed them  
256 on a Mettler AE 200 balance ( $\pm 0.0001$ g) to determine specific leaf area (leaf area per unit

257 biomass,  $\text{cm}^2/\text{g}$ ). On these understory shrubs, we collected both sun and shade leaves to quantify  
258 individual-level foliar traits more accurately and precisely. Some leaves had evidence of  
259 herbivory. To obtain the leaf area of undamaged leaves (without herbivory), we filled in internal  
260 holes in Image J and redrew leaf margins. Owing to low herbivore damage (mean  $\pm$  SD:  $2.1\% \pm$   
261  $3.4\%$  leaf area removed by herbivores in  $N=372$  genotypes of the 2005 cohort and  $3.1\% \pm 4.2\%$ ,  
262  $N=298$  genotypes in the 2006 cohort), it was straightforward to modify the leaf images to reflect  
263 leaf size prior to herbivory.

264 We quantified stomatal density by making epidermal impressions of the abaxial (lower)  
265 leaf surface with clear nail polish, mounting these impressions on microscope slides, and  
266 visualizing them under  $400\times$  magnification using a compound microscope. We calculated  
267 stomatal density by averaging the number of stomata across four distinct nonoverlapping  
268  $0.0352\text{ mm}^2$  areas of each impression. Additionally, we made stomatal peels of  $N=33$  samples on  
269 the adaxial (upper) surface of the leaf to examine the potential for adaxial stomata (Woodward  
270 1986), which are typically rare in shrubs (Muir 2015). As we were unable to detect any evidence  
271 of adaxial stomata in *V. elliotii*, we proceeded with quantification of abaxial stomatal density.

272

### 273 **Statistical analyses**

274 For all analyses, we first calculated family-mean trait values for each year of  
275 measurement as well as across all years (least square means; hereafter: LSMEANs) and fitness  
276 components as a function of transplant habitat by family in models that included block nested  
277 within transplant site as a random effect (Proc Mixed, SAS ver. 9.4). We standardized traits to a  
278 mean of zero and a standard deviation of one to facilitate comparison of selection on traits

279 measured on different scales. We analyzed the two cohorts separately because of differences in  
280 the duration of the monitoring.

281 ***Phenotypic plasticity***—We evaluated plasticity across habitat types through a repeated  
282 measures multivariate regression with a Kenward-Roger degree of freedom approximation. We  
283 analyzed family-level LSMEANs in all three foliar traits jointly as a function of transplant  
284 habitat type, year of measurement, source habitat, and all two and three-way interactions, with  
285 random effects for family and family by transplant habitat using the Mixed procedure in SAS  
286 (ver. 9.4). These multivariate repeated measures models specify the covariance structure of the R  
287 matrix using direct (Kronecker) product structures [type=UN@AR(1)] to fit multiple response  
288 variables (unstructured covariance matrix, UN) measured on the same plant genotypes across  
289 years [autoregressive covariance matrix, AR(1)] (Galecki 1994). A significant main effect of  
290 transplant habitat would indicate spatial plasticity, and a main effect of growing season would  
291 point to temporal plasticity. Interactions of transplant habitat and season would suggest that the  
292 degree of spatial plasticity depended upon the growing season. A main effect of source habitat or  
293 interactions with that factor would suggest genetic differentiation in phenotypes between upland  
294 and bottomland source populations.

295 ***Genotypic selection analyses***—Genotypic selection analyses (Rausher 1992) tested  
296 whether: 1) divergent selection favors different phenotypic optima under contrasting  
297 environmental conditions, and 2) phenotypic plasticity in foliar traits is adaptive. Many  
298 individuals died before foliar traits were measured. These individuals could have died due, at  
299 least in part, to limited phenotypic plasticity and trait values that were inappropriate for the  
300 transplant environment. Phenotypic traits of dead individuals can be estimated based on trait  
301 values of their surviving relatives (Hadfield 2008). Thus, for each family, we calculated

302 genotypic mean fitness based on data from every planted individual, and genotypic mean trait  
303 values from individuals that survived until trait measurement.

304 As very few individuals successfully flowered in the 8-9 years of this field experiment,  
305 we focused on survival as a critical component of fitness. For each individual plant, we  
306 calculated longevity as the number of elapsed days between planting and mortality. At the final  
307 census in April 2014, 479 individuals (40.2%) of the 2005 cohort and 195 individuals (42%) of  
308 the 2006 cohort remained alive. In the terminology of survivorship analysis, these individuals  
309 would be considered right-censored as they had not yet experienced mortality. However, it is not  
310 possible to analyze selection on plasticity within the framework of a survivorship analysis like  
311 Cox proportional hazards models because the genotypic selection analyses require family-level  
312 data on plasticity whereas survivorship models require individual-level data. Therefore, to  
313 include individuals that were alive on the final census in our analyses, we assigned them time of  
314 mortality of the final census. Survival was high between the penultimate and the final censuses;  
315 97% and 85% of individuals alive in March 2013 survived until April 2014 (2005 and 2006  
316 cohorts, respectively). Given that few plants died over the final year, we have not introduced bias  
317 into our analyses by coding living plants with the final census date. We also conducted  
318 complementary logistic regressions in a generalized linear mixed model framework, analyzing  
319 the number of individuals that survived until April 2014 over the number of individuals per  
320 family that were initially planted in the study (glmer function of the R package **lme4** ver. 1.1-21,  
321 Bates et al. 2015). This logistic regression approach treats all dead individuals identically,  
322 whether mortality happened early or late in the experiment.

323 *Divergent selection*—Divergent selection can be detected by a significant interaction  
324 between trait and transplant environment in genotypic selection analyses; therefore, we analyzed

325 relative fitness as a function of traits (specific leaf area, leaf size and stomatal density) by  
326 transplant habitat with a random effect of genotype.

327       Across the course of the experiment, mortality was significantly greater in the bottomland  
328 than in the upland transplant gardens (Anderson and Geber 2010, and this analysis), such that  
329 some families lack trait data for later years because all individuals died. For that reason, we  
330 analyzed selection using phenotypic data collected only during the first year of trait  
331 measurements (2006 traits for the 2005 cohort, and 2007 traits for the 2006 cohort) for which our  
332 trait dataset was the most complete (2005 cohort: N=173 families in upland gardens and N=48  
333 families in bottomland sites; 2006 cohort: N=70 families in upland gardens and N=69 families in  
334 bottomland gardens). We modeled viability through April 2014 as a function of these early  
335 phenotypic values; thus, we leveraged the full fitness dataset to evaluate selection on traits  
336 measured early in the study. Trait values from these years were within the range of trait values  
337 expressed in subsequent years (Fig. 1). A restricted dataset focused on the subset of clones for  
338 which we had trait data from all sampling time points (2005 cohort: 2006, 2007 and 2012 years;  
339 2006 cohort: 2007 and 2012 years) would lack data on families that died early in the experiment  
340 and may have been poorly adapted to upland or bottomland environments (2005 cohort: N=167  
341 families in upland gardens and N=33 families in bottomland gardens; 2006 cohort: N= 61  
342 families in upland gardens and N=36 families in bottomland gardens). Since we evaluated  
343 selection across two planting cohorts, we applied a corrected  $\alpha=0.025$  ( $=0.05/2$  non-independent  
344 datasets) to assess statistical significance.

345       In heterogeneous systems subject to demographic source-sink dynamics, analyses of  
346 absolute fitness may predominately detect selection in the more frequent or higher quality  
347 environment (upland forests) whereas analyses of relative fitness provide more robust

348 information on selection across each of the habitat types (Stanton and Thiede 2005). For that  
349 reason, we evaluated soft selection using relative fitness. To calculate relative fitness for  
350 longevity, we divided each family's absolute fitness by the mean fitness expressed by all families  
351 in that transplant environment. We tested for nonlinear selection by evaluating quadratic effects  
352 of traits and their interactions with environments; we removed any nonsignificant quadratic  
353 effects from the final models. For all analyses, we visualized selection using the R package  
354 **visreg** vers. 2.6-0 (Breheny and Burchett 2017) by plotting partial residuals from the multiple  
355 regressions while holding other explanatory variables at their median value (conditional plots).

356 ***Selection on plasticity***—To test whether plasticity is adaptive, we used across-environment  
357 multivariate genotypic selection analysis (Van Kleunen and Fischer 2001; Stinchcombe et al.  
358 2004), using the **glmer** (generalized linear mixed models with gamma distribution) functions of  
359 the R package **lme4** (ver. 1.1-21, Bates et al. 2015). As with our analyses of divergent selection,  
360 we tested whether selection favored spatial plasticity using the first year of trait data (N=41  
361 families for the 2005 cohort; N=60 families for the 2006 cohort), for which we have a larger  
362 sample size than if we restricted the dataset to families with data from all trait sampling points  
363 (N=24 families for the 2005 cohort; N=28 families for the 2006 cohort). For these analyses, we  
364 also used a corrected  $\alpha=0.025$  ( $=0.05/2$  sets of analyses) to assess statistical significance because  
365 we included two cohorts.

366 In multiple regression analyses, we modeled relative fitness as a function of family-mean  
367 trait values (averaged across environments) and plasticity in traits to identify selection on  
368 plasticity independent from selection on trait values. For each family, we quantified plasticity via  
369 a modified version of the phenotypic plasticity index ( $PI_{LSM}$ ), based on least square mean trait  
370 values for each clone in each environment (Valladares et al. 2006). The original  $PI_{LSM}$  metric is



371 calculated as the difference between maximum LSMEAN trait values and minimum LSMEAN  
372 trait values divided by the maximum LSMEAN trait value [ $PI_{LSM} = (LSMEAN_{\text{maximum}} -$   
373  $LSMEAN_{\text{minimum}}) / LSMEAN_{\text{maximum}}$ ]. This metric quantifies the magnitude, but obscures the  
374 directionality of plasticity. In our system, some genotypes express plasticity in the opposite  
375 direction from average trait changes across habitat types and seasons, which could be  
376 maladaptive. As we aim to test whether plasticity is adaptive, our modified plasticity index  
377 incorporates the directionality of plasticity into Valladares et al.'s (2006) framework by  
378 quantifying plasticity as:  $(LSMEAN_{E, \text{high}} - LSMEAN_{E, \text{low}}) / LSMEAN_{E, \text{high}}$ , where  $LSMEAN_{E, \text{high}}$   
379 is the family-mean trait value in the environment with a global average higher mean for that trait,  
380 and  $LSMEAN_{E, \text{low}}$  is the family-mean trait value in the environment with a global lower mean.  
381 This formula maintains positive expected plasticity values, but allows for negative values for  
382 families that shift their trait values in the opposite direction from the population as a whole. For  
383 example, for both cohorts across study years, specific leaf area (SLA) was significantly greater in  
384 the bottomland transplant environment than in the upland transplant environment (Fig. 1).  
385 Therefore, for this trait:  $\text{plasticity index} = (SLA_{\text{bottomland}} - SLA_{\text{upland}}) / SLA_{\text{bottomland}}$ . We used the  
386 same configuration for leaf area because it generated an average positive plasticity index for both  
387 cohorts. Upland transplants expressed higher stomatal density values than bottomland  
388 transplants, so we considered the upland environment to be the minuend in the numerator, and  
389 the factor in the denominator in the calculation of plasticity in this trait.

390 If plasticity confers a fitness advantage in the source (upland) habitat, selection could  
391 maintain adaptive plasticity across the landscape despite demographic source-sink dynamics. To  
392 test whether selection favors plasticity in the source habitat, we analyzed genotypic fitness  
393 (longevity) of clones transplanted into the upland gardens as a function of trait values expressed

394 within upland sites only and spatial plasticity for the first year of trait measurement for both  
395 cohorts. We focused on the first year of data to maximize statistical power to test our hypothesis  
396 with the largest datasets available. This analysis tests whether the most phenotypically labile  
397 plants had greater longevity within upland forests. We ran these generalized linear mixed models  
398 using a gamma distribution and log link, and including a random effect for source population, in  
399 the glmer function of the R package **lme4** (ver. 1.1-21, Bates et al. 2015).

400

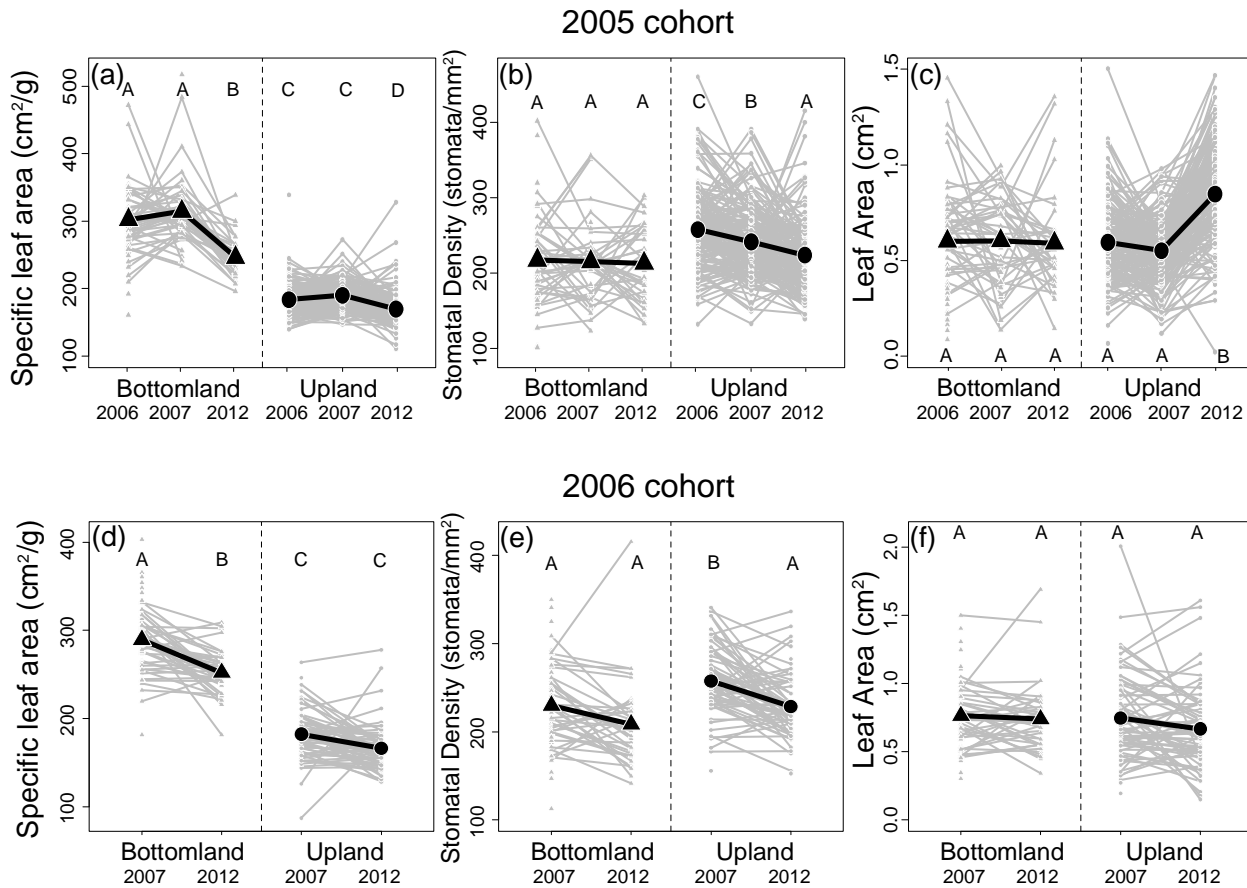
## 401 *Results*

### 402 **Phenotypic plasticity**

403 We found significant temporal and spatial plasticity for both cohorts (Table S2; Fig. 1).  
404 Furthermore, temporal variation was broadly concordant across cohorts. Nevertheless, trait  
405 variation was not always congruent with expectations. In the 2005 cohort, analyses confirmed  
406 previously reported plasticity in specific leaf area, as well as documenting plasticity in stomatal  
407 density and leaf area (Fig. 1a-1c; Table S2: trait  $\times$  transplant habitat  $\times$  season:  $F_{6,513}=38.4$ ,  
408  $p<0.0001$ ). Specific leaf area (SLA) was significantly lower in upland than bottomland  
409 environments across years and SLA values varied with year within habitats (Fig. 1a). Consistent  
410 with expectations, stomatal density was higher in upland than bottomland transplant habitats in  
411 two of three years; stomatal density varied across years in the upland environment but not in the  
412 bottomlands (Fig. 1b). Finally, leaf area was significantly greater in uplands than bottomlands in  
413 one year only (Fig. 1c). Significant source habitat by transplant habitat interactions and source  
414 habitat by growing season interactions indicated that the magnitude of spatio-temporal plasticity  
415 was slightly greater for bottomland than upland genotypes (Table S2), consistent with theoretical  
416 predictions of greater plasticity in marginal habitats (Chevin and Lande 2011).

417           The 2006 cohort also displayed a significant interaction among transplant habitat and  
418 growing season for all traits (Fig. 1d-1f; Table S2, trait  $\times$  transplant habitat  $\times$  season:  $F_{3,146}=2.68$ ,  
419  $p=0.049$ ). Concordant with expectations, experimental individuals expressed greater specific leaf  
420 area under the dense canopy of bottomland habitats than in the higher light environment of  
421 upland forests (Fig. 1d). Temporal variation in SLA was apparent in the bottomland but not the  
422 upland environment (Fig. 1d). Stomatal density was significantly greater in upland forests in one  
423 year (2007) than in all other transplant habitat by season combinations (Fig. 1e). However, leaf  
424 area did not differ across space or time (Fig. 1f). Finally, for leaf area only, our analyses found a  
425 significant effect of source habitat ( $F_{1,208.5}=68.58$ ,  $p<0.0001$ ), such that upland genotypes had  
426 larger leaves than bottomland genotypes (contrast in standardized trait values between  
427 bottomland and upland genotypes:  $-0.43 \pm 0.15$ ; Table S2).

428



### Transplant habitat and growing season year

429 **Figure 1:** Spatial and temporal variation in (a) stomatal density, (b) leaf area, and (c) specific  
 430 leaf area for the 2005 cohort measured in 2006, 2007 and 2012, and the same traits (d: SLA; e:  
 431 stomatal density; f: leaf area) for the 2006 cohort measured in 2007 and 2012. Data points in  
 432 grey represent LSMEAN trait values for N=183 (2005 cohort) and 79 (2006 cohort) clonal  
 433 families included in the transplant experiment. The black symbols and lines reflect the overall  
 434 means across families. We analyzed all traits simultaneously using repeated measures  
 435 multivariate regression. To achieve model convergence, we standardized traits to a mean of 0  
 436 and standard deviation of 1, but we present unstandardized values here. Letters within each panel  
 437 represent significant differences across habitat types and growing seasons for each trait  
 438 separately after Tukey's adjustment for multiple comparison.  
 439

### 440 **Divergent selection**

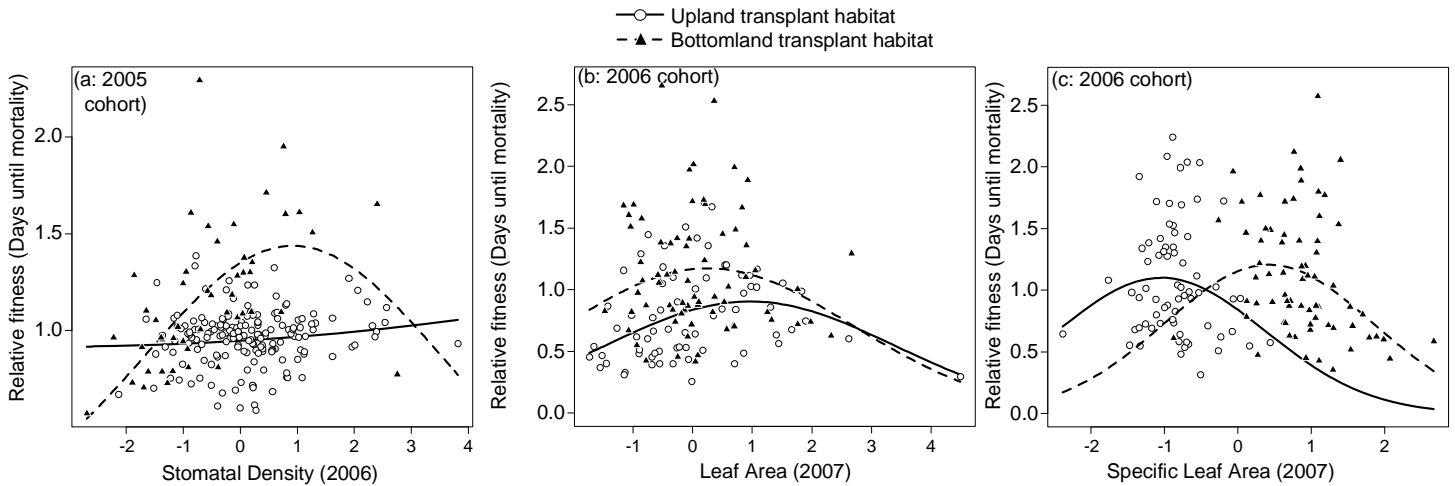
441 *Stomatal density*—Divergent selection in the 2005 cohort operated on stomatal density  
 442 (quadratic trait × transplant habitat interaction:  $\chi^2=9.56$ ,  $p=0.002$ , Fig. 2a, Table S3), with  
 443 stabilizing selection favored an intermediate stomatal density in bottomland forests and no

444 apparent selection within upland habitats. We found no evidence for divergent selection on this  
445 trait in the 2006 cohort. Contrary to expectations, logistic regression of survival revealed  
446 directional selection for low stomatal density in uplands and high stomatal density in  
447 bottomlands for the 2005 cohort (Table S4, Fig. S1a). Directional selection for increased  
448 stomatal density in the uplands in the 2006 cohort accorded with predictions (Table S4, Fig.  
449 S1b).

450 *Leaf area*— For the 2006 cohort: stabilizing longevity selection favored intermediate leaf  
451 sizes in the first year of measurement in both habitats (quadratic trait:  $\chi^2=12.98$ ,  $p=0.00031$ , Fig.  
452 2b, Table S3). Logistic regression revealed viability selection for increased leaf size in upland  
453 forests in both cohorts, and smaller leaf size in the bottomland forests in the 2006 cohort (Fig.  
454 S1c,d, Table S4).

455 *Specific leaf area (SLA)*— Concordant with expectations, selection favored lower  
456 specific leaf area in the upland forests and higher specific leaf area in the bottomland forests in  
457 the 2006 cohort (SLA  $\times$  transplant habitat interaction:  $\chi^2=5.15$ ,  $p=0.023$ ; quadratic trait:  
458  $\chi^2=10.14$ ,  $p=0.0015$ ; Fig. 2c, Table S3). We did not detect longevity selection on SLA for the  
459 2005 cohort (Table S3). However, for both cohorts, divergent viability selection assessed via  
460 logistic regression favored reduced SLA in uplands and increased SLA in bottomland forests  
461 (Fig. S1e, f, Tables S4), consistent with the direction of trait plasticity (Fig. 1a and d).

462  
463



**Figure 2:** Longevity selection on foliar traits in the 2005 and 2006 cohorts. Gamma regression analyses revealed selection operating on stomatal density (a) during the first year of trait measurement for the 2005 cohort. Stabilizing selection favored intermediate leaf area for (b) the 2006 cohort for both habitats. Finally, (c) divergent selection on specific leaf area in the 2006 cohort was consistent with the direction of plasticity. Bottomland data and predicted regression lines are displayed with closed triangles and dashed lines; upland data and regression lines are shown with open circles and solid lines. Traits were standardized to a mean of 0 and standard deviation of 1. Panels show partial residuals from multiple regressions while holding other traits at their median value.

464

465

466 **Selection on plasticity**

467 **Stomatal density**—Nonlinear selection favored intermediate plasticity in stomatal density

468 for the 2006 cohort (plasticity optimum: 0.10; quadratic effect:  $\chi^2=8.3$ ,  $p=0.0039$ ; Fig. 3a, Table

469 S5). We did not find viability selection on plasticity in stomatal density (Table S6).

470 **Leaf area**— We found no evidence for selection on leaf area plasticity in either cohort

471 (Tables S5 and S6).

472 **Specific leaf area (SLA)**— For the 2005 cohort, stabilizing selection operated on specific

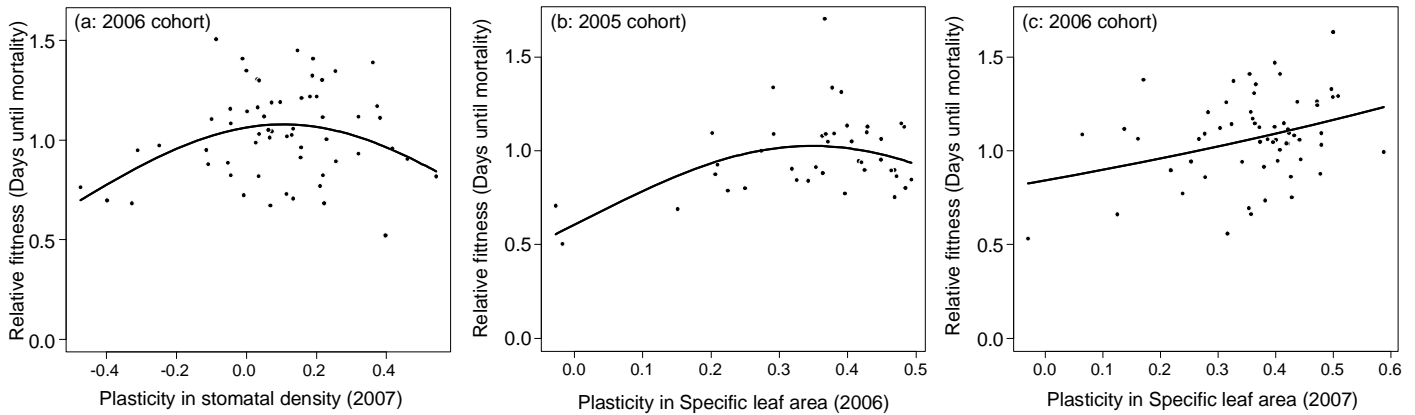
473 leaf area (plasticity optimum: 0.35 ;quadratic effect:  $\chi^2=6.35$ ,  $p=0.012$ ; Fig. 3b, Table S5). For

474 the 2006 cohort, directional selection favored increased plasticity in specific leaf area ( $\chi^2=6.34$ ,

475  $p=0.012$ ; Fig. 3c, Table S5).

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**Figure 3:** Longevity selection acted on plasticity in foliar traits in both cohorts. Stabilizing selection favored intermediate plasticity in (a) stomatal density for the 2006 cohort, and (b) specific leaf area in the 2005 cohort. Directional selection favored (c) increased plasticity in specific leaf area for the 2005 cohort. These multivariate genotypic selection analyses evaluated fitness as a function of mean trait values and plasticities in all three traits, with separate models for each planting cohort. Panels show partial residuals from multiple regressions while holding other traits at their median value.

487 *Selection on plasticity within the source environment*— Across both cohorts, longevity

488 selection favored greater plasticity in stomatal density and specific leaf area (Table S7, Fig. 4).

489 For the 2005 cohort, fitness increased with plasticity in stomatal density ( $\chi^2=8.65$ ,  $p=0.0033$ ; Fig.

490 4a). A negative quadratic curve suggested stabilizing selection on plasticity in stomatal density in

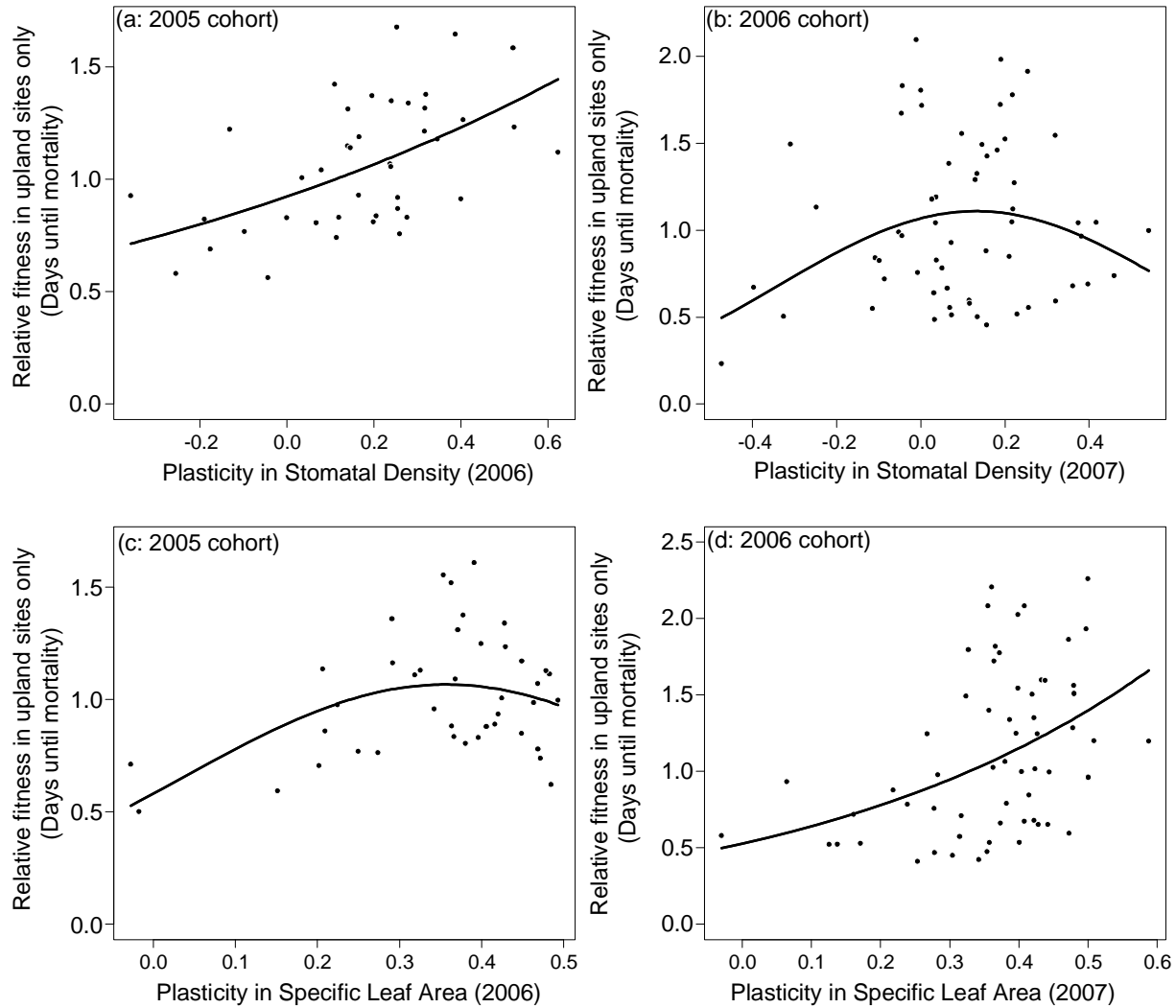
491 the 2006 cohort, favoring intermediate values (plasticity optimum: 0.13, quadratic term:  $\chi^2=5.34$ ,

492  $p=0.021$ ; Fig. 4b). We detected a trend that could reflect nonlinear selection for greater plasticity

493 in specific leaf area in the 2005 cohort (linear term:  $\chi^2=5.36$ ,  $p=0.021$ ; quadratic term:  $\chi^2=3.55$ ,

494  $p=0.06$ ; Fig. 4c). Directional selection within the uplands favored increased plasticity in specific

495 leaf area in the 2006 cohort ( $\chi^2=7.38$ ,  $p=0.0066$ ; Fig. 4d).



496

497 **Figure 4:** Selection favored plasticity in stomatal density (a: 2005 cohort; b: 2006 cohort) and  
498 specific leaf area (c: 2005 cohort; d: 2006 cohort) within upland forests. Panels show partial  
499 residuals from multiple regressions while holding other traits at their median value. Data points  
500 represent relative fitness based on genotype mean longevity as a function of spatial plasticity in  
501 traits measured in the first year for each cohort. Panels show partial residuals from multiple  
502 regressions while holding other traits at their median value.

503

#### 504 *Discussion*

505 When individuals express higher fitness in one habitat relative to another, selection is  
506 expected to favor trait values advantageous in the higher-quality or larger habitat at the expense  
507 of adaptations to the lower-quality habitat (Holt and Gaines 1992; Stanton and Thiede 2005;



508 Kawecki 2008). Similarly, if populations inhabiting a marginal environment produce fewer  
509 propagules than those from a higher quality habitat, evolution might not favor a plastic response  
510 to spatial heterogeneity (Holt and Gaines 1992; Sultan and Spencer 2002). Nevertheless, in our  
511 study, adaptive plasticity in morphological traits conferred a fitness advantage for *Vaccinium*  
512 *elliottii* across environments. In addition, selection favored adaptive plasticity in stomatal density  
513 and specific leaf area within upland forests (Fig. 4) where *V. elliotti* individuals have greater  
514 survival in our reciprocal transplant experiment, and natural populations are more fecund and  
515 abundant (Anderson and Geber 2010). We hypothesize that plasticity could be advantageous  
516 within upland forests because of microenvironmental spatial heterogeneity and temporal  
517 variation in conditions. Selection within upland forests could maintain adaptive morphological  
518 plasticity across populations in both habitat types. Further, we hypothesize that this plasticity -  
519 driven by selection in the uplands - could enhance survival within the lower-quality bottomland  
520 habitat, as is proposed in theoretical models (Chevin and Lande 2011). Our analyses support the  
521 hypothesis that selection can favor adaptive plasticity in a spatially and temporally  
522 heterogeneous landscape, despite demographic source-sink dynamics. Indeed, this adaptive  
523 plasticity could reduce maladaptation and enhance fitness in the marginal environment (Chevin  
524 and Lande 2011).

525 ***Divergent selection***—Based on trait variation across habitat types in this system (Fig. 1)  
526 in concert with clinal variation in other systems (Steinger et al. 2003; Wright et al. 2004; Carlson  
527 et al. 2015; Maire et al. 2015; Ramírez-Valiente et al. 2018), we expected selection to favor  
528 increased specific leaf area and leaf area, and reduced stomatal density in bottomland relative to  
529 upland forests. Indeed, concordant with predictions, we found divergent longevity (and viability)  
530 selection on specific leaf area. In contrast, selection on stomatal density did not follow

531 expectations. Rather, in the 2006 cohort, stabilizing selection favored intermediate stomatal  
532 density in bottomlands, but we found no evidence for selection in upland habitats.

533 We expected to find smaller leaves in upland than bottomland forests because leaf lamina  
534 area often declines with increasing aridity and light levels (Valladares et al. 2000; Carlson et al.  
535 2015; Ramírez-Valiente et al. 2018). Instead, we found the opposite pattern: leaves were similar  
536 in size across habitats in two years (2006 and 2007) and larger in upland than bottomland  
537 environments in another year (2012). Our analysis detected similar patterns of stabilizing  
538 selection on leaf size in both habitats. Not surprisingly, we found no evidence that selection  
539 favors plasticity in leaf area, which was the least plastic trait in the study and which is not subject  
540 to divergent selection across habitat types. Trait expression and selection on leaf area could be  
541 driven by factors other than aridity or understory light levels in this system. We note that our  
542 models incorporate indirect selection on focal traits mediated by unmeasured traits.

543 ***Selection for adaptive plasticity***—Spatial and temporal heterogeneity in environmental  
544 conditions can promote the evolution of adaptive plasticity when individuals experience multiple  
545 environmental conditions across their lifetimes or when the progeny disperse into non-parental  
546 habitat types (Baythavong and Stanton 2010; Baythavong 2011). Models suggest that even low  
547 levels of gene flow can favor the evolution of phenotypic plasticity (Sultan and Spencer 2002).  
548 In our system, asymmetric gene flow occurs predominately from upland to bottomland  
549 populations, yet rare gene flow in the reverse direction also connects populations (Anderson and  
550 Geber 2010). Given the high rates of gene flow across habitat types (Anderson and Geber 2010),  
551 *V. elliottii* seeds likely often germinate and establish in different environments than their  
552 maternal and paternal parents. In addition, water stress can vary inter- and intra-annually in both  
553 bottomland and upland habitats. Thus, in both habitat types, established individuals experience

554 multiple years of variable environmental conditions prior to reproduction, which could strongly  
555 favor the evolution of adaptive plasticity in functional traits.

556 Evolutionary studies that have explicitly tested the adaptive significance of plasticity in  
557 plants focus primarily on herbaceous systems (Dudley and Schmitt 1996; Scheiner and Callahan  
558 1999; Schmitt et al. 1999; Donohue et al. 2000; Steinger et al. 2003; Bell and Galloway 2007;  
559 Galloway and Etterson 2007; Baythavong 2011; Zhang et al. 2013; Wagner and Mitchell-Olds  
560 2018), even though woody plants represent ~45-48% of plant species globally (FitzJohn et al.  
561 2014). Woody plant species typically have reduced population genetic structure (lower  $F_{ST}$ )  
562 relative to annual or perennial herbaceous species, indicating greater rates of gene flow (Duminil  
563 et al. 2009). For those reasons, plasticity could be particularly adaptive for woody species,  
564 because they may be more likely than herbaceous species to experience temporal and spatial  
565 variation in environmental conditions. Even though our study of post-establishment survival did  
566 not capture the full extent of selection operating across the duration of the life cycle, we found  
567 that adaptive plasticity in stomatal density and specific leaf area confers a viability and longevity  
568 advantage across multiple years.

569 ***Stabilizing selection on plasticity***—An additional pattern emerged in our study:  
570 Stabilizing selection favored intermediate plasticity in stomatal density and specific leaf area.  
571 We propose that families with low levels of trait plasticity might not express appropriate  
572 phenotypes in response to spatial or temporal variation in environmental conditions. Similarly,  
573 families with very high levels of plasticity could be too labile, perhaps shifting phenotypes too  
574 readily or expressing exaggerated trait values. Thus, we might expect fitness to be maximized at  
575 some intermediate trait plasticity, just as stabilizing selection can favor intermediate trait  
576 expression in multivariate trait space (e.g., Brooks et al. 2005; Wadgymar et al. 2017; Taylor et

577 al. 2018). Logistical constraints often preclude the sample sizes necessary to gain sufficient  
578 statistical power for these analyses and the duration of the field studies from which to estimate  
579 plasticity and fitness. We suspect that as statistical tools become more powerful, researchers will  
580 uncover more examples of nonlinear selection on plasticity.

581 ***Selection varies across cohorts***—The magnitude and direction of selection can change  
582 through time. In several instances, the degree of selection differed across cohorts. In our  
583 experiment, the 2005 cohort experienced a large-scale flood event in the bottomland gardens  
584 during planting, which contrasted with the average conditions experienced by the 2006 cohort  
585 during and shortly after planting. Extreme events, such as the flood of 2005, can impose strong  
586 viability selection, which can restrict the number of individuals that survive these events and  
587 influence the distribution of trait and fitness values of the survivors. Our results indicate that  
588 conditions during initial establishment can set the stage for trait expression and selection later in  
589 life history.

590 ***Conclusions***—Our analyses suggest that spatial and temporal variation in environmental  
591 conditions favors phenotypic plasticity. Demographic source-sink dynamics pose challenges for  
592 conservation in contemporary landscapes, as small disconnected habitat patches can be  
593 associated with poor performance (Furrer and Pasinelli 2016) and habitat fragmentation could  
594 shift patches from sources to sinks. Understanding eco-evolutionary dynamics in source-sink  
595 systems could lead to better conservation outcomes. Asymmetrical gene flow from upland into  
596 bottomland forests likely constrains other adaptations to flooding such as adventitious roots,  
597 porous root tissue, and the formation of enlarged lenticels, which are present in other species of  
598 *Vaccinium* (Anderson and Geber 2010). Nevertheless, selection operating across this  
599 heterogeneous landscape can favor plasticity in key functional traits, which could enhance fitness

600 and population persistence within the marginal bottomland habitat type (Chevin and Lande  
601 2011). We suggest that phenotypic plasticity is likely to be advantageous in other systems when  
602 individuals encounter multiple environments over their lifetimes and progeny disperse to non-  
603 parental environments.

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## Supplemental materials for

778 Selection favors adaptive plasticity in a long-term reciprocal transplant experiment

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780 **Table S1:** Trait correlations in upland and bottomland transplant habitats for the 2005 cohort.

781 We find relatively low levels of correlations across traits in this experiment. Below, we include

782 the Pearson correlation coefficients and (uncorrected) p-values for trait correlations in both

783 transplant habitat types for the 2005 cohort. The three foliar traits are sometimes correlated with

784 each other, but these correlations vary substantially. We have not applied any corrections for

785 multiple testing in these tables. Trait correlations are similar for the 2006 cohort and can be

786 calculated from the Dryad datafile associated with this manuscript.

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788 **Upland transplant habitat (2005 cohort):**

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		Stomatal Density, 2006	Specific Leaf Area, 2006	Leaf Area, 2006	Stomatal Density, 2007	Specific Leaf Area, 2007	Leaf area, 2007	Stomatal Density, 2012	Specific Leaf Area, 2012	Leaf area, 2012
Stomatal Density, 2006	r	1								
	p-value									
Specific Leaf Area, 2006	r	-0.05	1							
	p-value	0.5495								
Leaf Area, 2006	r	-0.11	-0.22	1						
	p-value	0.1689	<b>0.0039</b>							
Stomatal Density, 2007	r	0.5	-0.01	-0.02	1					
	p-value	<b>&lt;0.0001</b>	0.866	0.8401						
Specific Leaf Area, 2007	r	-0.08	0.47	-0.12	-0.05	1				
	p-value	0.3089	0	0.1153	0.5115					
Leaf area, 2007	r	0.03	0.02	0.33	-0.07	0.13	1			
	p-value	0.7283	0.7668	<b>&lt;0.0001</b>	0.3278	0.0947				
Stomatal Density, 2012	r	0.26	0.04	0	0.28	0	0.05	1		
	p-value	<b>0.0006</b>	0.5834	0.9685	<b>0.0003</b>	0.9703	0.512			
Specific Leaf Area, 2012	r	0.05	0.31	-0.1	0.01	0.28	-0.05	0.18	1	
	p-value	0.5092	<b>&lt;0.0001</b>	0.1838	0.939	<b>0.0002</b>	0.5425	<b>0.0203</b>		
Leaf area, 2012	r	0	0	0.32	-0.05	0	0.39	-0.08	-0.22	1
	p-value	0.9567	0.9995	<b>&lt;0.0001</b>	0.5046	0.9834	<b>&lt;0.0001</b>	0.2723	<b>0.0043</b>	

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**Bottomland transplant habitat (2005 cohort):**

		Stomatal Density, 2006	Specific Leaf Area, 2006	Leaf Area, 2006	Stomatal Density, 2007	Specific Leaf Area, 2007	Leaf area, 2007	Stomatal Density, 2012	Specific Leaf Area, 2012	Leaf area, 2012
Stomatal Density, 2006	r	1								
	p-value									
Specific Leaf Area, 2006	r	0.12	1							
	p-value	0.4229								
Leaf Area, 2006	r	-0.04	-0.23	1						
	p-value	0.7674	0.0701							
Stomatal Density, 2007	r	0.41	0	-0.24	1					
	p-value	<b>0.0089</b>	0.9886	0.1341						
Specific Leaf Area, 2007	r	-0.34	0	-0.07	0.17	1				
	p-value	<b>0.0237</b>	0.9957	0.627	0.2699					
Leaf area, 2007	r	0.09	0.06	0.33	-0.1	-0.27	1			
	p-value	0.5693	0.6604	0.0209	0.5094	0.0512				
Stomatal Density, 2012	r	0.45	-0.15	0.03	0.25	-0.05	-0.22	1		
	p-value	<b>0.0083</b>	0.3936	0.851	0.16	0.754	0.1984			
Specific Leaf Area, 2012	r	-0.05	0.29	0	-0.17	0.21	-0.11	0.03	1	
	p-value	0.7828	0.0757	0.9858	0.3077	0.2015	0.5038	0.8764		
Leaf area, 2012	r	-0.04	0.06	0.19	0	-0.02	0.05	-0.44	0.05	1
	p-value	0.8142	0.7042	0.266	0.9798	0.8879	0.7651	<b>0.0089</b>	0.7562	

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808 **Table S2:** Repeated measures multivariate analyses of stomatal anatomy, specific leaf area, and  
809 leaf size from the 2005 and 2006 cohorts demonstrates phenotypic plasticity across time and  
810 space (habitat type). These models simultaneously evaluate all three traits and their interactions  
811 with growing season, transplant habitat, and source habitat (Proc Mixed, SAS ver. 9.4).  
812 Significant interactions between phenotype and other explanatory variables indicate that effects  
813 of habitat, season, life history and their interactions differ by trait. We used slice statements in  
814 SAS to examine plasticity separately for each trait. We assessed significance of the random  
815 effect of genotype and genotype by habitat via likelihood ratio tests by comparison of models  
816 with and without these effects ( $\chi^2$ , degrees of freedom = 1).

817 Our analysis of the 2005 cohort uncovered two unexpected interactions with source  
818 habitat. The interaction between transplant habitat and source habitat revealed that bottomland  
819 genotypes expressed greater spatial plasticity than upland genotypes for all three foliar traits.  
820 Here, we present plasticity as the contrast in standardized trait values between upland and  
821 bottomland transplant sites : stomatal density ( $F_{3,501.3}=7.89$ ,  $p<0.0001$ ; plasticity of bottomland  
822 genotypes :  $-0.60 \pm 0.16$ ,  $t_{472.8}=-3.83$ , Tukey's adjusted  $p=0.0009$ ; plasticity of upland genotypes:  
823  $-0.44 \pm 0.14$ ,  $t_{559.5}=-3.00$ ,  $p=0.015$ ); specific leaf area ( $F_{3,235.6}=198.5$ ,  $p<0.0001$ , plasticity of  
824 bottomland genotypes:  $1.96 \pm 0.1$ ,  $t_{237.1}=18.6$ ,  $p<0.0001$ ; than upland genotypes plasticity:  $1.65$   
825  $\pm 0.1$ ,  $t_{216.8}=15.8$ ,  $p<0.0001$ ); and leaf area ( $F_{3,513.3}=5.39$ ,  $p=0.0012$ ; plasticity of bottomland  
826 genotypes:  $-0.53 \pm 0.14$ ;  $t_{490}=-3.81$ ,  $p=0.0009$ ; plasticity of upland genotypes:  $-0.078 \pm 0.14$ ;  
827  $t_{458}=-0.57$ ,  $p=0.94$ ).

828 The interaction between source habitat and growing season revealed that bottomland  
829 genotypes expressed greater temporal plasticity than upland genotypes for two of the three foliar  
830 traits. For example, for stomatal density ( $F_{5,490}=3.81$ ,  $p=0.0021$ ), plasticity between the year with  
831 the greatest stomatal density (2006) and the year with the lowest average stomatal density (2012)  
832 was greater for bottomland genotypes ( $0.53 \pm 0.15$ ;  $t_{375.6}=3.58$ ,  $p=0.0051$ ) than upland genotypes  
833 ( $0.30 \pm 0.14$ ;  $t_{361.7}=2.24$ ,  $p=0.22$ ). Similarly, bottomland genotypes had greater temporal  
834 plasticity than upland genotypes in leaf area ( $F_{5,516.5}=10.1$ ,  $p<0.0001$ ; plasticity of bottomland  
835 genotypes from 2006 to 2012:  $-0.43 \pm 0.13$ ;  $t_{406.9}=-3.34$ ,  $p=0.012$ ; plasticity of upland  
836 genotypes:  $-0.31 \pm 0.12$ ;  $t_{415.3}=-2.51$ ,  $p=0.12$ ). Finally, the source habitat by season interaction  
837 for specific leaf area ( $F_{5,436}=48.18$ ,  $p<0.0001$ ) was not as straightforward. When we evaluated  
838 years separately, we found no difference in traits values between source habitats in 2006  
839 ( $t_{394.5}=0$ ,  $p=1$ ), 2007 ( $t_{445.8}=0.91$ ,  $p=0.94$ ), or 2012 ( $t_{566.1}=1.1$ ,  $p=0.88$ ). Therefore, this interaction  
840 may have arisen through only very slight shifts in the rankings of genotypes across seasons. For  
841 example, the shift in trait values between the year with the greatest SLA (2006) and the year with  
842 the lowest average SLA (2012) was slightly lower for bottomland genotypes (contrast in  
843 standardized trait values between 2006 and 2012:  $0.56 \pm 0.07$ ;  $t_{267.4}=7.6$ ,  $p<0.0001$ ) than upland  
844 genotypes (contrast in standardized trait values between 2006 and 2012:  $0.67 \pm 0.07$ ;  $t_{261.4}=9.42$ ,  
845  $p<0.0001$ ).

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Effect	2005 cohort		2006 cohort	
	F-value	p-value	F-value	p-value
Phenotype (P)	F <sub>3,421</sub> =49.4	< <b>0.0001</b>	F <sub>3,163</sub> =0.53	0.66
P × Season	F <sub>6,513</sub> =43.3	< <b>0.0001</b>	F <sub>3,146</sub> =17.22	< <b>0.0001</b>
P × Transplant habitat	F <sub>3,376</sub> =209.7	< <b>0.0001</b>	F <sub>3,111</sub> =62.7	< <b>0.0001</b>
P × Source habitat	F <sub>3,421</sub> =0.43	0.73	F <sub>3,163</sub> =3.33	<b>0.0211</b>
P × Transplant habitat × Season	F <sub>6,513</sub> =28.4	< <b>0.0001</b>	F <sub>3,146</sub> =2.68	<b>0.049</b>
P × Source habitat × Season	F <sub>6,513</sub> =2.48	<b>0.023</b>	F <sub>3,146</sub> =1.51	0.21
P × Transplant habitat × Source habitat	F <sub>3,376</sub> =3.3	<b>0.021</b>	F <sub>3,111</sub> =0.92	0.43
P × Transplant habitat × Source habitat × Season	F <sub>6,513</sub> =1.23	0.29	F <sub>3,146</sub> =0.41	0.75

	$\chi^2$	p-value	$\chi^2$	p-value
Genotype	8.3	<b>0.004</b>	12.8	<b>0.00035</b>
Genotype × Transplant habitat	18.2	< <b>0.0001</b>	0	1

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866 **Table S3:** Results of longevity selection (relative days until mortality) models of on foliar  
 867 phenotypes for the 2005 and 2006 cohorts. We assessed significance of the random effect of  
 868 genotype via likelihood ratio tests ( $\chi^2$ , degrees of freedom = 1). We used a corrected  $\alpha=0.025$   
 869 ( $=0.05/2$  sets of analyses) to assess statistical significance. For significant traits, we present  
 870 partial regression coefficients  $\pm$  standard errors to evaluate the magnitude of selection. It is  
 871 important to consider that we present unexponentiated coefficients from these Gamma  
 872 regressions, and undoubled quadratic coefficients. To estimate quadratic selection gradients ( $\gamma$ ),  
 873 partial regression coefficients and standard errors must be doubled (Stinchcombe et al. 2008).

	2005 cohort				2006 cohort			
	Partial regression coefficients $\pm$ S.E.	$\chi^2$	Df	p-value	Partial regression coefficients $\pm$ S.E.	$\chi^2$	Df	p-value
Transplant habitat	NA	7.795	1	<b>&lt;0.0001</b>	NA	3.703	1	<b>&lt;0.0001</b>
Specific leaf area	NA	0.007	1	0.932	See transplant habitat by Specific Leaf Area coefficients for each habitat	7.80	1	<b>0.005</b>
Stomatal Density	NA	0.347	1	0.556	NA	0.12	1	0.73
Leaf area	NA	0.579	1	0.447	0.10 $\pm$ 0.05	7.78	1	<b>0.005</b>
Transplant habitat $\times$ Specific Leaf Area	NA	1.276	1	0.259	Upland: -0.52 $\pm$ 0.18 Bottomland: 0.21 $\pm$ 0.17	5.15	1	<b>0.023</b>
Transplant habitat $\times$ Stomatal Density	Upland: 0.019 $\pm$ 0.032 Bottomland: 0.14 $\pm$ 0.034	9.027	1	<b>0.0027</b>	NA	1.82	1	0.18
Transplant habitat $\times$ Leaf Area	NA	2.839	1	0.092	NA	2.29	1	0.13
Stomatal density, quadratic effect	NA	0.026	1	0.873	NA	NA	NA	NA
Transplant habitat $\times$ Stomatal Density (quadratic)	Upland: 0.0026 $\pm$ 0.016 Bottomland: -0.074 $\pm$ 0.022	9.564	1	<b>0.0020</b>	NA	NA	NA	NA
Leaf area, quadratic effect	NA	NA	1	NA	-0.085 $\pm$ 0.023	12.984	1	<b>0.00031</b>
Specific Leaf area, quadratic effect	NA	NA	1	NA	-0.25 $\pm$ 0.078	10.138	1	<b>0.0015</b>
Genotype	NA	88.9	1	<b>&lt;0.0001</b>	NA	59.5	1	<b>&lt;0.0001</b>

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878 **Table S4:** Results of logistic regression models of viability (# individuals alive in April 2014/#  
 879 individuals planted per family) for the 2005 and 2006 cohorts. We assessed significance of the  
 880 random effect of genotype via likelihood ratio tests ( $\chi^2$ , degrees of freedom = 1). We used an  
 881 adjusted  $\alpha=0.025$  ( $=0.05/2$  traits) to correct for multiple testing.  
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	2005 cohort			2006 cohort		
	$\chi^2$	Df	p-value	$\chi^2$	Df	p-value
Transplant habitat	15.8	1	<b>&lt;0.0001</b>	0.98	1	0.32
Specific leaf area	2.06	1	0.15	6.93	1	<b>0.0085</b>
Stomatal Density	2.80	1	0.09	7.07	1	<b>0.0079</b>
Leaf area	9.61	1	<b>0.0019</b>	15.66	1	<b>&lt;0.0001</b>
Transplant habitat × Specific Leaf Area	10.71	1	<b>0.0011</b>	3.35	1	0.067
Transplant habitat × Stomatal Density	7.39	1	<b>0.0066</b>	4.33	1	0.037
Transplant habitat × Leaf Area	2.4	1	0.12	7.13	1	<b>0.0076</b>
Leaf area, quadratic effect	NA	NA	NA	4.26	1	0.039
Specific leaf area, quadratic effect	NA	NA	NA	4.57	1	0.033
Genotype	0.10	1	0.75	3.72	1	0.054

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901 **Table S5:** Selection on plasticity for the 2005 and 2006 cohorts. Analyses evaluated selection in  
 902 models that included trait values averaged across environments and plasticity in those traits. We  
 903 incorporated quadratic effects of plasticity terms if preliminary models indicated nonlinear  
 904 selection. We modeled population of origin as a random effect, assessing significance via  
 905 likelihood ratio tests ( $\chi^2$ , degrees of freedom = 1). We used an adjusted  $\alpha=0.025$  ( $=0.05/2$  traits)  
 906 to correct for multiple testing across two cohorts. For significant traits, we present partial  
 907 regression coefficients  $\pm$  standard errors to evaluate the magnitude of selection. It is important to  
 908 consider that we present unexponentiated coefficients from these Gamma regressions, and  
 909 undoubled quadratic coefficients. To estimate quadratic selection gradients ( $\gamma$ ), partial regression  
 910 coefficients and standard errors must be doubled (Stinchcombe et al. 2008).  
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	2005 cohort				2006 cohort			
	Partial regression coefficients $\pm$ S.E.	$\chi^2$	Df	p-value	Partial regression coefficients $\pm$ S.E.	$\chi^2$	Df	p-value
Mean Specific leaf area across environments	NA	1.011	1	0.315	NA	1.36	1	0.2
Mean Stomatal Density, across environments	NA	0.182	1	0.67	NA	1.17	1	0.2
Mean Leaf area, across environments	NA	0.128	1	0.721	NA	0.011	1	0.9
Plasticity in Specific Leaf Area	3.03 $\pm$ 1.12	7.339	1	<b>0.0067</b>	0.65 $\pm$ 0.26	6.38	1	<b>0.01</b>
Plasticity in Stomatal Density	NA	0.247	1	0.619	0.27 $\pm$ 0.15	3.21	1	0.07
Plasticity in Leaf Area	NA	2.542	1	0.111	NA	2.57	1	0.1
Plasticity in Specific Leaf Area, quadratic effect	-4.26 $\pm$ 1.8	6.347	1	<b>0.012</b>	NA	NA	NA	N.
Plasticity in Stomatal Density, quadratic effect	NA	NA	NA	NA	-1.30 $\pm$ 0.45	8.31	1	<b>0.003</b>
Source population	NA	1.89	1	0.17	NA	0	1	

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925 **Table S6:** Selection on plasticity for the 2005 and 2006 cohorts via viability (# individuals alive  
926 in April 2014/# individuals planted per family). Genotypic selection analyses evaluated selection  
927 in models that included mean trait values and plasticity in those traits. We incorporated quadratic  
928 effects of plasticity terms if preliminary models indicated nonlinear selection. We used an  
929 adjusted  $\alpha=0.025$  ( $=0.05/2$  traits) to correct for multiple testing across two cohorts.  
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	2005 cohort			2006 cohort		
	$\chi^2$	Df	p-value	$\chi^2$	Df	p-value
Specific leaf area	1.28	1	0.26	0.44	1	0.51
Stomatal Density	0.079	1	0.78	0.026	1	0.87
Leaf area	0.31	1	0.58	0.5	1	0.48
Plasticity in Specific Leaf area	1.33	1	0.25	0.15	1	0.7
Plasticity in Stomatal Density	0.003	1	0.96	1.97	1	0.16
Plasticity in Leaf Area	0.012	1	0.91	1.15	1	0.28
Source population	0	1	1	0	1	1

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956 **Table S7:** Selection on plasticity for the 2005 and 2006 cohorts within upland forests only.  
957 Analyses evaluated longevity within the uplands as a function of trait values averaged within the



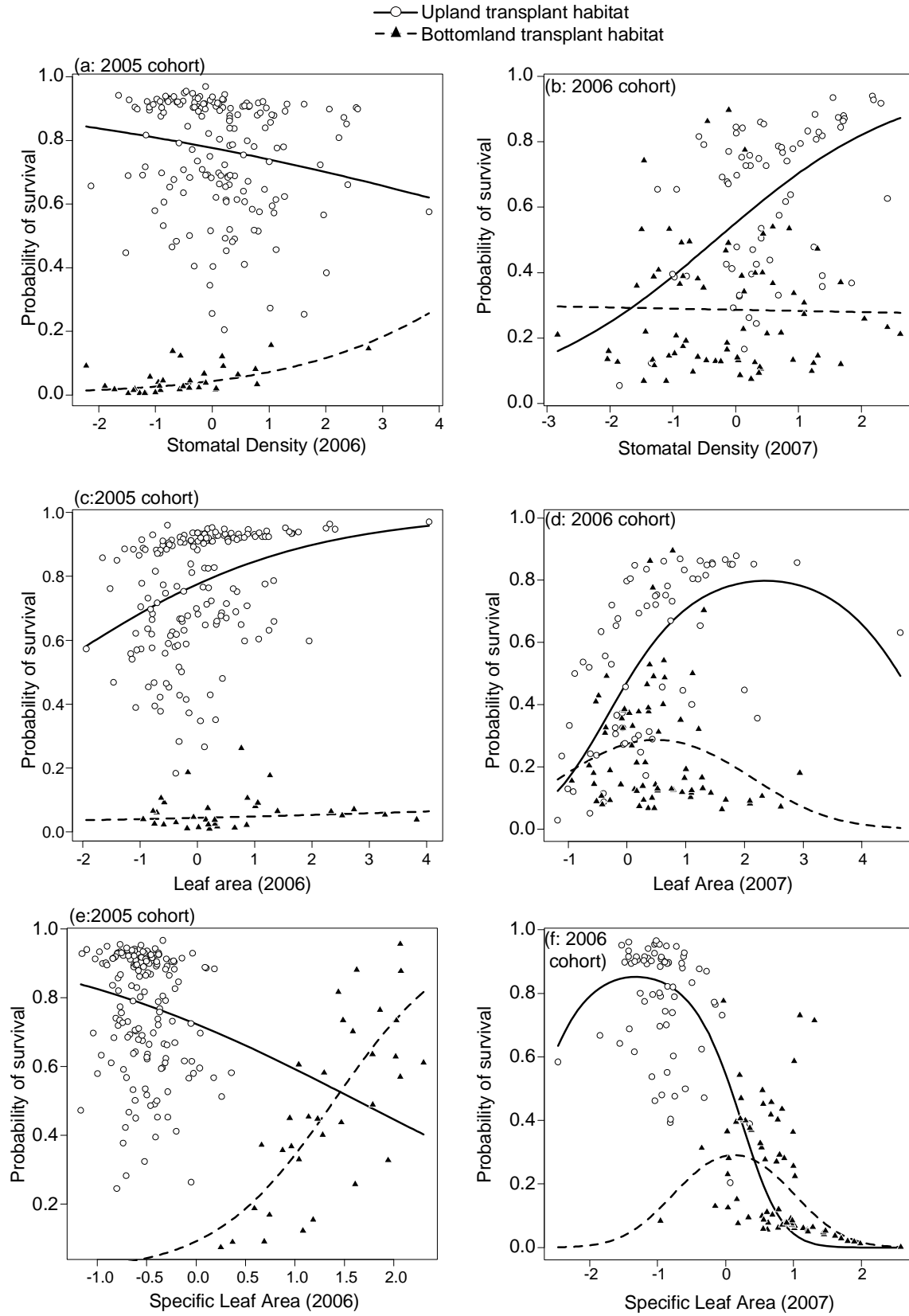
958 upland and spatial plasticity in those traits. We incorporated quadratic effects of plasticity terms  
 959 if preliminary models indicated nonlinear selection. We modeled population of origin as a  
 960 random effect, assessing significance via likelihood ratio tests ( $\chi^2$ , degrees of freedom = 1). We  
 961 used an adjusted  $\alpha=0.025$  (=0.05/2 traits) to correct for multiple testing across two cohorts. For  
 962 significant traits, we present partial regression coefficients  $\pm$  standard errors to evaluate the  
 963 magnitude of selection. It is important to consider that we present unexponentiated coefficients  
 964 from these Gamma regressions, and undoubled quadratic coefficients. To estimate quadratic  
 965 selection gradients ( $\gamma$ ), partial regression coefficients and standard errors must be doubled  
 966 (Stinchcombe et al. 2008).  
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	2005 cohort				2006 cohort			
	Partial regression coefficients $\pm$ S.E.	$\chi^2$	Df	p-value	Partial regression coefficients $\pm$ S.E.	$\chi^2$	Df	p-value
Specific leaf area, upland average	NA	2.5	1	0.114	NA	0.84	1	0.3
Stomatal Density, upland average	NA	1.71	1	0.19	NA	0.58	1	0.4
Leaf area, upland average	NA	0.37	1	0.544	NA	0.26	1	0.6
Plasticity in Specific Leaf area	$3.4 \pm 1.5$	5.36	1	<b>0.0206</b>	$1.95 \pm 0.72$	7.38	1	<b>0.006</b>
Plasticity in Stomatal Density	$0.72 \pm 0.24$	8.65	1	<b>0.0033</b>	$0.58 \pm 0.34$	2.93	1	0.087
Plasticity in Leaf Area	NA	2.61	1	0.106	NA	0.02	1	0.
Plasticity in Specific Leaf area, quadratic	$-4.8 \pm 2.5$	3.55	1	<i>0.0596</i>	NA	NA	NA	N.
Plasticity in Stomatal density, quadratic	NA	NA	NA	NA	$-2.2 \pm 0.95$	5.34	1	<b>0.020</b>
Population of origin	NA	2.95	1	<i>0.086</i>	NA	0	1	

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**Figure S1:** Logistic regressions evaluating viability selection on foliar traits in upland and bottomland forests for the 2005 and 2006 cohorts. For the 2005 cohort, (a) selection favored reduced stomatal density in the upland forests and increased stomatal density in the bottomlands, which contrasted with (b) the 2006 cohort when selection favored greater stomatal density in the uplands with no patterns in the bottomlands. For the 2005 cohort (c), selection favored larger leaves in both sites. Quadratic selection (d) favored large leaves in the uplands in the 2006 cohort, and intermediate sized leaves in the bottomlands during the first year of trait measurement. Divergent selection favored lower specific leaf area in uplands and higher specific leaf area in the bottomlands for both cohorts (e for the 2005 cohort and f for the 2006 cohort). This divergent selection on specific leaf area was congruent with patterns of trait plasticity (Fig. 1).