EVOLUTION OF FLOODING TOLERANCE IN A SPATIALLY AND TEMPORALLY HETEROGENEOUS LANDSCAPE

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EVOLUTION OF FLOODING TOLERANCE IN A SPATIALLY AND TEMPORALLY HETEROGENEOUS LANDSCAPE

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This dissertation explores plant adaptations to flooding and drought stress. In the first study, I assessed flooding tolerance as a function of life history stage in *Itea virginica*, a species of shrub from cypress tupelo forests of the United States. Results from this study indicate that limited flooding tolerance of juveniles restricts the distribution patterns of adults (Chapter 1).

In a complementary study, I assessed adaptive evolution in Elliott's blueberry (*Vaccinium elliottii*). In heterogeneous landscapes, natural selection can result in the evolution of locally adapted ecotypes. However, if habitats differ in size or quality, demographic source-sink dynamics can shape the evolutionary trajectory of species. I conducted a multiyear reciprocal transplant experiment to test whether *V. elliottii* is locally adapted to upland and floodplain forests in South Carolina. These contrasting habitats vary tremendously in water table depth, light levels and edaphic conditions. In the greenhouse, I exposed individuals to drought and flooding to assess selection on traits in response to disparate abiotic stresses. Finally, I quantified population differentiation and gene flow via microsatellite markers.

V.elliottii families exhibited significantly higher fitness in upland relative to floodplain forests, regardless of the habitat of origin. Similar results from the greenhouse show that *V. elliottii* is better adapted to drought than flooding. The

population density of this species is higher in upland than floodplain forests and upland populations harbor significantly greater genetic diversity. This disparity in population size produces asymmetrical gene flow from upland to floodplain populations. These patterns are consistent with genetic source-sink dynamics, in which adaptation to a marginal habitat is constrained by immigration from a benign habitat (Chapter 2).

V. elliottii exhibits significant phenotypic plasticity in foliar, ecophysiological and root-based traits. Theoretical models predict that under source-sink dynamics, species evolve traits that maximize fitness in the source habitat at the expense of fitness in the sink habitat. The phenotypic plasticity expressed by this species contradicts this expectation; in Chapter 3, I discuss three hypotheses that could resolve this paradox: phenotypic plasticity could be a phylogenetic legacy, this species could be undergoing niche expansion, or plasticity could be adaptive within upland forests.

BIOGRAPHICAL SKETCH

Jill Anderson was born in Chicago, Illinois in 1976. She developed an early interest in ecology through habitat restoration efforts in tallgrass prairies. She studied biology as an undergraduate at Brown University, where her honor's thesis focused on mating behavior of crab spiders (*Misumena vatia*, Thomisidae). After earning her Bachelor's in Science in 1998, she conducted research in tropical South America prior to beginning graduate school. During this time, she worked as a plant taxonomist in a 50 hectare plot in Yasuni National Forest, Ecuador for 1.5 years and near Cobjia, Bolivia for 6 months. Additionally, she served as a research assistant for 6 months on a project on behavioral ecology of capuchin monkeys (*Cebus capucinus*, Cebidae).

She joined the Ecology and Evolutionary Biology Department at Cornell University to pursue a Ph.D. in 2001. Her research focused on adaptive evolution and abiotic stress tolerance in woody plant species. While at Cornell, she also investigated seed dispersal by fruit-eating fishes (*Colossoma macropomum* and *Piaractus brachypomus*) and seedling regeneration in Pacaya-Samiria reserve, Peru. She hopes to continue her studies of constraints on adaptation, especially in relation to anthropogenic disturbance. She will begin a postdoctoral position at Duke University in January 2009.

To my husband, Thomas Pendergast,

and my mom, Rinda West,

for their love and support.

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CHAPTER 1

LIMITED FLOODING TOLERANCE OF JUVENILES RESTRICTS THE DISTRIBUTION OF ADULTS IN AN UNDERSTORY SHRUB (*ITEA VIRGINICA*)

Summary

Juvenile plants often have tight microhabitat associations due to specific requirements for germination and establishment. In contrast, adults are frequently more adept at coping with stress, which can result in ontogenetic expansions in niche breadth. In cypress-tupelo swamps of the United States, most understory plant species grow on microsites elevated above floodwaters; *Itea virginica* (Iteaceae) exemplifies this distributional pattern. In a field survey, we found that more than 98% of *Itea* seedlings occurred on elevated microsites. However, the strict microhabitat association of *Itea* relaxed through ontogeny; nearly 8% of subadults and adults were rooted directly on the forest floor. We hypothesized that flooding inhibits juvenile establishment, but not adult growth. In a series of greenhouse experiments, we investigated the effects of ontogenetic stage, substrate type and flooding on *Itea* performance. Seeds had similar germination rates on drained swamp soil and wood from cypress knees. Seedling growth was high on unflooded soil, but declined precipitously when seedlings were submerged. Finally, seedling, but not adult, performance decreased with flood severity. Our results indicate that the limited flooding tolerance of juveniles restricts adults to elevated microsites. Understanding distribution patterns may require explicit consideration of niche breadth and stress tolerance of various ontogenetic stages.

Introduction

Determining the causes and consequences of the distribution patterns and habitat associations of species is a fundamental goal of ecology. Limited distribution can be a function of constraints on both dispersal and establishment (e.g. Moore and

Elmendorf, 2006). Furthermore, niche breadth can expand or shift through life history, though little is known about how ontogenetic changes in niche influence the distribution of plant species (Parrish and Bazzaz, 1985; Dalling et al., 2001; Eriksson, 2002; Miriti, 2006). The paucity of empirical studies on the importance of life history in defining the niche of plants contrasts starkly with the numerous studies of animals, where ontogenetic shifts in diet, morphology and trophic interactions have been described (e.g. Sillett and Foster, 2000; Post, 2003). Nevertheless, the importance of the regeneration niche (sensu Grubb, 1977) is readily appreciated for plants, and it is clear that plants can experience substantially different microsite conditions at different sizes (Clark and Clark, 1992). In particular, juveniles may be more susceptible to abiotic or biotic stress than adults, or may require greater resource availability to transition successfully into older life history stages (e.g. Dalling *et al.*, 2001). Limitations to juvenile establishment could restrict adult distribution, even if adults are capable of tolerating a broader range of conditions (Dalling *et al.*, 2001).

Environmental conditions optimal for germination can be detrimental for seedling survivorship, leading to seed-seedling conflicts (Lamont *et al.*, 1993; Schupp, 1995; Battaglia *et al.*, 2000). Such ontogenetic conflicts may also be present during other life history transitions, such as that between juveniles and adults (Comita *et al.*, 2007; Menges and Marks, 2008), perhaps due to changing physiological needs. Indeed, plants can vary in light requirements and water-use through life history (Donovan and Ehleringer, 1992; Dalling et al., 2001). Size influences the way in which individuals interact with the environment for both animals and plants (Donovan and Ehleringer, 1992; Sillett and Foster, 2000). For example, in arid habitats, adults with deep taproots are more adept at exploiting stable water sources than are shallowly-rooted seedlings (Donovan and Ehleringer, 1992); thus conditions suitable for adults may be inhospitable for juveniles.

Juveniles often have strict microhabitat requirements, and microtopography in the landscape can facilitate establishment by alleviating environmental stresses (Dovčiak *et al.*, 2003; Flinn, 2007). In this study, we hypothesize that the regeneration niche restricts the distribution patterns of a species of shrub in cypress-tupelo swamps of the southeastern United States. Floodplain forests are good study systems for assessing ontogenetic shifts in microhabitat association because of the high degree of microtopography and seasonal fluctuation in floodwaters (Huenneke and Sharitz, 1986; Battaglia and Sharitz, 2006). These factors expose plants to varying levels of flooding intensity (duration, depth and frequency) based on whether they are rooted on microsites elevated above the water or on the forest floor (Battaglia and Sharitz, 2006). Indeed, understory species can escape long-term flooding by growing on fallen logs, living cypress knees, tree stumps and other elevated microsites.

Flooding is a severe stress for plants. It deprives roots of oxygen, resulting in a shift from aerobic respiration to anaerobic fermentation, the accretion of large quantities of toxic byproducts of fermentation and a reduction in photosynthesis and stomatal conductance (Keeley, 1979; Blokhina *et al.*, 2003; Mielke et al., 2003; Visser et al., 2003). Plants have evolved a variety of adaptations to mitigate these stresses by increasing oxygen availability to the roots. Flooding can induce the formation of traits such as adventitious roots with high porosity (Parolin, 2001; Li *et al.*, 2006) and hypertrophied lenticels to enhance gas exchange (Kozlowski, 2002). Additionally, flooding can alter the root to shoot ratio as deep roots senesce and new roots are produced above floodwaters and at the air-water interface (Keeley, 1979; Megonigal and Day, 1992; Blom and Voesenek, 1996). Similar to plants in arid environments, the size of individuals in floodplain forests influences tolerance to water stress (Nabben *et al.*, 1999). Indeed, juveniles could be particularly flood intolerant if they

are submerged or mostly submerged during floods. Complete submergence virtually eliminates gas exchange and can result in rapid mortality (Nabben *et al.*, 1999; Souther and Shaffer, 2000; Visser et al., 2003; Jackson, 2008). Submergence and flooding are strong ecological filters that influence community composition (Battaglia *et al.*, 2000). To date, however, no study has investigated whether the relative flooding tolerance of juveniles and adults affects microhabitat associations of floodplain species.

We focused this study on *Itea virginica* L. (Iteaceae; hereafter *Itea*), a perennial understory shrub that occurs in cypress-tupelo swamps in the southeastern United States and reaches a stature of 1 – 2 m tall (Radford *et al.*, 1968). When cultivated in gardens, it grows well under a wide range of moisture conditions and across a variety of soil types (Scheiber *et al.*, 2008, also see record in United States Department of Agriculture: The PLANTS database http://plants.usda.gov/). This flexibility raises questions about the more restricted distribution of *Itea* in the wild. Specifically, mature individuals typically grow on microsites that are elevated above the floodwaters, such as on cypress knees (living root structures of cypress trees, Figure 1.1), fallen logs, and tree buttresses (Schlesinger, 1978; Huenneke and Sharitz, 1986). However, the roots of adult plants often extend from elevated sites through flood waters into forest floor soil (pers. obs.); as such, it is clear that roots of adults can withstand prolonged flooding. We hypothesize that flooding tolerance increases through life history, and that the germination and establishment requirements of juveniles limit adults to elevated sites. To explore these hypotheses, we: 1) quantified the distribution patterns of several life history stages of *Itea* in the field; 2) tested seed germination and seedling growth on different substrates and flooding conditions; and 3) compared the performance of seedlings and cuttings under various degrees of water stress in the greenhouse.

Figure 1.1: *Itea virginica* adult growing on a living cypress knee (*Taxodium distichum* var. *distichum*) in Francis Beidler Forest (Four Holes Swamp, S.C.). Adults are 1-2 m tall. Photo credit: Alicia Landi.

Materials and Methods

Study System—Four Holes Swamp is a diffuse brown-water floodplain system with no discernable river channel in the coastal plain of South Carolina; the system is approximately 97 km long and averages 1.5 to 2.5 km wide (Porcher, 1981). We conducted the field component of this study at Francis Beidler Forest in Four Holes Swamp (33°13'N 80°20'W), which contains one of the largest stands of original-growth cypress-tupelo swamp forest in the United States (Porcher, 1981). Standing water covers the swamp floor for most of the year to a depth of >1 m, but there is an appreciable drop in water level during the summer (30 year unpublished water level data at Beidler forest, N. Brunswig and M. Dawson). When water level is low, the forest floor is partially exposed and colonized by woody seedlings and herbaceous plants until the floods return (J. Anderson, pers. obs.). Bald cypress (*Taxodium*

distichum var. *distichum*) and water tupelo (*Nyssa aquatica*) provide the structural framework underlying the distribution and abundance of other plant species in the community (Porcher, 1981; Huenneke and Sharitz, 1986). Their trunks, roots, knees and buttresses as well as fallen logs create the various elevated conditions present in the system, on which numerous understory shrub and herbaceous species grow (Huenneke and Sharitz, 1986).

Distribution pattern—To quantify distribution patterns, we established 15 transects of 100 m × 10 m in five sites on the Beidler forest property (2-4 transects per site). At 1 m increments, we dropped a surveyor's pin 0.75 m to the right of the transect tape and recorded whether the pin fell on an elevated microhabitat, or on the forest floor. We recognized five microsites as elevated: tree stumps, cypress knees, fallen logs, fallen branches, and the trunks of living trees. We extensively censused the entire 1000 m^2 area of each transect to locate all *Itea* individuals from newly-germinated seedlings to adults. We recorded the life history stage and the microsite on which each individual was rooted. *Itea* plants were classified as: seedlings (cotyledons present, 0-5 true leaves, n=6912), saplings (height >5cm, but <50cm, n=266), subadults and adults (height >50 cm, often with reproductive structures, n=201). When an individual was found growing directly from the forest floor, we surveyed the surrounding area to determine whether there was any evidence of a decaying log or cypress knee; such evidence could suggest that the individual germinated on an elevated microsite, which subsequently degraded. All analyses were conducted in SAS/STAT (v. 9.1.3, SAS Institute, Cary, North Carolina) unless otherwise noted. We performed a Chi-square goodness-of-fit test (Proc Freq) to test whether *Itea* plants were distributed randomly given the frequency of elevated microsites vs. forest floor and a Chi-square test of independence to determine whether seedlings were more likely to grow on elevated sites than adults.

Seed Experiment 1: Substrate—To investigate the effects of substrate on germination rate, we planted seeds in Petri dishes in three treatments at Cornell University: moist paper towel control, swamp soil, and pieces of intact epidermis and vascular tissue from cypress knees. We collected soil and dead cypress knees from the field and kept them at room temperature with ample light and water for three weeks to ensure that any seeds they contained would germinate. In no case did we recover *Itea* germinants. We spread each substrate in ten Petri dishes and placed twenty *Itea* seeds collected from the field directly on bark, soil, or control substrate in each dish (n=600 seeds total distributed evenly in 30 Petri dishes). The substrates were kept moist and monitored for 65 days. The number of germinants was assessed four times before the termination of the experiment.

Seed Experiment 2: Flooding—The second seed experiment investigated the effects of flood duration on germination. We flooded *Itea* seeds for: 1) seven weeks, 2) four weeks, or 3) zero weeks (control). Flooded seeds (treatments 1 and 2) were wrapped in a moist paper towel and sunk under water in a vial for the specified amount of time. After the initial treatment, all seeds were placed on moist paper towel in Petri dishes (n=20 seeds per Petri dish, 10 Petri dishes per treatment, overall n=600 seeds). We monitored these seeds for 69 days. Survivorship analyses (Proc TPHREG, SAS v. 9.1.3) were used to address whether germination rate was a function of substrate (seed experiment 1) or duration of flooding (seed experiment 2).

Seedling establishment: Substrate by flooding—To test the combined effects of flooding and substrate on seedling establishment, we planted seeds on forest floor substrate and pieces of cypress knees in plastic containers in March 2007 (4 cm deep; 7.5 cm diameter; n=13 seeds/ container; 20 containers/substrate). When approximately 3 seedlings had germinated in each container (end of April 2007), we flooded half of the experimental units and continued to water the other half normally

(n=10 containers in each treatment: soil flooded, soil control, wood flooded, wood control). At the initiation of flooding, seedlings were uniformly small (under 0.5 cm in height), and had two cotyledons, but no true leaves. We monitored the seedlings for 160 days after imposing the flooding treatment. This experiment allowed us to assess whether flooding inhibits early seedling establishment, and whether soil from the forest floor promotes seedling establishment. We used an unequal variance mixed model (Proc Mixed) to assess final seedling biomass as a function of soil substrate, water level, and their interaction, with block (i.e. container) as a random effect.

Life history stage by flooding experiment—It was not logistically possible to transplant adult *Itea* individuals to elevated and forest floor microsites in the field because of their large size (>1-2 m tall). Instead, we conducted a greenhouse experiment to compare the flooding tolerance of seedlings and cuttings made from adults. Similar to adults in nature, the cuttings had adult tissue and were large enough to maintain most of their biomass above the water level in both the waterlogged and the flooded treatments. We collected cuttings from reproductive individuals in the field in October 2005, applied rooting hormone (Rhizopon AA #2, 0.3% IBA, Rhizopon bv, Hazerswoude, Holland) and placed them under a misting system at Cornell University until their roots established (approximately 30 days). Cuttings were then grown in favorable greenhouse conditions for up to 4 months until the beginning of the experiment. Seedlings were germinated from seeds collected at the field site and grown for 3-4 months before beginning the experiment.

We hypothesized that seedlings and adults would show differences in response to flooding either because smaller individuals have lower stress thresholds than larger individuals regardless of life history stage, or because adults are more adept at producing flood-induced phenotypes than seedlings regardless of size. We therefore varied both life history stage (seedling vs. adult) and initial height within life history

stage. Prior to the initiation of the experiment, we sorted the plants by initial height, which ranged from 3 to 58 cm for cuttings and from 1 to 8.5 cm for seedlings. Experimental plants were randomly assigned to one of three treatments to maximize variation in initial height in each treatment: 1) control (well-watered, but drained), 2) waterlogged (water level 1 cm above soil), and 3) flooded (water 6 cm above soil level for seedlings; 9 cm for cuttings). There was no significant difference between treatments in the initial heights of seedlings ($F_{2,195}$=0.27, p=0.76) or cuttings ($F_{2,128}$=0.76, p=0.47).

Seedlings and adults were planted in conetainers of different sizes (seedlings: SC10 Super Cell conetainers: volume=164 mL, diameter =3.8 cm, depth=21 cm; adults: Deepot D25L: volume =410 mL, diameter=5 cm, depth =25cm, cut to 21 cm for this study, Stuewe and Sons, Inc., Corvallis, Oregon, USA). Due to the different sized conetainers, we placed seedlings and cuttings in separate trays, but on the same bench in the greenhouse. Each block consisted of a seedling- or adult-sized conetainer tray cut to fit within a 14 gallon plastic storage bin (Rubbermaid Home Products, Fairlawn, Ohio USA). We drilled drainage holes into the bottom of the control bins, but not the other treatments. We planted 33-34 seedlings and 21-24 cuttings in two bins for each treatment (n=131 adults and 200 seedlings distributed among 2 adult and 2 seedling control bins, 2 adult and 2 seedling waterlogged bins, and 2 adult and 2 seedling flooded bins, n=12 bins total).

To simulate increasing flood levels in the field, we incrementally raised the water level in the waterlogged and flooded treatments by 7 cm every two days; the treatments reached their final flood level within a week and these levels were maintained throughout the experiment. Control plants were watered daily and water was added to the waterlogged and flooded bins routinely to maintain treatment levels and oxygenate the water. We fertilized all of the treatments, rotated, drained and

cleaned the bins every two weeks. The adult experiment was started July 10-13 2006, 50 days before the seedling experiment (August 28, 2006) because seedlings were not ready for transplantation from germination flats at the earlier date. At the end of the experiment (144-147 days adults, 94-95 days seedlings), we recorded mortality, harvested above- and below-ground biomass, and quantified the proportion of stem covered by enlarged lenticels (relative to the final height of the plant), and adventitious root biomass.

We calculated relative growth rate (RGR) as: RGR= (ln(final biomass)-ln(initial biomass))/elapsed time, where initial biomass was estimated based on allometry determined from seedlings and cuttings sacrificed before the beginning of the experiment. Representative plants were measured and sacrificed to obtain a relationship between stem diameter, height and biomass. Many cuttings had two main stems; there was a significant relationship between diameters 1 and 2, and total biomass ($F_{2,25}$= 64.68, $p<0.0001$, R^2=0.825, n=28):

$$(\text{biomass of cutting})^{0.25} = 0.19 + (0.212 \times \text{diameter of largest stem}) + (0.0708 \times \text{diameter of second largest stem})$$

We measured the diameters at the base of both stems before the initiation of the experiment, and used these values to estimate initial biomass of adults included in the study. For seedlings, biomass varied significantly with height, diameter at the base of the stem, and the number of leaves ($F_{3,64}$= 104.4, $p<0.0001$, R^2=0.83, n=68):

$$(\text{seedling biomass})^{0.5} = 0.037 + (0.052 \times \text{diameter}) + (0.023 \times \text{height}) + (0.00213 \times \text{leaves})$$

Data analysis: life history stage by flooding experiment—*Mortality*—We conducted a logistic regression with block as a random statement to test the effects of treatment, life history stage, and their interaction on mortality (Proc Glimmix). This model did not converge due to quasi-separation of data points; therefore, we

implemented Firth's procedure (Heinze and Schemper, 2002) in LogXact (version 8.0, Cytel Inc., Cambridge, Massachusetts, USA). LogXact is not capable of accommodating random effects while performing Firth's procedure, so we included block as a fixed effect.

Growth and phenotype—We conducted multivariate analyses of variance (MANOVA, Proc Mixed) to test the effects of treatment, life history stage and the two-way interaction on several response variables: 1) RGR, 2) lenticel height (relative to final height), 3) adventitious root biomass (relative to total root biomass), and 4) the ratio of root to shoot biomass (natural log transformed). The transformation was made to improve normality and homoscedasticity of the residuals. We included a random statement for life history by treatment nested within block and modeled this random effect with a compound symmetry covariance matrix (Littell, Henry, and Ammerman, 1998). Since the MANOVA was highly significant, we subsequently ran a series of univariate ANOVAs (Proc Mixed) for each of the four response variables (Scheiner, 2001). Tukey's multiple comparison tests were performed to compare traits across the treatments and life history stages. We were also interested in whether performance under flooding increased with plant size. An ontogenetic shift in flooding tolerance could occur because larger individuals maintain a greater proportion of their biomass above the water level and can oxygenate their roots more effectively. Therefore, we also tested the effect of initial height, life history stage, treatment, and all two and three way interactions on RGR.

Results

Field Study—*Itea* showed a highly significant association with elevated microsites (χ^2 =2598.6, d.f.=1, p<<0.0001). Elevated microsites comprised only 26.7% of the swamp landscape, yet > 99% of the 7379 *Itea* plants were rooted on this microhabitat (Figure 1.2). Furthermore, there was a significant change in distribution

pattern through life history, such that older life history stages were more likely to occur on the forest floor than seedlings (χ^2 =272.7, d.f.=2, p<<0.0001, Figure 1.2). We found no evidence for decaying logs in the immediate vicinity of individuals growing from the forest floor. Rather, these individuals appeared to have germinated and established directly on the forest floor.

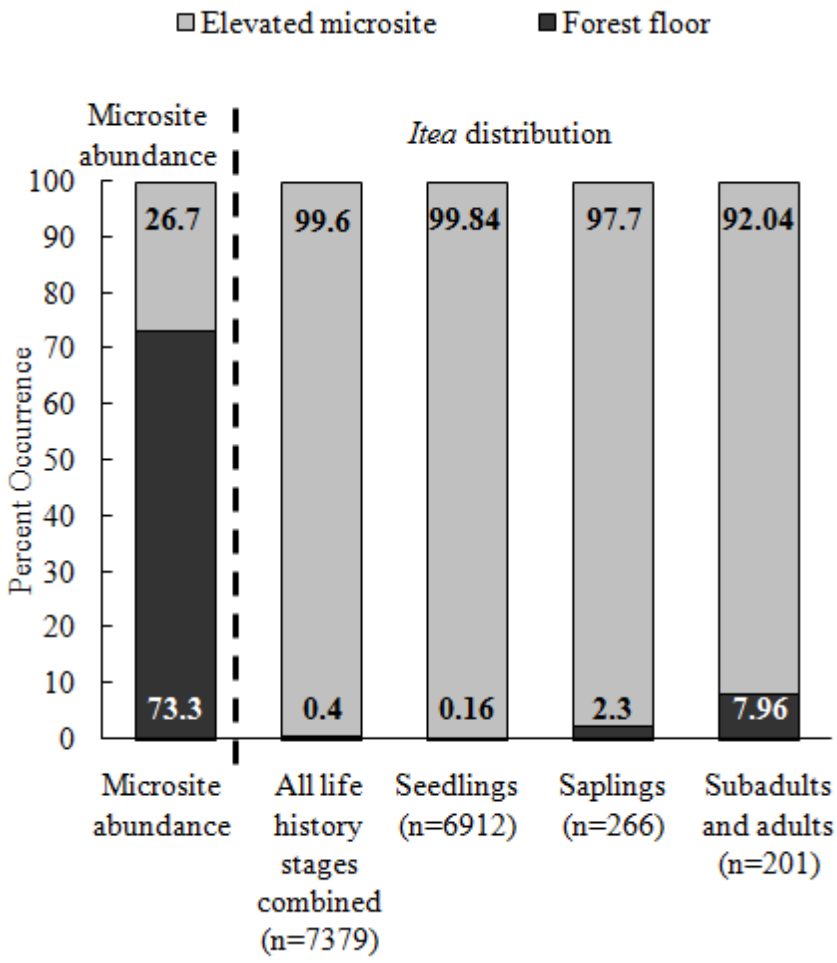

Figure 1.2: Microsite abundance and distribution patterns of *Itea virginica* seedlings, saplings, and subadults and adults, sampled in 15 transects (1000 m^2/transect).

Seed Experiment 1:Substrate—There was no significant difference in germination of seeds planted on cypress knee wood, forest floor soil, or the control substrate (χ^2 =0.14, d.f.=2, p=0.93, Figure 1.3a).

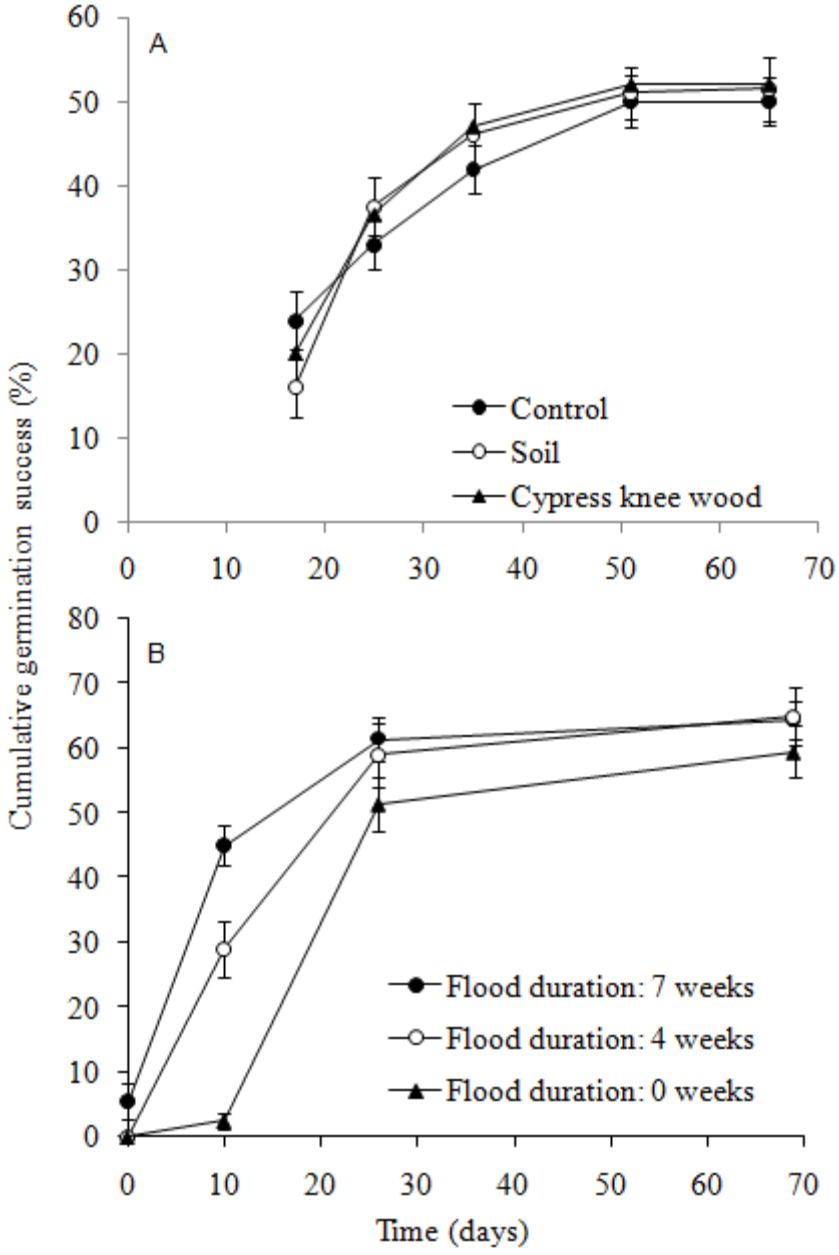

Figure 1.3: The effects of substrate (A; seed experiment 1) and flooding duration (B; seed experiment 2) on *Itea* germination success. The means (± S.E.) across Petri dishes are plotted for both seed experiments 1 and 2.

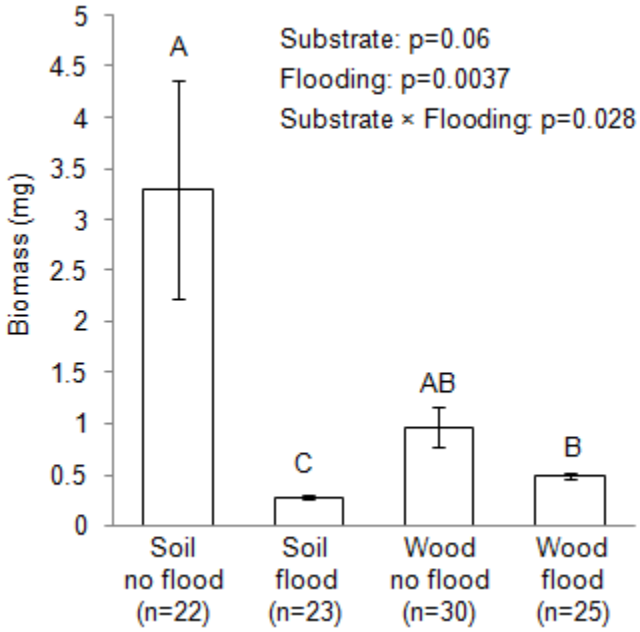

Figure 1.4: The effect of substrate and flooding treatment on growth (mean biomass ± S.E.) of newly emerged seedlings. Letters represent significantly different contrasts after correction for multiple tests.

Seed Experiment 2:Flooding—Although there was no significant difference in final germination success across treatments (χ^2 =1.58, d.f.=2, p=0.45), seeds soaked for 4 or 7 weeks germinated significantly faster than control seeds (χ^2 =12.98, d.f.=2, p=0.0015, Figure 1.3b).

Seedling establishment: Substrate by flooding—Mortality in this experiment was very low; only two seedlings died, and they were both in the flooded treatment on soil substrate. Similarly, very few seeds germinated after flooding was imposed; only 3 germinated on flooded wood substrate, and 5 germinated on unflooded soil of 417 potentially viable seeds. Newly recruited individuals were not included in the analysis of final seedling biomass because they had a shorter period for growth. However, the results are qualitatively similar if we leave the new recruits in the dataset. Final seedling biomass was significantly influenced by flooding treatment ($F_{1,23}$=10.5, p=0.0037) and the interaction between substrate and treatment ($F_{1,23}$=5.5, p=0.028),

but the effect of substrate was marginal ($F_{1,23}$=3.8, p=0.06, Figure 1.4). Flooding depressed seedling growth more on soil than on wood substrate. The contrast between seedlings growing on wood and soil in the unflooded treatment was nonsignificant after Tukey's adjustment (p=0.13).

Life history stage by flooding experiment—*Mortality Analysis*—Mortality in the greenhouse experiment was low (5.2%), but was distributed nonrandomly between treatments (χ^2 =357, d.f.=5, p<0.0001, Table 1). There were significant effects of treatment (natural log of odds ratio $\pm$ SE: -7.1$\pm$ 2.4, p=0.0025), life history stage (-6.9$\pm$ 2.4, p=0.0047) and the interaction between treatment and life history stage (7.7 $\pm$ 2.6, p=0.0031). Seedling mortality increased with flooding level, whereas adult mortality was greatest in the control treatment. Only one cutting died in the flooded treatment and it was initially short (3 cm) and submerged.

Table 1.1: Mortality results from the life history stage by flooding experiment. Mortality is expressed as the percentage of individuals that died relative to the number included in each life history stage by treatment block.

Life history stage	Treatment		
	Control	Waterlogged	Flooded
Adult	6.4	0	2.1
Seedling	0	7.5	11.9

Performance and trait-based analyses—The MANOVA indicated a significant effect of treatment, life history stage, and the interaction between life history and treatment on relative growth rate, lenticel height, adventitious root production, and root:shoot (Table 1.2). Below, we present the results of univariate analyses for these traits (Table 1.3, Figure 1.5).

Relative Growth Rate—Whereas RGR did not differ significantly across

treatments for cuttings, seedling RGR declined with increasing flood stress (Figure 1.5a). Seedlings in the control treatment had significantly greater RGR than seedlings in the waterlogged treatment (t_6=9.9, p=0.0005), which in turn outperformed flooded seedlings (t_6=5.6, p=0.01).

Table 1.2: MANOVA conducted in Proc Mixed examining the effect of treatment (flooded, waterlogged or control), life history stage (cutting vs. seedling), and the interaction between treatment and life history on *Itea viriginica* growth rate and phenotype. We included block (treatment × life history stage) as a random effect.

Explanatory variable	F	P
Treatment	$F_{2,24}$= 213.8	<0.0001
Life history stage	$F_{1,24}$= 16.9	0.0004
Treatment × life history stage	$F_{2,24}$= 11.0	0.0004

Table 1.3: Univariate ANOVA exploring the effect of treatment, life history stage, and the interaction on relative growth rate (RGR), lenticels height (relative to final stem height), allocation to adventitious roots (adventitious roots to total root biomass) and the root:shoot ratio (natural log transformed) of *Itea virginica*. We nested treatment × life history stage within block as a random effect, which resulted in denominator degrees of freedom of six.

Source		RGR		Lenticel height		Allocation to adventitious roots		Root:Shoot	
	df	F	p	F	p	F	p	F	p
Treatment	2	60.7	0.0001	47.0	0.0002	27.7	0.0009	39.7	0.0003
Life history stage	1	33.7	0.0011	106.4	<0.0001	29.3	0.0016	23.8	0.0028
Treatment × life history stage	2	54.6	0.0001	51.3	0.0002	15.5	0.0042	2.57	0.16

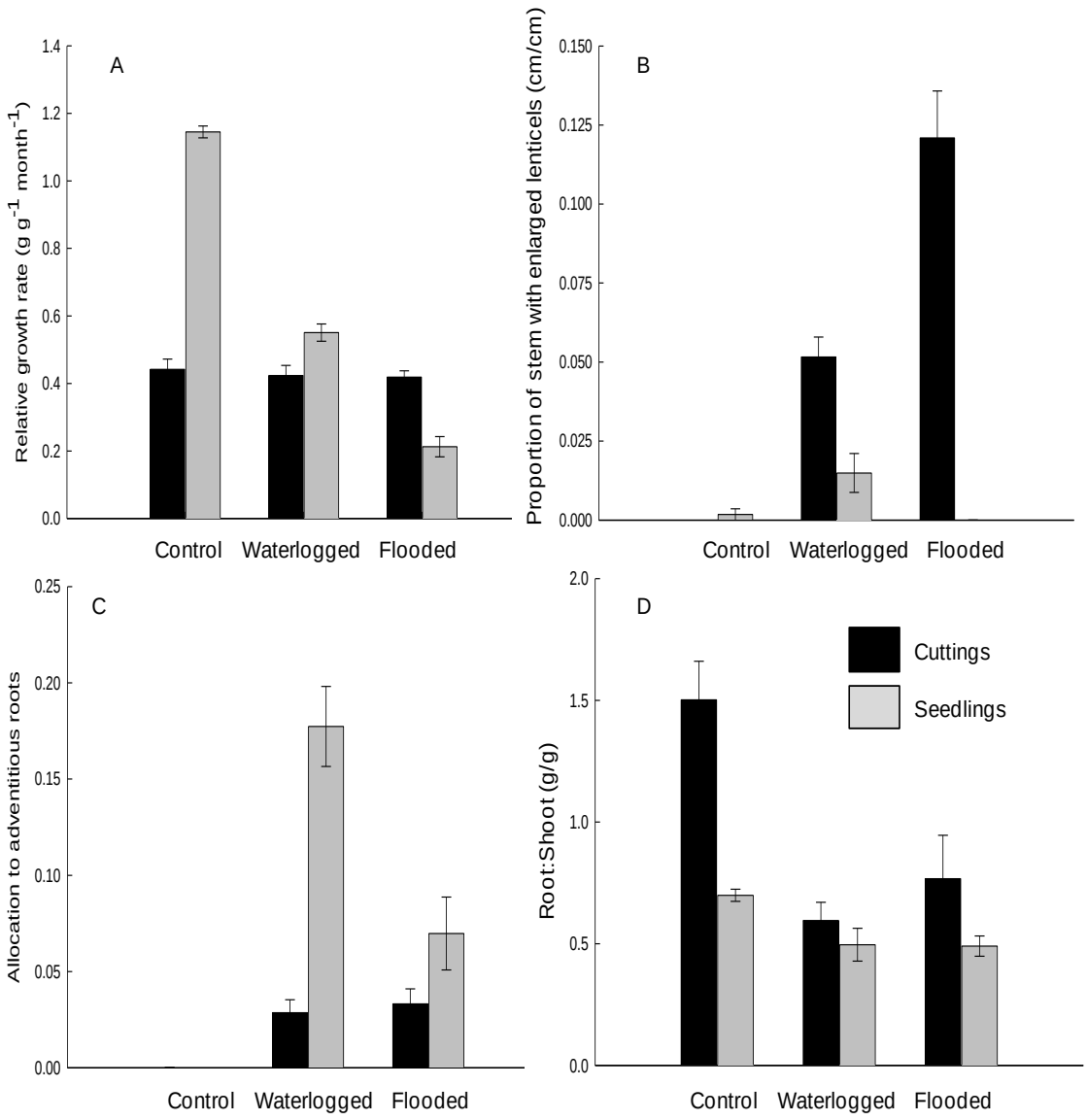

Figure 1.5: Performance and phenotypic traits of cuttings and seedlings in control, waterlogged and flooded treatments in the greenhouse. Relative Growth Rate (A), lenticel height relative to total stem height (B), adventitious root biomass relative to total root biomass (C), and root to shoot ratio (D) all varied by treatment, life history stage and their interaction. Means (± S.E.) of untransformed data are plotted.

When initial height was included in the model, the effect of life history stage becomes nonsignificant (p=0.75). In that model, initial height ($F_{1,296}$= 4.6, p=0.03), treatment ($F_{2,6}$= 55.2, p=0.0001), treatment by life history stage ($F_{2,6}$= 36.7, p=0.0004), initial height by life history stage ($F_{1,296}$= 13.3, p=0.0003), initial height by treatment ($F_{2,296}$= 16.6, p<0.0001), and initial height by life history stage by treatment ($F_{2,296}$= 14.1, p<0.0001) all significantly predicted RGR. For seedlings in the flooded and waterlogged treatments, there was a positive effect of initial size on RGR; thus flooding tolerance does appear to increase with seedling size (Figure 1.6). In contrast, initial size was unrelated to performance for cuttings.

Enlarged Lenticels—Enlarged lenticel production increased significantly with flood stress for cuttings, but did not differ across treatments for seedlings (Figure 1.5b). Cuttings in the control treatment did not exhibit enlarged lenticels and thus had significantly lower lenticel production than waterlogged cuttings (t_6=5.5, p=0.012), which had lower values than flooded cuttings (t_6=7.2, p=0.0028).

Adventitious Roots—Flooding also induced adventitious root production (Figure 1.5c). Waterlogged seedlings had significantly greater allocation to adventitious roots than control (t_6=10.1, p=0.0004) and flooded (t_6=5.95, p=0.0076) seedlings. Contrasts revealed no significant differences between treatments for adults.

Root to Shoot Ratio—Root:shoot declined similarly for both seedlings and adults in response to flooding (Figure 1.5d) . For both life stages, root:shoot was significantly greater in control plants than waterlogged (t_6=8.0, p=0.0005) and flooded (t_6=7.4, p=0.0008) plants, but did not differ between waterlogged and flooded plants (p=0.83). Cuttings also had higher root:shoot ratios than seedlings (t_6=4.9, p=0.0028).

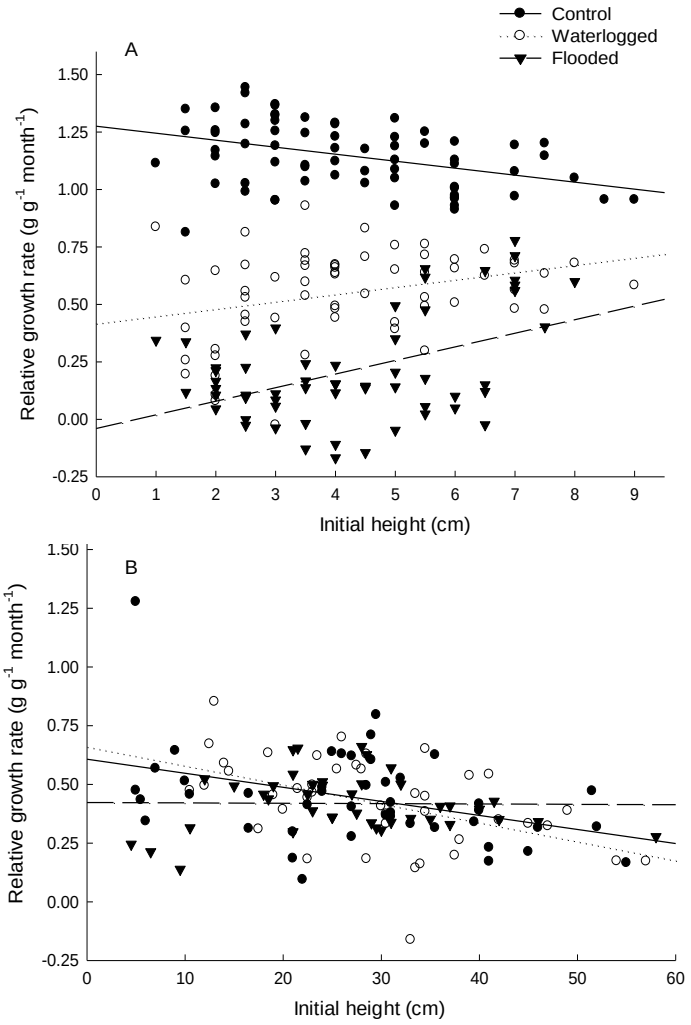

Figure 1.6: Relative growth rate as a function of initial size for both seedlings (A) and cuttings (B) grown under different water regimes in the greenhouse. Water levels were maintained at 1 cm above the soil surface for all waterlogged treatments and 6 cm and 9 cm, respectively, for flooded seedlings and cuttings.

Discussion

In cypress-tupelo swamps, *Itea* plants are located almost exclusively on microsites elevated above the floodwaters. Nonetheless, adults are capable of growing directly from the forest floor. Additionally, adults located on elevated microsites

extend their roots through floodwaters to the forest floor. The reason for the strict microhabitat association of this species is unclear without explicit consideration of the niche requirements of early life history stages. Our field and greenhouse studies support the hypothesis that the regeneration niche of *Itea* constrains adult distribution patterns within these wetland forests.

Both dispersal and establishment can limit distribution (e.g. Moore and Elmendorf, 2006). Although we did not study it, dispersal is unlikely to confine *Itea* to elevated microsites. For one, *Itea* seeds are very small (<1mm in diameter, Schneider and Sharitz, 1986), are primarily wind dispersed, and secondarily dispersed by water. These dispersal modes likely broadcast *Itea* seeds across the forest floor and understory. Although *Itea* seed capsules typically dehisce during high waters in the winter, we have collected numerous viable seeds from capsules during the summer when water levels are low and unflooded forest floor is available. In a study on seed banks, Schneider and Sharitz (1986) found *Itea virginica* seeds in soil cores taken from the floor of cypress-tupelo swamps and drier bottomland hardwood forests, even though their study was biased against recovering these small seeds. At our field site, bottomland hardwood forests exist as slightly elevated islands in a matrix of cypress-tupelo swamp (Porcher, 1981). *Itea* does not reach adult status in this drier habitat (Porcher, 1981), but we have noted *Itea* juveniles growing from the bottomland hardwood forest floor. These observations strongly suggest that dispersal limitation is not the primary determinant of *Itea* distribution.

Forest floor and elevated microsites differ in two primary ways: substrate and the extent of flooding duration and depth. The tight microhabitat association of *Itea* seedlings could result from either low flood tolerance or enhanced performance on woody substrates. In our study, germination rates were indistinguishable on forest floor, cypress knee, and control substrates, indicating that seeds can germinate equally

well on all substrates under drained conditions. Additionally, there was a strong trend for enhanced seedling growth on forest floor soil in our substrate by flooding experiment ($p=0.06$, Figure 4). Fallen logs, and other woody structures, have low nutrient availability, which can inhibit seedling growth (Harmon *et al.*, 1986; Takahashi *et al.*, 2000). In the absence of flooding, *Itea* plants may be better able to access nutrients directly from the forest floor than from the woody substrate of elevated microsites. Finally, flooding inhibited the growth of newly emerged seedlings (substrate by flooding experiment) and the survivorship and growth of older seedlings (life history stage by flooding experiment). Our results are consistent with the hypothesis that flooding restricts *Itea* to the relatively infrequent elevated microhabitats.

In our life history stage by flooding experiment, cuttings (i.e. large plants with adult tissue) had a greater ability to cope with flooding than seedlings in terms of both survivorship and growth. The performance and phenotypic differences that we observed between adults and seedlings were likely due to the relative extent of flooding. Only 3 (7%) of the cuttings in the flooded treatment had no above-water biomass, whereas 83% of the flooded seedlings were completely submerged. Larger sizes enables plants to maintain biomass above the floodwaters, which can substantially increase performance (Nabben *et al.*, 1999; Souther and Shaffer, 2000). Indeed, among seedlings, there was a positive correlation between initial height and growth rate in the flooded and waterlogged treatments, whereas height did not influence adult growth in any treatment. Thus, under water stress, larger seedlings exhibited enhanced performance. Individuals that maintain biomass above the floodwaters can effectively transport oxygen to flooded roots, whereas aerial oxygen is not available to submerged plants (Laan *et al.*, 1990; Jackson, 2008). In our study, cuttings were more efficient at producing hypertrophied lenticels than seedlings.

Lenticels promote gas exchange between the stem and the roots (Kozlowski and Pallardy, 2002); it is, therefore, not surprising that flooded seedlings did not enlarge lenticels because lenticels would serve no gas exchange purpose for partially or completely submerged seedlings. Similarly, allocation to adventitious roots was greater in waterlogged than flooded seedlings, which is expected because adventitious roots are generally produced at the air-water interface and production would not be beneficial in completely submerged plants.

Itea mortality rates were small under controlled conditions. The flooding that we imposed in the greenhouse (6-9 cm depth) is far more benign than the flooding that plants experience in the field (up to 1 m). Nevertheless, *Itea* seedlings showed evidence of depressed mortality and growth even under this moderate flooding stress. Additionally, *Itea* seedlings exposed to flooding in the greenhouse had very frail leaves and stems when harvested. Indeed, the only experimental individuals with negative growth rates were submerged or partially submerged seedlings and one cutting in the waterlogged treatment. Negative growth often results in future mortality and the negative correlation between growth and mortality is a fundamental component of many models of forest dynamics (e.g. Long *et al.*, 2007, and references therein). Even seedlings of *Taxodium distichum* var. *distichum* (bald cypress), a canopy dominant, do not survive complete submergence in nature (Demaree, 1932; Souther and Shaffer, 2000). We attempted a complementary field experiment in 2004 to assess survivorship on fallen logs and the forest floor; however, a large flood later that year eliminated almost all individuals, except three plants that survived on fallen logs (of 30 initial pairs of cuttings planted on the forest floor and elevated microsites).

An increase in flooding tolerance through life history and size is likely common in plants that grow in extensively flooded habitats, especially when juveniles remain completely submerged for prolonged periods. For example, germination of

bald cypress seeds (*T. distichum* var. *distichum*) and survivorship of seedlings require either elevated microsites or a series of dry years, despite the high flood tolerance of later life history stages (Demaree, 1932; Souther and Shaffer, 2000). Similar temporal dynamics likely underlie *Itea* distribution patterns. We propose that adult *Itea* individuals rooted in the forest floor established there during periods of low water. Further, we hypothesize that as these individuals grew, they became more flood tolerant, which allowed them to survive future flooding and continue to thrive on the forest floor. Indeed, in our field survey, adults found on the forest floor were in good condition and were often reproductive, suggesting that adult performance is not greatly diminished by flooding. *Itea* seedlings are unlikely to establish directly on the forest floor in average flooding regimes due to their low flood tolerance. Ontogenetic changes in resource needs and stress tolerance of plants are likely common in other abiotically stressful systems as well (Donovan and Ehleringer, 1992).

Elevated microsites such as fallen logs play important roles in plant regeneration in unflooded forests as well (e.g. Marx and Walters, 2008). Nurse logs can enhance seed germination and seedling establishment by reducing competition, providing enemy-free space, and/or improving abiotic conditions (Harmon and Franklin, 1989; Santiago, 2000; O'Hanlon-Manners and Kotanen, 2004). In many systems, fallen logs decompose as the establishing tree or shrub grows, and adults are found rooted on the forest floor (e.g. Harmon and Franklin, 1989). Adults are unlikely to need the advantages nurse logs provide to juveniles; similar to *Itea*, these species would exhibit a relaxation in microhabitat association through life history.

***Conclusions*—**In some species, adults exhibit higher degrees of habitat association than juveniles, presumably because widespread dispersal leads to initial juvenile establishment in unsuitable sites within the landscape (Paoli *et al.*, 2006). Here, we demonstrate the opposite pattern: juveniles of *Itea* showed tighter

microhabitat associations than adults, due to expansion in flooding tolerance through life history. Seedlings that establish on the forest floor during relatively dry years could develop into adults with high fitness because of greater access to nutrients present in the forest floor than on elevated microsites. Results from our study, and others (e.g. Demaree, 1932; Souther and Shaffer, 2000), suggest that an increase in flooding tolerance with size is probably common in woody wetland plants. Furthermore, our results highlight the importance of assessing niche breadth over multiple life history stages because limitations of juveniles can constrain future ontogenetic stages. Incorporating ontogeny into studies of species coexistence and habitat associations will also illuminate the extent of niche differentiation between plant species within communities (e.g. Comita *et al.*, 2007).

Finally, our results are important to consider in habitat conservation and restoration plans. Bald cypress (*Taxodium distichum*) is prized for its high quality wood, and was heavily logged throughout the coastal plain of the Southeastern United States in the 19th century (Souther and Shaffer, 2000). This species has failed to regenerate naturally in some logged cypress-tupelo forests, due, in part, to the effect of prolonged flooding on newly germinated seedlings (Souther and Shaffer, 2000). Most of the understory plant diversity in cypress-tupelo forests occurs on elevated sites, which shield vulnerable seeds and seedlings from flooding (Huenneke and Sharitz, 1986, and pers. obs.). During restoration efforts, an attempt must be made to increase the structural complexity of regenerating cypress-tupelo swamps to capture plant diversity and the regeneration niche of perennial woody species (e.g. Young *et al.*, 2005).

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CHAPTER 2

DEMOGRAPHIC SOURCE-SINK DYNAMICS RESTRICT LOCAL ADAPTATION IN ELLIOTT'S BLUEBERRY (*VACCINIUM ELLIOTTII*)

Summary

In heterogeneous landscapes, divergent selection can result in the evolution of locally adapted ecotypes, especially when interhabitat gene flow is minimal. However, if habitats differ in size or quality, source-sink dynamics can shape the evolutionary trajectory of species. I conducted a multiyear reciprocal transplant experiment to test whether Elliot's blueberry (*Vaccinium elliottii*) is locally adapted to contrasting environments in a spatially variable landscape (upland vs. bottomland forests). This species spans a wide range of habitats that differ in water table depth, light penetration to the understory and edaphic conditions, all of which could result in divergent selection across the landscape. In addition to the field experiment, I exposed individuals of two life history stages to prolonged drought and flooding in the greenhouse to assess fitness responses to abiotic stress. Contrary to predictions, *V.elliottii* families in the field experiment consistently exhibited significantly greater fitness (survivorship and growth) in upland relative to bottomland forests, regardless of the habitat of origin. Similar results from the greenhouse experiment suggest that *V. elliottii* is better adapted to drought stress than flooding. The population density of this species is higher in the uplands and upland populations harbor significantly greater genetic diversity (unique alleles). The disparity in population sizes likely results in asymmetric gene flow from upland to bottomland forests. Furthermore, neutral population differentiation as measured by microsatellite loci is extremely small both for seeds and adults. These results are consistent with genetic source-sink dynamics, in which adaptation to a marginal habitat is constrained by continual immigration from a more benign habitat.

Introduction

Environmental heterogeneity results in varying patterns of natural selection across the landscape (e.g. Dudley, 1996; Heywood, 1991; Nagy and Rice, 1997). Strongly divergent selection in alternate habitats promotes specialization when environmental conditions change slowly relative to the lifespan of an individual (e.g. Alpert and Simms, 2002; Hedrick, 1986). Interhabitat gene flow is thought to constrain the evolution of specialization because immigrants introduce maladapted alleles and decrease the frequency of locally-adapted ecotypes (e.g. Hendry *et al.*, 2002; Holt and Gomulkiewicz, 1997; Langerhans *et al.*, 2003; Storfer *et al.*, 1999). Indeed, specialization is particularly likely if gene flow between populations in contrasting environments is limited (Lenormand, 2002). For example, Hendry and colleagues have demonstrated that lake and stream sticklebacks exhibit genetically-based divergent phenotypes when gene flow is low, but maladapted intermediates occur in areas with increased lake to stream migration (Hendry and Taylor, 2004; Hendry *et al.*, 2002; Moore *et al.*, 2007).

This framework assumes that the quality of local habitat patches is relatively similar across the landscape; however, if fitness varies with habitat or habitat size differs, source-sink dynamics can influence the evolutionary trajectory of a species (Pulliam, 1988). In this case, a larger proportion of the population occurs in the source habitat, and natural selection favors traits that maximize fitness there (Kawecki, 1995). Source-sink dynamics can hinder adaptive evolution in marginal habitats and result in the persistence of maladapted forms (Dias, 1996; Holt and Gaines, 1992; Kawecki, 1995). Even when habitat patches are equal in quality, asymmetrical gene flow can establish source-sink dynamics (Kawecki and Holt, 2002). For example, Dias and Blondel (1996) studied Mediterranean blue tits (*Parus caeruleus*) in evergreen and deciduous forests in two separate regions. The habitats differ in area at

the two sites, and in each landscape the birds are locally adapted to the more common habitat (Dias and Blondel, 1996). This species, therefore, has the genetic variation necessary to adapt to both habitats, but source-sink dynamics result in local maladaptation to the less common habitat within a region (Dias and Blondel, 1996). Thus, evolution in heterogeneous landscapes depends on the strength of divergent selection, the extent of genetic isolation of populations in contrasting habitats, and differences in habitat quality and area (Kawecki and Ebert, 2004). To understand adaptive evolution in this situation, it is important to assess fitness and phenotypic responses to contrasting habitat types as well as quantify interhabitat migration (Kawecki and Ebert, 2004).

This study examined whether the scale of environmental variation influences adaptive and neutral population differentiation in a woody perennial shrub. *Vaccinium elliottii* Chapm. (Ericaceae) inhabits both upland and bottomland forests in the Southeastern United States (Godfrey and Wooten, 1981; Radford *et al.*, 1968). Bottomland forests are dynamic systems that experience annual floods (Burke *et al.*, 1999). In contrast, drought-stress can be pronounced in the sandy soils of upland forests (Burke *et al.*, 1999; Megonigal *et al.*, 1997). Thus, divergent selection may lead to different phenotypic optima in the two habitats. The study was designed to test whether: 1) spatial heterogeneity favors local adaptation to contrasting habitats or 2) interhabitat gene flow restricts adaptive population differentiation. Within each habitat, I selected upland and bottomland populations that abutted a sharp ecotone, as well as remote populations 0.75 – 3.7 km from the nearest ecotone; my intention was to sample along a gradient of interhabitat gene flow. This design permitted me to test whether remote populations expressed a greater degree of local adaptation than ecotonal populations, where interhabitat gene flow is likely to be high. To address the objectives, I: 1) conducted a multi-year reciprocal transplant experiment across a

complex gradient in hydrology, soil chemistry and light availability; 2) isolated the effects of flooding and drought on plant fitness in a greenhouse experiment; 3) analyzed genetic population differentiation using neutral microsatellite markers; and 4) implemented a small demographic study.

Materials and Methods

Focal species—*Vaccinium elliottii* Chapm. (Ericaceae), a species of highbush blueberry, is widely distributed throughout the Southeast in seasonally flooded bottomland forests and xeric upland forests (Godfrey and Wooten, 1981; Radford *et al.*, 1968). The U.S. Fish and Wildlife Service lists *V. elliottii* as a facultative plus wetland species, i.e., one that is slightly more likely to occur in wetland than non-wetland systems (http://www.fws.gov/nwi/bha/downloads/1996/national.pdf, see also Wilen and Bates, 1995). *Vaccinium* flowers are insect-pollinated and the seeds are animal-dispersed (Martin *et al.*, 1951); therefore, substantial gene flow between populations is possible, leading to low levels of population genetic differentiation (Loveless and Hamrick, 1984). Individuals from upland and bottomland forests exhibit distinct phenotypes, which may be related to water-stress. For example, small adventitious roots emerge from the stem on individuals in bottomlands, but not on plants in the uplands, and may represent adaptations to soil flooding (Anderson, pers. obs.). Preliminary work revealed that specific leaf area (SLA) is significantly lower (indicating thicker leaves) in remote upland populations of naturally-recruited *V. elliottii* individuals than in ecotonal uplands and remote and ecotonal bottomlands (mean $\pm$ S.E.: remote uplands= 157 $\pm$ 5.6 cm^2/g, n=28; ecotonal uplands =185$\pm$5.4, n= 30; ecotonal bottomland = 192.8$\pm$4.26, n=49; remote bottomland = 193.5$\pm$ 4.6, n=41; $p<0.0001$).

Study system—The primary field site, Beidler Forest (N 33°12.13, W 080°18.50) in the Four Holes Swamp watershed of South Carolina, consists of 5260

hectares of bottomland hardwood and swamp forests with conservation easements in xeric upland forests. I also sampled populations in the Pee Dee and Santee watersheds for experimental work; however, molecular work with microsatellites was restricted to the Four Holes Watershed. All three watersheds lie within the Coastal Plain of South Carolina and have similar average temperatures and rainfall levels (NOAA, 2002). In these watersheds, I sampled 7-9 populations from each of four forest types (32 total populations): remote bottomlands (0.75-1.04 km from the nearest upland forest), remote uplands (0.8-3.7 km from the nearest bottomland forest), ecotonal uplands immediately adjacent to bottomland forests and ecotonal bottomland adjacent to upland forests. The bottomland is 1-1.5 km wide, so I was unable to locate remote bottomland populations farther than this distance from upland forests. In statistical analyses, I distinguished between the effects of the habitat of origin (hereafter: habitat), population proximity to the alternate habitat type (hereafter: proximity; coded as ecotone or remote) and their interactions. I located all populations with the aid of GIS-based maps from the U.S. Fish and Wildlife Service, which identify habitats using National Wetland Indicator habitat codes (http://wetlandsfws.er.usgs.gov/wtlnds/launch.html); this strategy minimized abiotic and biotic differences between sites within each habitat. The plant species composition of upland and bottomland forests differs substantially (Porcher, 1981) and *V. elliottii* is the only species that was abundant enough in both habitats for this study.

Interannual variation in flooding duration is high in bottomland hardwood forests. The Audubon Society has maintained a daily record of water levels since 1977. Since that time, flood duration in bottomland forests has ranged from a total of 3 - 139 days/ year (average ± S.D.: 43.6 ± 36.1 days/year; Brunswig, N. and Dawson, M., unpublished data). This habitat experiences relatively shallow water levels during

flooding events, which are generally only several cm deep. Precipitation records at the field site also show periods of very infrequent rainfall, which can result in drought stress in upland forests (Brunswig, N. and Dawson, M., unpublished data). Rainfall is quite variable and can range from 0-122.9 mm/month during the growing season (average ± S.D.: 125.3 ± 79.8 mm/month).

Abiotic differences between habitats—To assess temporal and spatial variation in abiotic stresses, I quantified hydrological, edaphic, and light conditions in upland and bottomland forests. Typically, bottomland forests in the Coastal Plain flood annually in the late winter or early spring and for a shorter duration in the summer (Burke *et al.*, 1999). To test for habitat and seasonal differences in soil moisture and bulk density (i.e., soil compaction), I collected 3 soil cores of known volume in October 2006, March 2007 and March 2008 from 6 sites (plus 5 sites monitored only once). I measured wet and dry soil weight to quantify soil moisture [(wet weight – dry weight)/wet weight)], volumetric water content [(wet weight – dry weight)/(density of water at 22°C × soil volume)], and bulk density (dry weight/soil volume). Mixed model ANOVAs with site as a repeated statement were used for analyses (Proc Mixed, SAS ver. 9.2). Additionally, I determined depth to the water table by excavating to a maximum depth of 1.34 m at four upland and four bottomland sites in April 2008.

Soil samples from eight sites collected in October 2006 were made for analysis of pH, exchangeable acidity, organic matter (LOI), Morgan extractable P, K, Ca, Mg, Mn, Zn, Al, NO_3. These samples were collected, air dried and stored in paper bags before analysis by the Cornell University Nutrient Analysis Laboratory (CNAL). This facility follows protocols detailed in the Soil Survey Laboratory Methods Manual (*National Soil Survey Center*, National Resources Conservation Service, United States Department of Agriculture; http://soils.usda.gov/technical/lmm/). These assays resulted in undetectable levels of available nitrate. Since I was interested in habitat-

based differences in the C:N ratio, I collected additional soil samples from 5 upland and 4 bottomlands sites in March 2008 for total C and N determination at CNAL. Due to small sample sizes and a large number of soil nutrient variables, I was unable to conduct a multivariate ANOVA (MANOVA); instead, I performed univariate ANOVAs on nutrient data. I used the Satterwaithe approximation for degrees of freedom for the analysis of soil C:N because of differences in variance between upland and bottomland forests.

In April 2008, I took two hemispherical canopy photos at each of 4 bottomland and 4 upland sites to assess light level using Gap Light Analyzer ver. 2.0 (Frazer *et al.*, 1999). I used a Nikon N70 equipped with a fish-eye lens to take the photographs under overcast conditions. Hemispherical photography quantifies the degree of canopy openness, the effective leaf area index, and direct and diffuse solar radiation transmitted through the canopy (Frazer *et al.*, 1999). As with the soil nutrient analyses, I conducted univariate Mixed model ANOVAs on these four metrics of light penetration to the understory (Proc Mixed, with a random statement for site).

Demography— To determine whether population size and reproductive fitness varied by habitat, I established two 50 m × 10 m transects per site in each of two remote and ecotonal upland and bottomland habitats in March and April 2008 (n= 8 sites; 13 total transects; only 1 transect was used at each of three sites). *Vaccinium elliottii* can spread vegetatively; therefore, I was careful to count stems that represented distinct individuals (not clones). I recorded the abundance of adult plants in both transects per site (>50 cm tall, with stems of >0.5 cm diameter at the base); this size is the smallest at which individuals flower in the field. In one transect per site, I also quantified the total number of reproductive structures (flower buds, flowers, and developing fruits) on each adult. The results of these transects accord with my observations from 2004-2007, suggesting that spring 2008 was not abnormal. I used

mixed model ANOVAs to test the effects of habitat (bottomland vs. upland), proximity (ecotone vs. remote), and their interaction on adult abundance (Poisson distribution, Proc Glimmix) and reproductive output (sum of all reproductive structures per individual, Proc Mixed). I included site nested within habitat by proximity as a random effect in both analyses. Reproductive output was right-skewed, but a Poisson distribution showed poor fit to these data. Instead, I used a natural logarithm transformation of counts (+ 0.5 due to 0 values).

Reciprocal transplant experiment—To assess the potential for local adaption, I conducted a multiyear reciprocal transplant experiment. If *V. elliottii* exhibits local adaptation, but there is interhabitat gene flow, adults could show greater adaptive population divergence than juveniles; therefore, I included both seeds and cuttings made from adults. In 2004 and 2005, I collected 4000 cuttings from adult *V. elliottii* individuals in 32 populations (n=9 remote upland populations; 7 remote bottomland; 8 ecotonal upland; 8 ecotonal bottomland). Cuttings were made from new growth and were 10 cm in length. I removed all but 2-3 leaves, applied rooting hormone (Rhizopon AA #3, 0.8% IBA, Rhizopon bv, Hazerswoude, Holland) to each cutting, and placed cuttings under an automated misting system at Beidler forest. In the fall of both years, I transported cuttings to Cornell University and maintained misting until roots established (2-3 months). Cuttings were grown in the greenhouse until May (2005 and 2006) when I transported them back to Four Holes Swamp. They were approximately 20 cm tall at planting. *V. elliottii* seeds are difficult to monitor in the field due to their small size, so I planted seedlings. In 2005, seeds were collected from plants in 16 populations (4 remote upland, 3 remote bottomland, 5 ecotonal upland, and 4 ecotonal bottomland populations) and were germinated in the laboratory. Seedling families, which likely consist of a mixture of half- and full-siblings, were transplanted into the field in 2006 when individuals were approximately 12 cm tall.

I transplanted individuals into two upland and two bottomland sites to represent the environmental variation present in these habitats. The sites within a habitat did not vary appreciably in abiotic characteristics (Appendix 1); however, they did differ in plant density in the understory (pers. obs.). Upland site one had a less dense understory than upland site 2; bottomland site 1 had a more abundant understory population of *Sabal minor* (dwarf palmetto, Arecaceae) than bottomland site 2. These differences allowed me to test plant performance across a range of biotic conditions.

In 2005, I planted cuttings at 1 m intervals in grids in the two upland and two bottomland transplant sites (n=1685 cuttings from 412 genotypes and 22 populations). In 2006, I outplanted cuttings (n=548 from 106 genotypes and 22 populations) and seedlings (n=814 from 81 families and 16 populations) in the same transplant sites. There were 12 populations in common between the 2005 and 2006 cuttings and 10 populations unique to each transplant year. In both years, I planted 2-3 individuals per clone or seedling family in both upland and bottomland habitats; however, in some cases, I had limited numbers of plants from each family and could plant only 1 individual per habitat. Individuals within a family were randomly assigned to bottomland or upland transplant sites. Within a site, planting was done haphazardly so that clones or seedlings from the same family were not spatially clumped. Prior to planting, I measured the stem diameter(s) at the base of each individual, which correlates well with total biomass ($biomass^{0.5} = -0.29 + 0.59 \times$ total stem diameter $- 0.013 \times$ total stem diameter $^2 + 0.17 \times$ life history stage; $F_{3,88}=410$, $p<0.0001$, $R^2=0.94$, n=39 cuttings + 50 seedlings). I watered all individuals for the first two weeks.

In October 2006, I measured the diameter of all clones planted in 2005 to calculate relative growth rate (RGR) and collected leaves to assess foliar traits (Chapter 3). At that point, I did not measure 2006 transplants because they still

retained leaves produced in the greenhouse prior to transplanting. In October 2007, I repeated those measurements and leaf collections for all plants included in the study (cuttings and seedlings planted in 2005 and 2006).

***Greenhouse experiment*—**A subset of cuttings and seedlings collected in 2005 were used in a greenhouse experiment to quantify plant performance in response to prolonged drought and flooding. Prior to the experiment, these individuals were well-watered and grown under supplemental lighting for 12 hours/day and at temperatures of 80-85 °C for 4-6 months. Before the experiment began, I transferred all individuals from smaller (SC10 Super Cell conetainers: volume=164 mL, diameter =3.8 cm, depth=21 cm) to larger conetainers (Deepot D25L: volume =410 mL, diameter=5 cm, depth =25cm, Stuewe and Sons, Inc., Corvallis, OR, USA) to provide space for continued growth. I used a 1:1 mixture of peat moss and sand as the potting soil to mimic the naturally acidic soil in the field.

In October 2006, I randomly allocated multiple individuals per family to two treatments: 1) flooded (water level 5 cm above soil) and 2) drought (watered only once per week). Here, drought refers to a sustained period of limited and infrequent water availability. I measured initial plant diameter to estimate biomass. Plants were divided into 54 blocks (26 flooded and 26 drought blocks). Each block consisted of a conetainer tray cut to fit in a 14 gallon plastic storage bin (Rubbermaid Home Products, USA). I drilled drainage holes in the bottom of the drought bins. Competition between plants was minimized by placing an average of 16 plants in trays that could hold 40. Within each bin, I included seedlings and cuttings, as well as individuals of multiple populations from all habitat by proximity configurations.

The experiment began October 30, 2006 and continued until May 4, 2007. To ensure that individuals had adequate time to respond to changing environmental conditions, plants experienced four incremental levels of stress separated by 7-10 days

to mimic increasing (or decreasing) soil saturation in the field. The treatments reached their final flooding or drought levels by November 27, 2006 and mortality censuses occurred every 7-10 days after that date. I excluded individuals that died prior to November 27th from statistical analyses because these plants did not experience final stress levels. This experiment included 201 families of seedlings and cuttings (n=87 seedling families and 271 seedlings; n= 133 cutting families and 458 cuttings) from multiple populations (n= 16 populations for seedlings and 25 for cuttings). The 16 seedling populations were a subset of the populations of cuttings. The populations represented all habitat by proximity configurations (n=5 remote bottomland, 7 ecotonal bottomland, 6 remote upland, and 7 ecotonal upland populations). I included multiple individuals per family when available to assess performance of related individuals under different experimental conditions and to facilitate genotypic selection analyses within each treatment (number of individuals per family: mean ± S.E.: 3.65 ± 0.17; range 1-14). All statistical analyses accounted for correlation between individuals from the same family and population.

After final treatments were imposed, I added water 3-4 times weekly to the flooded treatment to maintain the water level and to oxygenate the water. During the experiment, I watered drought plants to saturation once per week (~150 mL of water, resulting in a volumetric water content of ~32% mL water/mL soil + water). The soil dried almost completely within three days of watering. Additionally, I drained the flooded treatment weekly and removed any accumulated algae with a coarse scrub (no soap). Before refilling the flooded bins, I rotated all bins to eliminate any effects of abiotic gradients within the greenhouse. This procedure took approximately 4 hours. Over the course of the experiment, each bin experienced all positions in the greenhouse. Finally, I added fertilizer for acid-loving plants to all individuals four times during the experiment (November, December, February and March; Miracle-

Gro® Water Soluble Azalea, Camellia, Rhododendron Plant Food, Scotts Miracle Gro Inc., 30:10:10 N:P:K). To ensure that plants in both treatments received the same amount of fertilizer, I watered the drought treatment thoroughly and drained the flooded plants. After fertilizing, I waited until all pots were drained before re-initiating flooding. During the experiment, I harvested dead individuals only if they were recorded as dead in 4 sequential mortality censuses; only two individuals recorded as dead resprouted before harvesting. At the end of the experiment, I harvested all individuals, and dried the leaves, stems and roots of each plant in separate collections at 50-60 °C for 4-5 days.

I designed the greenhouse experiment to assess plant response to extreme water stress and the treatments are relevant to conditions that plants can encounter in the field. During the 30 year period for which the Audubon Society has maintained water level data, one year (2003) experienced 139 days of almost continual flooding during the growing season (N. Brunswig and M. Dawson), which is only slightly shorter than the 150 days of this experiment. It is likely that the drought I imposed in the greenhouse was more severe than most droughts that this species experiences in nature because plants in the greenhouse do not have access to a water table and are exposed to elevated temperatures and full light, which increase soil water loss through evapotranspiration. Indeed, wilting was observed weekly on many plants.

Fitness—Local adaptation can be detected through genotype by environment interactions (G × E) in fitness, where genotype refers to the habitat of origin of a genotype or family and environment refers to either transplant environment (field experiment) or treatment (greenhouse experiment). If divergent selection favors local adaptation, I expect bottomland genotypes to have greater performance in bottomland habitats and flooded conditions, and upland genotypes to show the opposite pattern (habitat of origin × transplant environment or treatment interaction). If interhabitat

gene flow restricts adaptive population divergence, I predict that individuals from ecotonal populations (where gene flow is likely high) will have reduced fitness relative to individuals from remote populations both within and across environments, which would result in significant effects of proximity and proximity by environment interactions. Survivorship and relative growth rate (RGR) were the fitness components for both experiments because relatively few plants flowered (55 during the entire course of the reciprocal transplant experiment). Fitness was analyzed as a function of habitat of origin (G), transplant habitat or treatment (E), proximity (ecotone vs. remote), life history stage (2006 transplants only), their interactions, and initial plant size (a covariate).

Fitness component: growth—In the field experiment, RGR was calculated as $(\ln(\text{diameter}_t)-\ln(\text{diameter}_i))/t$, where t is elapsed time (in months), diameter_t is the total diameter at the base of each stem at time t, and diameter_i is the initial diameter. I have three sets of RGR measurements: 1) first year growth for 2005 transplants (measured in 2006), 2) second year growth for 2005 transplants (measured in 2007), and 3) first year growth for 2006 transplants (measured in 2007). I conducted a repeated measures ANOVA (Proc Mixed) on the first and second year RGR values from the 2005 transplants with a repeated statement for year and a random statement for family nested within population of origin to account for family-level correlation. I analyzed RGR of the 2006 transplants separately in a mixed model with a random statement for family nested within population (Proc Mixed). In both analyses, I included transplant site nested within transplant habitat as a fixed effect.

In the greenhouse experiment, RGR was calculated as (ln(final biomass)-ln(initial biomass))/t, where t is elapsed time (in months) and initial biomass was estimated from initial diameter measurements. I used a mixed model ANOVA (Proc Mixed) for this analysis. To account for correlation between plants from the same

family and block, I used random statements for family nested within population of origin and block nested within treatment.

Fitness component: survivorship—Survivorship was monitored 10 (2005 transplants) and 8 (2006 transplants) times for the field experiment and 16 times in the greenhouse experiment. I conducted discrete-time survivorship analyses (Cox proportional hazards models) separately for the two transplant years and the greenhouse experiment. Random effects (frailty) modeling permits the analysis of clustered survivorship data (Cortinas Abrahantes *et al.*, 2007; Kelly, 2004; Liu and Huang, 2008). Such clustering arises when time-to-event data are collected on multiple individuals within a family or population. I implemented a Bayesian approach using WinBUGS ver. 1.4.3 (Bayesian Analysis using Gibbs Sampling) (Lunn *et al.*, 2000) because standard statistical software (SAS, R, STATA) cannot accommodate multiple random effects in survivorship analysis (Kelly, 2004). I modeled time until death as a function of initial size (standardized diameter in the field experiment, standardized initial biomass in the greenhouse experiment), habitat of origin (bottomland or upland), proximity (ecotone or remote), transplant habitat (field experiment) or treatment (greenhouse experiment), transplant site (not applicable for the greenhouse experiment), and all two and three-way interactions between habitat, proximity, and transplant habitat or treatment. For the 2006 plantings and the greenhouse experiment, I also included life history stage as a fixed effect and modeled the interactions between this predictor and transplant habitat or treatment, habitat and proximity. I included family by population of origin as gamma-distributed random variables and assumed these random statements operated multiplicatively on the baseline hazard, which is appropriate for clustering at the family level (Koissi and Hognas, 2005; Sastry, 1997). In the greenhouse analysis, I incorporated an additive random effect for block. I used uninformative priors with 40,000 iterations following

a burn-in of 10,000 iterations. Convergence was assessed visually and with convergence diagnostics in BOA (Smith, 2005). The WinBugs code is modified from WinBugs example volume I (Leuk with frailties) and Koissi and Hognas (2005) (greenhouse experiment code is presented in Appendix 2). Cox proportional hazards models assume that the difference in hazard rates between treatments remains similar across time periods; when this assumption was violated, I included time-dependent predictor variables in the model. Prior to analysis in Winbugs, I assessed the proportionality assumption using Proc PHREG in SAS (ver 9.2).

Population genetic differentiation—Genetic differentiation can increase during ontogeny, when maladapted seedlings that are the product of gene flow are eliminated by selection (Kalisz *et al.*, 2001; Kittelson and Maron, 2001). Changes in genetic structure can, therefore, illuminate the action of selection at different ontogenetic stages (Kalisz *et al.*, 2001; Kittelson and Maron, 2001; McCue and Holtsford, 1998; Tonsor *et al.*, 1993). To examine changes in interhabitat gene flow and genetic diversity through ontogeny, we haphazardly sampled 315 adult individuals from 17 populations in Four Holes Watershed (mean $\pm$ S.E., n= 18.5 ± 1.3 individuals per population, see Appendix 3 for information on sampling locations and sample sizes). Leaf samples of distinct individuals were either collected in the field and stored in silica gel until DNA extraction with Qiagen plant kits at Cornell University (Qiagen Inc., Valencia, CA), or collected from living cuttings in the greenhouse (prior to use in the field experiment) and ground fresh in liquid N_2. Naturally-recruited seedlings and juveniles of *V. elliottii* are rare at these field sites (pers. obs.); I was, therefore, only able to compare the genetic population structure of adults with that of seeds. During June and July 2006 and 2007, I collected seeds directly from adults in 15 populations at Four Holes Swamp, germinated them in the lab, and extracted DNA from fresh leaf tissue of 174 individuals (n= 11.6 ± 0.73 individuals / population).

These pre-dispersal seeds provide information on pollen movement throughout the landscape.

Microsatellites are highly informative in estimating gene flow (Hamilton *et al.*, 1999) because they are codominant, (putatively) selectively neutral, and highly variable regions of DNA (Jarne and Lagoda, 1996). I used eight primers from a published microsatellite library developed for the heterospecific *V. corymbosum* (NA961, NA398,CA94F, CA23F, CA787F, CA169F, CA855F, and CA190R) (Boches *et al.*, 2005). I used polymerase chain reaction (PCR) annealing temperatures in Boches et al. (2005), but optimized the $MgCl_2$ concentrations for NA398 and CA94F. The 5' end of each primer was fluorescently labeled with NED (CA169F, NA398, CA94F), 6-FAM (CA190R), PET (CA23F, CA855F), or VIC (NA961, CA787F). Genotypes at each locus were resolved by electrophoresis using an ABI 3100 Capillary Sequencer in the Evolutionary Genetics Core Facility (EGCF) at Cornell University. Data were collected and scored with GeneMapper v. 3.0.

I also assessed each allele score manually, which allowed me to distinguish between near neighbor heterozygotes and homozygotes at the only locus with stutter (CA94F) (Dewoody *et al.*, 2006). Additionally, following Dewoody et al.'s (2006) suggestion, I reamplified and rescored samples with potentially problematic peaks. Furthermore, I compared genotypes of adults with their corresponding offspring for 86 pairs in the program CERVUS 3.0 (e.g. Hoffman and Amos, 2005) to assess the number of cases in which mother and offspring do not share a common allele; in all such cases, both individuals were reamplified and rescored. Following the recommendations of Selkoe and Toonen (2006), I assessed gametic disequilibrium among loci in GENEPOP (Rousset, 2008), selective neutrality in FDIST2 (Beaumont and Nichols, 1996), and null alleles and scoring errors in Micro-checker using individuals that amplified at all loci (n=465) to eliminate samples with potentially degraded DNA

(only a problem for a subset of leaves stored in silica gel) (Gardner *et al.*, 2007; Van Oosterhout *et al.*, 2004).

I assumed an infinite allele mutational model because it is more robust to violation than the stepwise mutational model (Selkoe and Toonen 2006) and because one highly polymorphic locus (CA855F) contained a compound repeat consisting of a long dimer and slightly shorter trimer for which the stepwise model is not appropriate. I tested for deviation from Hardy-Weinberg equilibrium for each population and locus combination for seeds and adults using GENALEX (Peakall and Smouse, 2006). I used FSTAT (Goudet, 1995, ver. 2.9.3.2) to calculate observed (Ho) and expected heterozygosity (H_E), *F*-statistics and pairwise F_{ST} using the estimators of Weir and Cockerham (1984).

I used the program TFPGA (Miller, 1997) to conduct exact tests of population differentiation in allele frequency (Raymond and Rousset, 1995) between bottomland and upland populations for both seeds and adults (1000 dememorization steps, 20 batches, 10000 permutations per batch). Partial Mantel tests using the program zt (100,000 iterations, Bonnet and Peer, 2002) assessed the correlation between pairwise genetic differentiation ($F_{ST}/(1- F_{ST})$) and geographic distance (natural logarithm transformed) (Rousset, 1997) in a habitat context for both seeds and adults. The habitat matrix included values of 1 for population pairs located in the same habitat, and 2 for population pairs in opposing habitats. This analysis can detect whether habitat presents a barrier to gene flow. Standardized pairwise population differentiation (G'st) values proposed by Hedrick (2005) and Nei's (1978) unbiased genetic distance produced similar results (not shown). I also used a Mantel test to assess difference in pairwise F_{ST} values between seeds and adults for the 15 populations for which I had data at both life history stages. Finally, I conducted analysis of molecular variance (AMOVA) in Arlequin (ver. 3.1) to test for genetic

homogeneity among populations and between habitats.

Population genetic diversity—I calculated standard metrics of within population genetic diversity using HP-RARE (Kalinowski, 2005): allelic richness rarefied to a sample of 10 genes, which was the minimum sample size per locus per population after correction for null alleles (mean sample size $\pm$ S.E.: 29.8 $\pm$ 0.79 genes; maximum: 56 genes); rarefied private allele richness, which measures the number of unique alleles within a population; and expected heterozygosity (He), which represents the probability that two randomly chosen alleles from a population are different (Kalinowski, 2004; Vellend, 2004). I conducted a mixed multivariate analysis of variance (MANOVA, Proc Mixed) to test the effects of life history stage (seed vs. adult), habitat (bottomland vs. upland), proximity (ecotone vs. remote), and two- and three-way interactions on these response variables; population and locus were included as random effects. Since the MANOVA produced significant results (Appendix 4), I implemented univariate mixed ANOVAs to test each response variable independently. I selected Proc Glimmix for this analysis because it allowed me to fit two R-sided covariance structures (equivalent to a repeated statement) for the random effects of locus within population and life history stage within population (Proc Glimmix: http://support.sas.com/rnd/app/papers/glimmix.pdf).

The relatedness between parents can influence survivorship and reproductive success of offspring (e.g. Amos *et al.*, 2001; Hoffman *et al.*, 2004). Amos et al. (2001) proposed a metric of parental genetic similarity, internal relatedness, which weights individual heterozygosity by allele frequency at each locus. Internal relatedness (IR) ranges from -1 to 1, with negative values indicating that parents are more distantly related. IR is strongly correlated with individual-level, multilocus heterozygosity (results not shown). I restricted our analysis to plants with alleles scored at all six loci (n=281 adults and 165 seeds after correction for null alleles) and

conducted a mixed model ANOVA (Proc Mixed) to test the effects of life history stage, habitat of origin, population proximity, and all interactions on IR. I included a repeated statement for life history stage nested within genotype to control for the non-independence of mother-offspring pairs. Family nested within population was also incorporated as a random statement.

***Gene flow*—**Finally, I assessed the potential for asymmetrical gene flow using Bayesian inference in MIGRATE ver. 3.0 (Beerli and Felsenstein, 2001), which provides estimates of both effective population size (N_e) and migration. One simulation was performed with two concurrent independent chains and adaptive heating with four temperatures for seeds. I tested for habitat-based differences in effective population size using ANOVA and assessed the symmetry of interhabitat migration with a paired t-test. This paired t-test was used to test whether the number of migrants ($4N_em$) was significantly greater from upland into bottomland populations than the reverse for upland-bottomland pairs of populations.

Results

***Abiotic differences between habitats* —**Bottomland forests have significantly greater soil moisture and bulk density than upland forests (Tables 2.1 and 2.2), which is not surprising because bottomland soils are primarily clay, whereas upland soils consist of sand. Additionally, soil moisture is higher in the spring in both habitats. In the spring of 2008, I was unable to reach the water table in holes 1.34 m deep at four upland sites (2 holes/site). In contrast, the water table was near the surface at four bottomland sites (mean ± S.D. water table depth: 0.24 ± 0.14 m deep). Finally, bottomland forests had significantly more nutrient rich soils than uplands (Table 2.3).

Light level in the understory was significantly greater in upland than bottomland sites (Table 2.4). Bottomland forests are composed primarily of deciduous trees and shrubs, with very few evergreen species (e.g. *Pinus taeda*); in

contrast, upland forests consist primarily of pines, evergreen oaks and understory plants (e.g. *Vaccinium arboreum*) (Porcher, 1981). In early April, when hemispherical photographs were taken, the bottomland forest was not completely leafed out, whereas most species in the uplands had a complete set of leaves (Anderson, pers. obs.). It is highly likely that our results underestimate the difference in light level between habitats. By May, upland forests have relatively similar light levels, whereas bottomland forests likely receive even less light in the understory.

Table 2.1: Soil moisture, volumetric water content and bulk density in the fall (2006) and spring (2007). Averages (± S.D.) are presented

Habitat	Season	Soil moisture (%)	Volumetric water content (%)	Bulk density (g/cm^3)
Upland	Fall	6.6 ± 2.7	25.1 ± 12.5	0.915 ± 0.12
Upland	Spring	10.2 ± 0.86	42.8 ± 12.5	0.85 ± 0.10
Bottomland	Fall	11.29 ± 1.77	57.1± 6.1	1.14 ± 0.079
Bottomland	Spring	22.7 ± 2.4	153.4 ± 26.2	1.12 ± 0.073

Table 2.2: Results of univariate ANOVAs on bulk density and soil moisture. Site was included as a repeated effect in these analyses.

	Bulk Density		Soil Moisture	
	F	p-value	F	p-value
Habitat	$F_{1,10}$=25.0	0.0005	$F_{1,9}$=58.6	<0.0001
Season	$F_{1,3}$=6.69	0.08	$F_{1,3}$=11.49	0.043
Habitat × Season	$F_{1,3}$=0.16	0.72	$F_{1,3}$=6.34	0.086

Table 2.3: Univariate ANOVA results of soil nutrient levels (means ± standard deviation). Pooled samples from four upland and four bottomland sites were assessed, except for C:N for which 5 upland and four bottomland sites were used ($F_{1,4.7}$).

	Bottomland	Upland	$F_{1,6}$	p-value
Available Phosphorus (mg/Kg)	1.83 ± 0.53	0.9 ± 0.42	7.4	0.035
Available Potassium (mg/Kg)	40.75 ± 5.12	24.75 ± 4.19	23.4	0.0029
Available Magnesium (mg/Kg)	66.55 ± 29.4	7.13 ± 3.86	16.1	0.007
Available Calcium (mg/Kg)	1088.3 ± 451.5	83.5 ± 102.5	18.8	0.005
Available Iron (mg/Kg)	14.33 ± 6.89	6.35 ± 3.68	4.2	0.087
Available Aluminum (mg/Kg)	21.7 ± 5.56	73.6 ± 46.5	4.9	0.069
Available Manganese (mg/Kg)	22.93 ± 7.9	2.78 ± 2.99	22.7	0.0031
Available Zinc (mg/Kg)	0.60 ± 0.36	0.21 ± 0.11	4.4	0.081
Available Copper (mg/Kg)	1.85 ± 1.03	0.4 ± 0.32	7.3	0.037
pH	5.32 ± 0.16	4.86 ± 0.58	2.3	0.18
Percentage Organic Matter	2.28 ± 0.78	0.93 ± 0.58	7.63	0.033
C:N	16.9 ± 0.4	25.6 ± 1.3	41.0	0.002

Table 2.4: LSMEANS (± SE) of light levels at four bottomland and four upland sites (April 2008). Canopy openness is the percentage of open sky visible from the understory. Dimensionless LAI5 represents the effective leaf area index integrated over zenith angles 0-75°; higher values indicate more closed canopies. Direct and diffuse solar radiation refer to the amount of transmitted light (Frazer et al. 1999).

	Bottomland	Upland	$F_{1,8}$	p-value
Canopy openness (%)	33.3 ± 2.08	44.8 ± 2.08	15.3	0.005
Leaf area index (LAI5)	1.13 ± 0.05	0.75 ± 0.05	28.0	0.0007
Direct radiation (Mols m^{-2} day^{-1})	8.77 ± 0.38	11.98 ± 0.4	36.6	0.0003
Diffuse radiation (Mols m^{-2} day^{-1})	7.95 ± 0.40	11.1 ± 0.4	29.8	0.0006

***Demography*—**Plant density of non-reproductive and reproductive adults was higher in upland than bottomland populations (mean ± S.E. population size of non-reproductive and reproductive adults / transect: upland: 29 ± 5.1; bottomland: 5.4 ± 1.1). Additionally, upland populations produced more reproductive structures than bottomland populations (mean ± S.E. number of reproductive structures: upland: 222.7 ± 7.8; bottomland: 16.1 ± 5.9; Table 3). There was no effect of population proximity or habitat by proximity in either analysis (Table 2.5). Similar patterns resulted when I analyzed total plant density (juveniles, as well as non-reproductive and reproductive adults).

Table 2.5: *V. elliottii* abundance and reproductive success (flower and fruit production) of naturally-recruited plants in upland and bottomland forests.

	Population density		Reproductive output	
	$F_{1,4}$	p-value	$F_{1,4}$	p-value
Habitat	18.3	0.013	14.2	0.02
Proximity	0.26	0.64	0.01	0.92
Habitat × Proximity	0.27	0.63	0.15	0.72

***Reciprocal transplant experiment: Survivorship*—**In the field experiment, survivorship declined precipitously in bottomland transplant sites for both 2005 and 2006 transplants. Cox proportional hazards models indicate a significant and strong effect of habitat on the time until mortality (Table 2.6, Figure 2.1). Additionally, seedlings had significantly lower survivorship in the 2006 transplants than cuttings (Figure 2.2) and had very poor success in the bottomlands. The significant effect of transplant site in both years reflected enhanced performance at Upland Site 1,

Table 2.6: Results of Cox proportional hazards survivorship analyses implemented in Winbugs for the 2005 and 2006 transplants. Predictors whose 95% credible intervals do not span 0 have a significant influence on survivorship and are highlighted in bold. The proportionality assumption was violated for several predictors, which necessitated the inclusion of time dependence (i.e. predictor × natural log of time). Additional interaction terms were evaluated, but are not shown because they were not significant.

Explanatory variable	2005 transplants	2006 transplants
	95% credible interval	95% credible interval
Initial diameter	**(-1.7, -1.2)**	**(-1.03, -0.77)**
Initial diameter × Time	**(0.08, 0.24)**	
Habitat of origin	(-0.17, 0.024)	(-0.4, 0.09)
Proximity	(-0.034, 0.362)	(-0.32, 0.17)
Habitat × Proximity	**(10.5, 20.6)**	(-0.75, 0.23)
Habitat × Proximity × Time	**(-3.1, -1.6)**	
Transplant environment	**(-13.1, -10.2)**	**(-1.8,-0.98)**
Transplant environment × Time	**(1.1, 1.6)**	
Habitat × Transplant environment	(-0.32, 0.36)	(-0.47,0.20)
Proximity × Transplant environment	(-0.12, 0.57)	(-0.58, 0.12)
Habitat × Proximity × Transplant environment	**(17.1, 37.8)**	(-0.57, 0.88)
Habitat × Proximity × Transplant environment × Time	**(-5.5, -2.4)**	
Life history stage		**(0.10, 0.58)**
Site	**(-0.65, -0.24)**	**(-0.53, -0.18)**
Variance of population frailty	**(0.048, 0.14)**	**(0.0009,0.3)**
Variance of family frailty	**(0.052, 0.18)**	**(0.002, 0.13)**

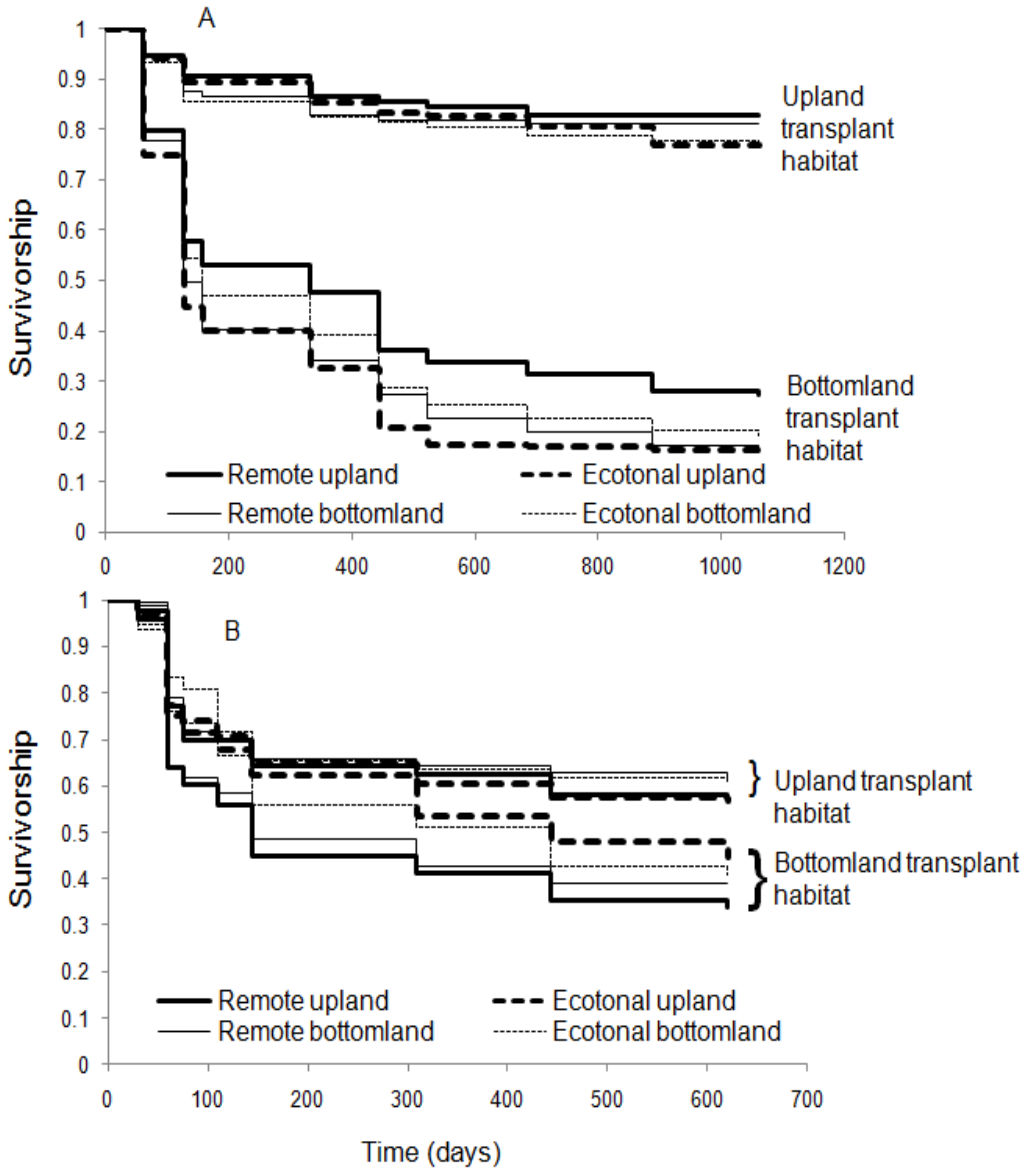

Figure 2.1: Kaplan-Meier survivorship curves for (a) 2005 and (b) 2006 transplants. Both panels indicate: transplant habitat, as well as habitat of origin and population proximity to the ecotone.

and relatively equivalent performance at Upland Site 2 and Bottomland Site 2, and depressed performance at Bottomland Site 1 (Appendix 5).

For the 2005 transplants, there were additional effects of habitat by proximity and habitat by proximity by transplant environment (as well as time-dependent factors

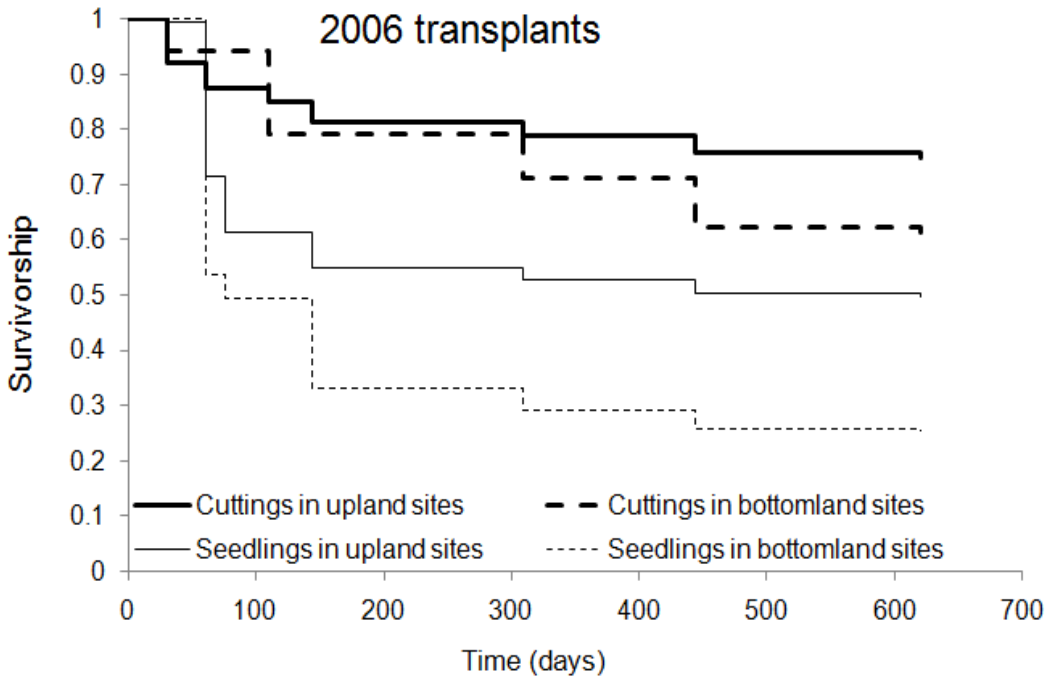

Figure 2.2: Kaplan-Meier survivorship curves for seedlings and cuttings from the 2006 transplant experiment.

of both of these predictors). These interactions were driven by enhanced performance of individuals from remote upland populations within the bottomland transplant sites (Figure 2.1), but equivalent survivorship of individuals within upland transplant sites.

Due to the long generation time of *V. elliottii*, it was not possible to rear multiple generations of adults in the greenhouse prior to experimental manipulations. I minimized potential maternal or latent environmental effects by growing all individuals in benign greenhouse conditions for 6-8 months before experimentation. Initial size did not differ between plants from bottomland and upland habitats or ecotonal and remote populations ($p > 0.12$ for the effects of habitat, proximity, and habitat × proximity and their interactions with transplant site on initial size). These results suggest that plants from remote upland populations did not have a systematic initial advantage.

Table 2.7: Survivorship analysis from the greenhouse experiment, conducted using Bayesian statistics in Winbugs. Statistically significant predictors of survivorship, whose 95% credible intervals do not span 0, are highlighted. I included a time-dependent predictor for treatment (treatment × natural log of time) because otherwise the model violated the proportionality assumption. Additional three and four-way interactions were nonsignificant and were dropped from the model.

	mean (standard deviation)	95% credible interval
Initial biomass	-0.069 (0.068)	(-0.20, 0.06)
Habitat of origin	-0.22 (0.15)	(-0.52,0.08)
Proximity	-0.03 (0.15)	(-0.33,0.28)
Habitat × Proximity	0.15 (0.30)	(-0.44, 0.75)
Treatment	**-5.7 (2.3)**	**(-5.7,-1.4)**
Treatment × Time	**0.97 (0.45)**	**(0.11, 1.9)**
Habitat × Treatment	-0.13 (0.27)	(-0.67, 0.40)
Proximity × Treatment	0.015 (0.28)	(-0.53, 0.56)
Habitat × Proximity × Treatment	0.4 (0.55)	(-0.67, 1.5)
Life history stage	**-23.2 (2.5)**	**(-28.1, -18.5)**
Life history stage × Treatment	-0.24 (0.30)	(-0.82, 0.34)
Life history stage × Proximity	**-0.55 (0.26)**	**(-1.05, -0.04)**
Life history stage × Habitat	**-0.59 (0.26)**	**(-1.11, -0.09)**
Life history stage × Habitat × Proximity × Treatment	-0.33 (0.99)	(-2.3, 1.6)
Life history stage × Time	**4.58 (0.5)**	**(3.65, 5.5)**
Variance of population frailty	**0.11 (0.04)**	**(0.05, 0.22)**
Variance of family frailty	**0.11 (0.04)**	**(0.05, 0.21)**
Variance of block frailty	**0.32 (0.08)**	**(0.17, 0.48)**

Greenhouse experiment: Survivorship—In the greenhouse, there were significant effects of treatment and treatment by time on survivorship (Figure 2.3, Table 2.7). Mortality was significantly greater in the flooded treatment than the drought treatment. A life history stage by habitat interaction from the greenhouse experiment shows that seedlings from bottomland populations had significantly greater survivorship than seedlings from upland populations; cuttings did not exhibit this pattern (Table 2.7). Finally, a life history stage by population proximity interaction (Table 2.7) indicates that seedlings from remote populations outperformed seedlings from ecotonal populations, while the opposite pattern was true for cuttings.

Reciprocal transplant experiment: Relative growth rate—Across years, individuals in upland transplant sites outgrew those in bottomland sites (Tables 2.8 and 2.9, Figure 2.4). For the 2005 transplants, there were additional effects of habitat

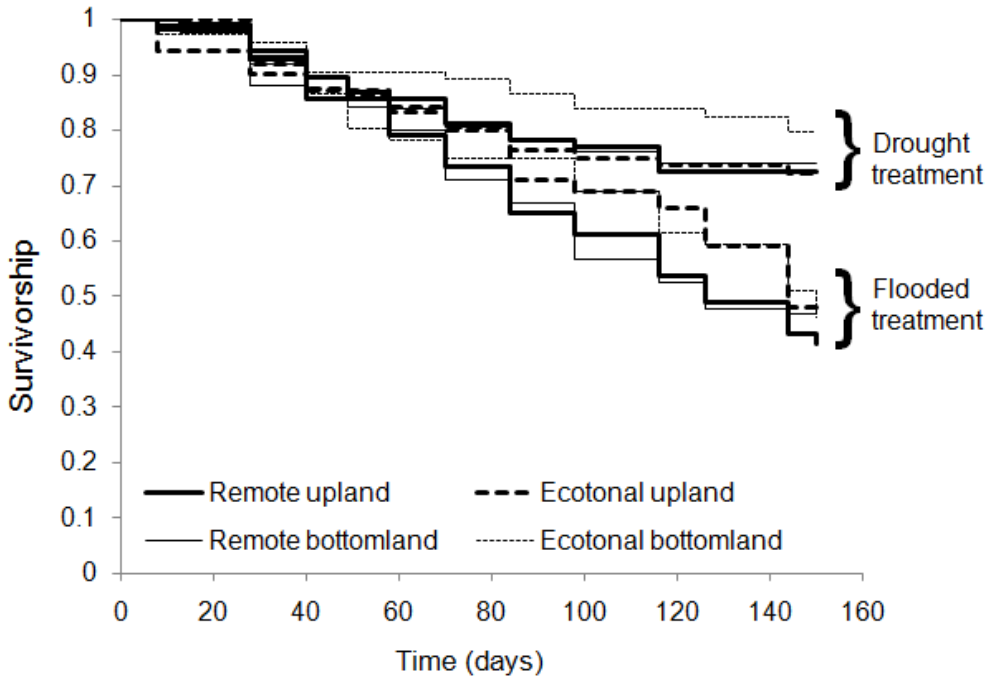

Figure 2.3: Kaplan-Meier survivorship curves from the flooded and drought treatments in the greenhouse experiment.

of origin, habitat of origin by transplant environment, year of measurement, year by transplant environment, and year by habitat of origin. Growth rate during the second

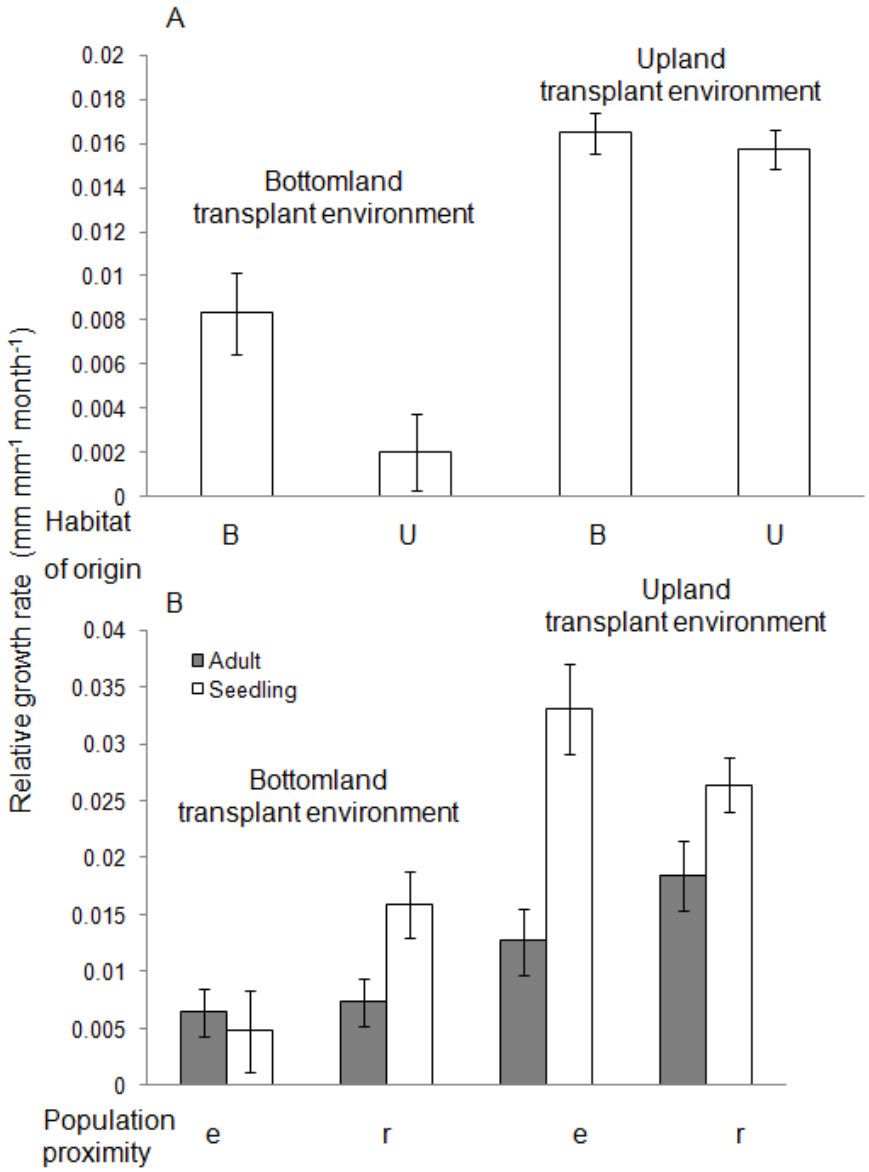

Figure 2.4: Relative growth rate analyses for (a) 2005 and (b) 2006 transplants. Refer to tables 2.8 and 2.9 for statistical results. Habitat of origin is indicated by B (bottomland) and U (upland) in panel (a) and population proximity is indicated by e (ecotonal population of origin) and r (remote population) in panel (b).

Table 2.8: Repeated measures ANOVA on relative growth rate (RGR) of 2005 transplants measured in October 2006 and 2007. These results are from the reduced model; all other effects and interactions were non-significant (p>0.1).

	$F_{1,1377}$	p-value
Habitat of origin	7.75	0.0054
Transplant environment	48.7	<0.0001
Habitat × Transplant environment	4.06	0.025
Year of measurement	112.9	<0.0001
Year × transplant environment	22.9	<0.0001
Year × Habitat of origin	4.9	0.027
Site(transplant environment)	39.9	<0.0001

Table 2.9: Mixed model ANOVA on first year relative growth rate (RGR) of 2006 transplants (measured in October, 2007). These results are from the reduced model; all other effects and interactions were non-significant (p>0.1). I retained proximity (ecotone or remote) due to the significant interaction between this effect, life history stage and transplant environment.

	F	p-value
Transplant environment	$F_{1,346}$=45.1	<0.0001
Life history stage	$F_{1,346}$=14.9	<0.0001
Life history stage × Transplant environment	$F_{1,346}$=7.56	0.0063
Proximity	$F_{1,346}$=1.66	0.20
Life history stage × Transplant environment × Proximity	$F_{3,346}$=3.65	0.013
Site(transplant environment)	$F_{1,346}$=8.97	0.0029

year was significantly lower than during the first year (LSMEANS ± S.E.: first year: 0.0194 ± 0.0011 mm mm^{-1} $month^{-1}$; year 2: 0.00187 ± 0.0012 mm mm^{-1} $month^{-1}$). Individuals from bottomland populations outperformed those from upland populations within the bottomland forest (t_{895}=2.8, p-value adjusted for multiple comparisons = 0.026); this is the only evidence for local adaptation in this dataset. There was no effect of habitat of origin or interaction between habitat and transplant environment for the 2006 transplants (measured in 2007). In addition to the main effect of transplant environment, life history stage, transplant environment by life history stage and transplant environment by population proximity all significantly predicted RGR. Seedlings had greater relative growth rates than cuttings (t_{468}=4.77, p-value adjusted for multiple comparisons <0.0001). The transplant environment by proximity by life history stage interaction indicated increased performance of cuttings from remote populations in upland transplant sites relative to cuttings from both remote and ecotonal populations in the bottomland site. In contrast, seedlings from remote populations had significantly greater RGR than ecotonal seedlings in the bottomland environment, but this pattern does not hold in the uplands.

Table 2.10: Mixed model ANOVA results of relative growth rate data from cuttings and seedlings in the greenhouse experiment. All other main effects and interaction terms were nonsignificant (p>0.1) and were removed from the model.

	F	p-value
Treatment	$F_{1,52}$=52.9	<0.0001
Life history stage	$F_{1,206}$=13.0	0.0004
Life history stage × Treatment	$F_{1,206}$=41.0	<0.0001

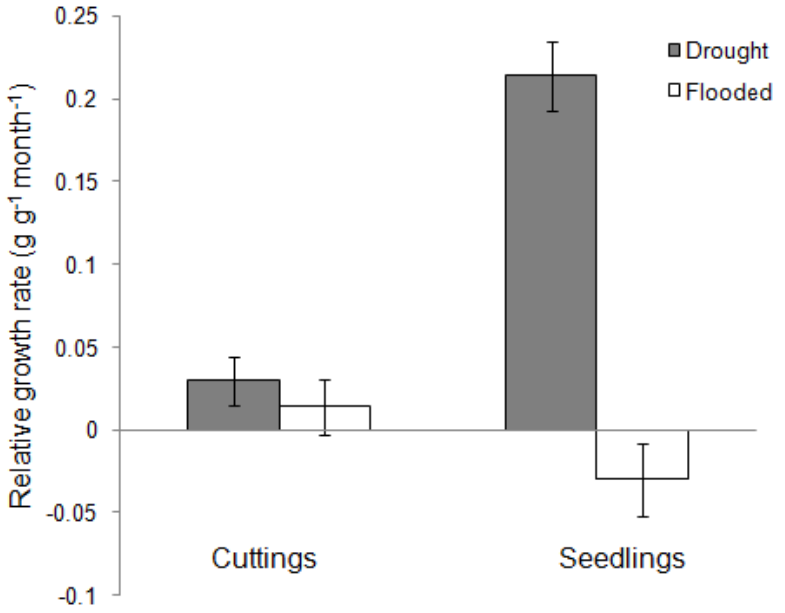

Figure 2.5: Effects of treatment and life history stage on relative growth rate in the greenhouse experiment.

***Greenhouse experiment: Relative growth rate*—**Relative growth rate was significantly greater in the drought than the flooded treatment in the greenhouse; seedlings also had greater RGR than adults (Table 2.10, Fig.2.5). An interaction between life history stage and treatment was driven by significantly greater performance of seedlings in the drought treatment than the flooded treatment and relatively equivalent performance of cuttings in both treatments.

***Population genetic differentiation*—**I detected no evidence for selection at any of the 8 loci tested (Appendix 6); however, GENEPOP revealed significant linkage disequilibrium between two loci (CA23F and CA787F) and three others; I removed these two loci from the dataset and resolved the disequilibrium problem. The remaining 6 loci appeared to be in Hardy-Weinberg equilibrium; I detected only 7 significant deviations out of 88 tests for adults (loci were monomorphic at 14 population-locus combinations) and 3 deviations out of 80 tests for seedlings (10

monomorphic population-locus combinations). Microchecker found no evidence for scoring errors due to stutter, or large allele drop out. Several loci (CA94F in 3 populations, and CA855F, NA961 and CA190R in one population each) showed an excess of homozygotes, suggesting the presence of null alleles. Null allele frequencies were estimated (Brookfield 2) and used in downstream analyses (Brookfield, 1996). Overall genotyping error rate for mother offspring pairs was low (only13 alleles mistyped out of 1368 reactions, see Appendix 7 for locus-specific error).

Multilocus estimates of F-statistics indicate that adults and seeds were more homozygous than would be expected under random mating, both across populations (adult F_{IT}: 0.108, 95% CI=0.074, 0.127; seedling F_{IT}: 0.080, 95% CI=0.034, 0.094) and within populations (adult F_{IS}: 0.078, 95% CI=0.044, 0.091; seedling F_{IS}: 0.043, 95% CI=-0.013, 0.067). F_{ST} values were relatively low, indicating little population differentiation. Furthermore, overall F_{ST} did not differ between adults (F_{ST}: 0.032, 95% CI=0.022, 0.045) and seeds (F_{ST}: 0.038, 95% CI=0.027, 0.052). A Mantel test also revealed no significant difference between pairwise population differentiation (F_{ST}) for adult and seed populations (r=0.21, p=0.2). Per-locus and weighted multilocus *F*-statistics, and observed and expected heterozygosities are presented in Appendix 8 (adults) and Appendix 9 (seeds).

An exact test for differentiation in allele frequency between upland and bottomland populations was significant both for adults (χ^2=28.1, d.f.=12, p=0.005) and seeds (χ^2=21.03, d.f.=12, p=0.05). Inspection of allele frequencies suggests that bottomland populations contain a subset of the alleles present in upland populations. However, in the analysis of molecular variance (AMOVA), I found significant variation within populations, and among populations, but not between upland and bottomland populations for both seeds and adults (Table 2.11).

Table 2.11: Analysis of molecular variance (AMOVA) results for seeds and adults.

	Adults				Seeds			
		Variance component				Variance component		
	d.f.	Absolute	%	P	d.f.	Absolute	%	P
Between habitats	1	0.0013	0.16	0.32	1	-0.006	-0.46	0.78
Among populations within a habitat	15	0.028	3.6	<0.0001	13	0.057	4.24	<0.0001
Within populations	613	0.76	96.3	<0.0001	333	1.29	96.2	<0.0001
Total	629	0.79			374	1.34		

Table 2.12: Partial Mantel tests of geographic distance and habitat (between or within habitat comparison) on pairwise genetic differentiation for seeds and adults. Note: The program zt provides one-tailed p-values, but I report two-tailed values.

	Adults		Seeds	
Mantel test	r	P-value†	r	P-value
Geographic distance	0.42	0.0014	-0.05	0.75
Habitat	0.02	0.68	-0.04	0.69
Geographic distance and Habitat (Simple Mantel test)	-0.11	0.08	-0.14	0.08

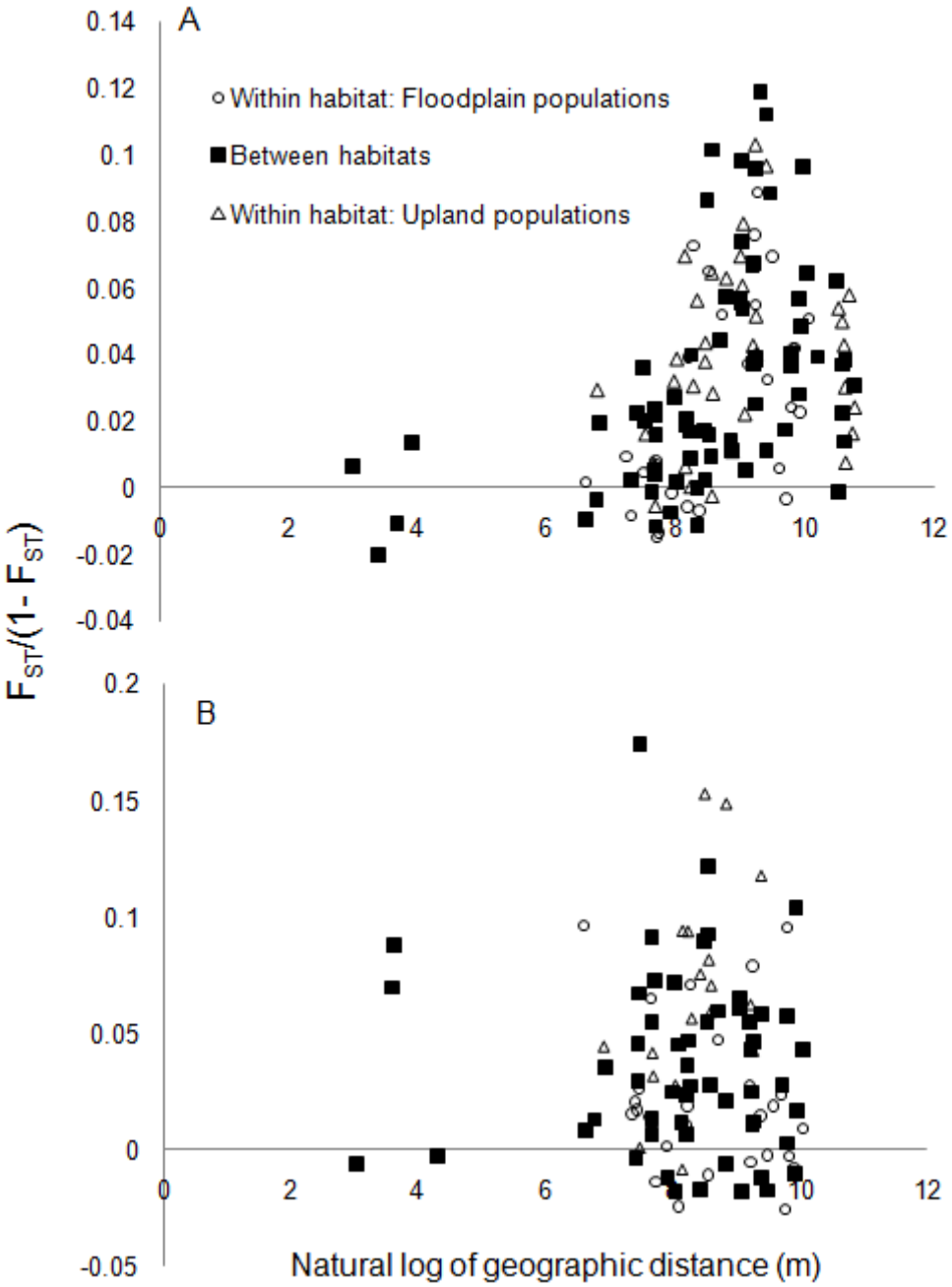

Figure 2.6: Genetic population divergence as a function of geographic distance for (a) adults and (b) seeds. Symbols indicate whether population pairs occurred in the same habitat or in different habitats.

Partial Mantel tests revealed that genetic population differentiation in adults increased significantly with geographic distance, but habitat was not significant (Figure 2.6, Table 2.12). For seeds, there was no effect of geographic distance or habitat on genetic divergence, suggesting relatively unrestricted gene flow via pollen.

Table 2.13: Analyses of private allelic richnesss (PAR) and internal relatedness using microsatellite markers. PAR is assessed at the population level. I sampled 17 populations for adults, but only 15 populations for seeds. Internal relatedness was measured at the individual level.

	Private Allelic richness		Internal relatedness	
	F	P	F	P
Life history stage	$F_{1,11}$=4.09	0.068	$F_{1,60}$=1.15	0.29
Habitat	$F_{1,13}$=7.01	**0.0201**	$F_{1,60}$=1.40	0.24
Proximity	$F_{1,13}$=5.00	0.043	$F_{1,60}$=0.34	0.56
Habitat × Proximity	$F_{1,11}$=10.3	**0.0068**	$F_{1,60}$=7.01	**0.010**
Habitat ×Life history	$F_{1,11}$=0.68	0.43	$F_{1,60}$=1.46	0.23
Proximity × Life history	$F_{1,11}$=2.67	0.13	$F_{1,60}$=3.01	0.088
Habitat × Proximity × Life history	$F_{1,11}$=7.79	**0.018**	$F_{1,60}$=1.66	0.202

Population genetic diversity—An analysis accounting for private (unique) alleles uncovered significant effects of habitat, proximity, habitat × proximity, and habitat × proximity × life history stage (Table 2.13). Upland populations had significantly greater private allelic richness (PAR) than bottomland populations, and remote populations in either habitat had significantly greater PAR than ecotonal populations. Similarly, remote upland populations had significantly greater PAR than all other habitat by proximity configurations (Fig. 2.7). Internal relatedness was significantly higher for genotypes from remote bottomland populations than remote

upland populations (Table 2.13, Fig 2.8). I found no effect of life history stage, habitat, proximity, or their interactions on rarefied allelic richness or expected heterozygosity (results not shown).

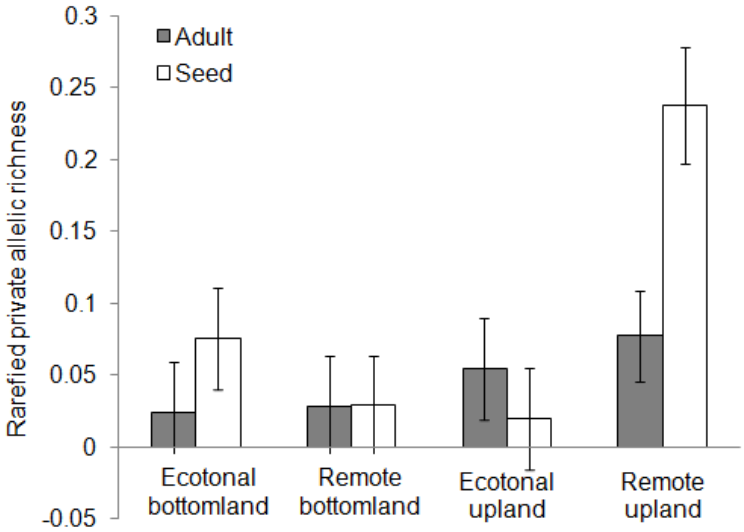

Figure 2.7: Genetic diversity (private allelic richness) as a function of life history stage, habitat, and proximity to the ecotone.

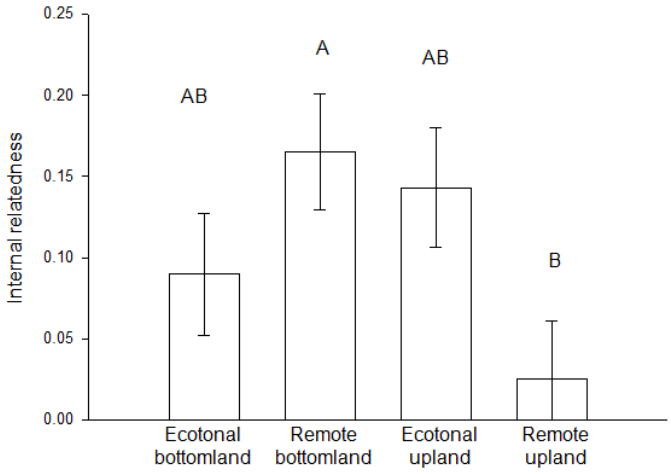

Figure 2.8: Internal relatedness as a function of habitat and proximity to the ecotone. Internal relatedness is a measure of parental similarity that ranges from -1 (distantly related) to 1 (completely inbred). Plotted are LSMEANS ± S.E. Letters represent significant differences between groups of populations.

***Gene flow*—**I found no effect of habitat or proximity on effective population size or interhabitat migration rate at the seed life history stage. However, significantly more migrants ($4N_em$) move from upland into bottomland populations (3.6 ± 0.28) than from bottomland into upland populations (2.7 ± 0.29, $t_{55}=3.45$, $p=0.0011$).

Discussion

Despite the steepness of the environmental gradient between upland and bottomland forests, *Vaccinium elliottii* showed virtually no evidence for adaptive or neutral population differentiation. Instead, *V. elliottii* individuals consistently had greater survivorship and growth (reciprocal transplant experiment), as well as flower and fruit production (demographic study) in upland than bottomland habitats. Additionally, this study provides strong evidence that *V. elliottii* individuals are better adapted to long-term drought than flooding. In the greenhouse, individuals in the drought treatment had significantly greater fitness than their relatives in the flooded treatment. Furthermore, our molecular study revealed very little population genetic differentiation, especially at the seed level where we did not even detect isolation by distance. Finally, remote upland populations harbored significantly greater genetic diversity in terms of unique alleles than all other populations. These characteristics are consistent with genetic source-sink dynamics, which could potentially limit adaptation to the marginal bottomland habitat (Dias and Blondel, 1996; Pulliam, 1988; Stanton and Galen, 1997).

The higher relative fitness of individuals in upland forests likely causes asymmetrical gene flow into the bottomlands. The flowers of *Vaccinium* spp. (including *elliottii*) are pollinated primarily by bees and the seeds are dispersed by a variety of birds and mammals (Javorek *et al.*, 2002; Mahoro, 2003; Nuortila *et al.*, 2002; Siitari *et al.*, 1999; Vander Kloet and Austin-Smith, 1986; Yang *et al.*, 2008). I have observed a similar suite of pollinators and seed dispersers in both upland and

bottomland forests. I hypothesize that, when foraging on *V. elliottii* flowers and fruits, they focus their efforts on the more productive upland populations and make limited visits to bottomland populations. Furthermore, genetic diversity is significantly greater and internal relatedness (parental similarity) is lower in the robust upland populations of *V. elliottii* relative to the less abundant bottomland populations. I do not have pedigree data sufficient to calculate the inbreeding coefficient (Pemberton, 2004; Slate *et al.*, 2004). However, individual-level metric of multilocus heterozygosity (internal relatedness) suggests that inbreeding may be lower in remote upland populations than remote bottomland populations. Hedrick et al. (2001) demonstrated a strong negative relationship between microsatellite heterozygosity and the inbreeding coefficient for a population of captive wolves. Other studies have also shown that heterozygosity is a good predictor of the inbreeding coefficient (e.g. Jensen *et al.*, 2007). I suggest that the ability of bottomland populations to adapt to local conditions is diminished by directional gene flow into bottomland forests, coupled with reduced fitness, depressed genetic variation and greater internal relatedness in bottomland forests. This hypothesis accords well with theoretical predictions that source-sink dynamics can constrain adaptive evolution in marginal habitats (Kawecki and Holt, 2002).

In a classic study, Stanton and Galen (1997) found no evidence for local adaptation in the snow buttercup across a snowmelt gradient. Rather, plants from high quality microsites produced high quality seeds, presumably due to greater resource availability; differences in seed quality resulted in asymmetric gene flow and the creation of source-sink dynamics (Stanton and Galen, 1997). Due to the small size of seeds, I was unable to assess fitness at the seed to seedling transition; however, I have seen no evidence that upland seeds were consistently larger, or had greater germination rates in the laboratory (pers. obs.). Interestingly, in our 2005 reciprocal

transplant experiment, plants from remote upland populations had significantly greater survivorship than individuals from bottomland and ecotonal upland populations within bottomland transplant sites. I did not see this effect, however, in the 2006 transplants. The year 2005 was unusual because of an extensive flood event that occurred after transplanting approximately half of the cuttings. Abiotic stress can augment inbreeding depression (Armbruster and Reed, 2005). It is possible that the greater genetic diversity, or reduced internal relatedness and inbreeding, of remote upland populations allowed them to exhibit enhanced performance beginning in a stressful year. Nevertheless, genotypes from remote upland populations did not have a fitness advantage in the prolonged drought and flooded treatments of the greenhouse experiment. The source-sink dynamics present in this system are likely due to the differences in the number of pollen grains and seeds produced in the two habitat types, but may be compounded by differences in genetic diversity and fitness between upland and bottomland genotypes.

I found very low levels of population genetic differentiation and no increase in population differentiation through ontogeny, which suggests that selection against immigrants is likely weak (Kalisz *et al.*, 2001). Additionally, there was no systematic evidence that the extent of local adaptation increased through life history. The only indication of local adaptation was the enhanced growth rates of bottomland genotypes in bottomland transplants sites for the cuttings planted in 2005. I am hesitant to conclude that cuttings are more locally adapted than seedlings because seedlings were not transplanted in 2005. However, in several instances, seedlings did appear more poorly adapted to bottomland and flooded conditions than cuttings. For example, seedling growth rate in the greenhouse experiment was actually negative in the flooded treatment, whereas drought-stressed seedlings had the greatest growth rates, exceeding those of cuttings in either treatment. Additionally, in the field experiment,

seedlings had very low survivorship in bottomland transplants sites. All seedlings died at bottomland transplant site 1 and the vast majority of seedlings died at bottomland transplant site 2. The relatively low performance of seedlings in bottomland habitat and under flooding likely contributes to low population densities and low genetic diversity in bottomland relative to upland habitats.

In the absence of migration from source to sink populations, the sink could either go extinct if mortality exceeds birth and survivorship (absolute sink), or adapt to local conditions if sufficient genetic variation exists (pseudosink in which immigration elevates population sizes above non-zero carrying capacity) (Dias, 1996; García-Ramos and Kirkpatrick, 1997; Kawecki and Holt, 2002; Pulliam, 1988). I do not know whether bottomland populations of *V. elliottii* would persist in the absence of interhabitat migration. *Vaccinium* is not monophyletic and a complete phylogeny of the hundreds of species in this genus is not yet available (Kron *et al.*, 2002; Powell and Kron, 2002). Without more detailed phylogenetic data, it is difficult to resolve the ancestral niche breadth of this species. Nevertheless, other species of *Vaccinium* and other genera in the Ericaceae (e.g. *Leucothoe*) sustain populations in bottomland habitats and five species of *Vaccinium* are considered obligate wetland species (http://www.fws.gov/nwi/bha/downloads/1996/national.pdf). Since other *Vaccinium* species have adapted to wetland conditions (Braendle and Crawford, 1999), *V. elliottii* may harbor the genetic potential to adapt to these conditions as well.

Abiotic and biotic factors appear to contribute to fitness differences of *V. elliottii* in upland and bottomland forests. For one, there is a steep flooding gradient from bottomland to upland forests (Burke *et al.*, 1999). The mean monthly rainfall in 2005 was 51 mm greater than average growing season levels (N. Brunswig and M. Dawson, unpub. precipitation records). A flood event that year led to high mortality in bottomland transplants. In contrast, 2006 was much drier (monthly precipitation

was 10 mL less than the average growing season value), which could have contributed to overall increases in survivorship in the bottomlands and decreases in the uplands. Indeed, survivorship declined more rapidly in bottomland sites for the 2005 transplants than the 2006 transplants, which have still not experienced a flood event. Flooded individuals from the greenhouse study also showed poor performance. Thus, flooding is a severe stress for this species; however, flooding is clearly not the sole determinant of plant performance, or the fitness of 2006 transplants would not vary with habitat. Leaf herbivory is significantly greater in bottomland than upland forests (Chapter 3). This difference in biotic stress likely reduces the relative fitness of plants in the bottomland system. Interspecific competition is an additional biotic stress that could influence survivorship and growth in the bottomlands. Upland forests have a sparse understory, whereas a dense layer of dwarf palmetto (*Sabal minor*, Arecaceae) covers the forest floor in bottomland forests (Porcher, 1981, Anderson unpub. data). One of our bottomland transplant sites (F2) had a lower abundance of *S. minor* than the other (F1); the significant effect of transplant site in the survivorship and growth analyses was due, in part, to enhanced performance of cuttings and seedlings at F2 relative to F1. Thus, a complex suite of factors, including flooding, herbivory and interspecific competition, probably reduces the fitness of *V. elliottii* individuals in the bottomlands. Differences in soil texture, bulk density, and nutrient content, and even ericoid mycorrhizal fungi abundance could also influence plant performance in the bottomlands, although we have no direct evidence for the effect of these abiotic and biotic factors on *V. elliottii* fitness.

***Conclusions*—**Results from this study show that *V.elliottii* is poorly adapted to bottomland and flooded conditions at multiple life history stages. Despite temporal variation in rainfall, in both years of the reciprocal transplant experiment, survivorship and growth rate were significantly greater in upland relative to bottomland forests.

Additionally, overall plant performance was relatively low in the flooded compared to the drought treatment in the greenhouse (see also Chapter 3). Upland populations have higher plant density, produce more reproductive structures per capita, and have greater genetic diversity than bottomland populations. Spatial genetic analyses indicated that gene flow is likely very high across the landscape and may be primarily from upland to bottomland populations. I propose that interhabitat gene flow reduces the potential for bottomland populations to adapt to local conditions.

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CHAPTER 3

PHENOTYPIC RESPONSE OF ELLIOTT'S BLUEBERRY (*VACCINIUM ELLIOTTII*) TO A COMPLEX ENVIRONMENTAL GRADIENT

Summary

Species that exhibit adaptive phenotypic plasticity alter their phenotypes in response to environmental conditions, thereby maximizing fitness across spatially and temporally heterogeneous landscapes. However, when individuals of a species show significantly greater fitness in one habitat than another, selection is thought to favor traits that enhance fitness in the high quality or source habitat at the expense of fitness in the marginal habitat. Phenotypic plasticity is not expected to evolve under these conditions. Elliott's blueberry (*Vaccinium elliottii*) occurs in dry upland and flood-prone bottomland forests through the Southeastern United States. These contrasting habitats differ in water table depth, soil texture and nutrient availability, plant species composition, and light penetration to the understory. These differences likely impose strongly divergent natural selection, which could favor alternate phenotypic optima. Nevertheless, I found limited evidence for local adaptation to bottomland and upland forests in a previous study. Instead, *V. elliottii* exhibited patterns consistent with source-sink dynamics: higher fitness in upland relative to bottomland forests and asymmetrical gene flow from upland into bottomland populations. In the current study, I assessed whether families of *V. elliottii* seedlings and cuttings displayed a phenotypically plastic response to a complex environmental gradient in a reciprocal transplant experiment. Additionally, a greenhouse experiment tested the effects of long-term drought vs. flooding on *V. elliottii* phenotypes, thus isolating one of the main abiotic differences between upland and bottomland habitats. In contrast to predictions from source-sink models, I found a high degree of phenotypic plasticity in foliar traits in the field and some root traits in the greenhouse. Nevertheless, this

species exhibited few traits consistent with flooding tolerance and long-term flooding appears to be a severe stress. Furthermore, phenotypic plasticity in foliar traits was greater in the field experiment than the greenhouse experiment, which suggests that differences in water stress were not the primary drivers of the foliar plasticity that was observed in the field. I hypothesize that foliar plasticity is in response to differences in light level and potentially edaphic conditions, and could actually be favored within upland habitats. Additionally, phenotypic plasticity could allow *V. elliottii* individuals to establish in the stressful bottomland forests. However, interhabitat gene flow probably also reduces the potential for bottomland populations to adapt to local conditions.

Introduction

Adaptive phenotypic plasticity, where phenotypes shift in response to changing environmental conditions, is a strategy that maximizes fitness across heterogeneous landscapes (van Tienderen, 1997). Phenotypic plasticity can evolve when individuals experience multiple environments during their lifetime (Moran, 1992; Stratton and Bennington, 1998). Even if parents only experience one set of environmental conditions, phenotypic plasticity can be advantageous if their offspring establish in a non-parental habitat (Alpert and Simms, 2002). Thus, interhabitat gene flow can enhance selection for plasticity (Hollander, 2008; Scheiner, 1998; Sultan and Spencer, 2002; van Tienderen, 1997). Phenotypic plasticity seems like an ideal response to heterogeneity because individuals express the appropriate phenotype for a given environment; however, not all species that encounter multiple abiotic and/or biotic stresses exhibit plastic phenotypes. The evolution of plasticity hinges on the requirements that individuals can adequately track environmental changes, can overcome the costs of a plastic response and are at a fitness advantage if they shift phenotypes in different habitats or under different environmental conditions (Alpert

and Simms, 2002; DeWitt *et al.*, 1998; Moran, 1992; Poulton and Winn, 2002; Sultan and Spencer, 2002; van Tienderen, 1997; Via and Lande, 1985). If these criteria are met, a plastic response can be generated in a spatially variable landscape when habitats are connected by gene flow (van Tienderen, 1997; Via and Lande, 1985).

In a previous study, I documented high gene flow between populations of Elliot's blueberry (*Vaccinium elliottii*) in contrasting upland and bottomland habitats and limited to no adaptive or neutral population differentiation (Chapter 2). These conditions would appear to promote the evolution of phenotypic plasticity. However, fitness (survivorship and growth) was significantly higher in dry uplands than flood-prone bottomland forests, consistent with source-sink dynamics. Source-sink dynamics present an additional constraint on the evolution of phenotypic plasticity. When habitat quality or size varies, source-sink models predict that populations will adapt to conditions in the higher quality or larger habitat (Holt and Gaines, 1992; Kawecki, 1995). Phenotypic plasticity is not predicted to evolve under these conditions because a relatively low proportion of the population experiences the sink habitat; therefore, selection for a plastic response should be minimal (Dias, 1996). However, plasticity can facilitate range or niche expansion (e.g. Schlichting and Smith, 2002). Phenotypic plasticity could enhance establishment and persistence in sink or marginal habitats. Indeed, plasticity could be particularly important for sessile organisms that cannot search for more hospitable habitats. Thus, the objective of this study was to determine whether *V. elliottii* exhibits fixed phenotypic traits that enhance fitness in upland habitats, as predicted by source-sink models, or whether phenotypic plasticity could evolve despite asymmetrical gene flow from higher to lower quality habitats.

To assess phenotypic evolution in an ecologically-relevant context, I conducted a multi-year reciprocal transplant experiment to quantify phenotypic

responses to a complex environmental gradient, which varies in water table depth, light level, and edaphic characteristics. In a separate greenhouse experiment, I isolated the effects of flooding and drought to assess whether phenotypic traits that varied in the field were a response to water stress. In both experiments, I measured phenotypic traits related to drought- and flooding-tolerance. Flooding deprives roots of oxygen and can result in a reduction in photosynthesis and stomatal conductance, greater leaf senescence, necrosis and abscission, and ultimately death (Blokhina *et al.*, 2003; Keeley, 1979; Mielke *et al.*, 2003; Pereira and Kozlowski, 1977; Visser *et al.*, 2003). Plants have evolved constitutively-expressed and inducible phenotypic traits that enhance gas exchange between above-water biomass and the roots (Kozlowski, 2002). For example, flooding can induce the formation of aerenchyma (gas-filled porous tissue, Evans, 2003), hypertrophied lenticels and adventitious, lateral or superficial roots, all of which help oxygenate the roots (Benz *et al.*, 2007; Fenster, 1997; Parolin, 2001). Drought is also a severe stress that can inhibit photosynthesis and stomatal conductance and can lead to a reduction in turgor pressure in the cells and potential xylem cavitation (Griffin *et al.*, 2004; Warren *et al.*, 2004). Some species develop small, thick leaves with low specific leaf area (SLA) and increased water-use efficiency (WUE) to optimize photosynthesis per unit of water transpired (Donovan and Ehleringer, 1994; Dudley, 1996; Fonseca *et al.*, 2000; Wright *et al.*, 2002). Additionally, deep taproots can exploit water at depth in the soil (e.g. Wildy *et al.*, 2004). In contrast, flood-adapted plants tend to develop shallow root systems to access oxygen at the water-air interface (Baker III *et al.*, 2001). Furthermore, highly porous roots are more likely to desiccate under dry conditions than roots of low porosity (Fenster, 1997). Since selection may favor opposing traits in upland and bottomland forests, I quantified a series of root and foliar traits, including: root porosity, root tissue density and rooting architecture (i.e. allocation to shallow roots);

root-to-shoot ratio; specific leaf area; leaf size; leaf retention; photosynthesis, stomatal conductance, and instantaneous WUE; stable carbon isotope ratios, which can reflect WUE and physiological function of leaves (e.g. Farquhar *et al.*, 1989); elemental content of leaves (%N), which correlates with photosynthetic capacity (e.g. Evans, 1989); and the extent of foliar herbivory, as herbivores influence plant fitness and can vary spatially (e.g. Sork *et al.*, 1993).

To understand the evolution of phenotypic plasticity, local adaptation, and local maladaptation in heterogeneous landscapes, it is important to quantify natural selection on traits and plasticity, and to test whether there are costs associated with phenotypic plasticity (DeWitt, 1998b; Dorn *et al.*, 2000). Costs and limitations can restrict the evolution of adaptive plasticity (DeWitt *et al.*, 1998). In addition to documenting patterns of phenotypic plasticity, I used selection analyses to assess whether plasticity is adaptive and to quantify the costs of maintaining phenotypic plasticity (sensu Caruso *et al.*, 2006; DeWitt, 1998b; Dorn *et al.*, 2000; van Tienderen, 1991). This study addresses an important evolutionary question: In source-sink systems, is the evolution of plasticity hindered by the inherent costs of plasticity, or is selection for plasticity weak?

Materials and Methods

I describe the study site and focal species and provide details on the reciprocal transplant and greenhouse experiments in Chapter 2. Briefly, in the springs of 2005 and 2006, I transplanted cuttings (both years) and seedlings (2006 only) into two upland and two bottomland transplants sites at Beidler forest (n=1685 cuttings from 412 genotypes and 22 populations in 2005; in 2006 n=548 cuttings from 106 genotypes and 22 populations and n=814 seedlings from 81 families and 16 populations). Bottomland and upland populations were sampled from both ecotonal areas where the two habitats come into contact and interhabitat gene flow is likely to

be high and remote areas (>0.5 km from the alternative habitat type) (also see Chapter 2). Multiple individuals per family were planted haphazardly in both transplant habitats. In October 2006 and 2007, I collected leaves from the surviving individuals to assess leaf area, specific leaf area, foliar N content and stable carbon isotope ratios. In November 2006, I established a greenhouse study at Cornell University to assess the effects of prolonged flooding and drought on survivorship, growth and phenotype of *V. elliottii* cuttings and seedlings from different populations (n= 271 seedlings and 458 cuttings from 201 families; 16 populations for seedlings and 25 for cuttings). I imposed experimental treatments gradually to provide adequate time for plants to sense and respond to changing conditions (Chapter 2). In both experiments, I included cuttings made from reproductive adults as well as seedlings generated from seeds collected in the field. These seedlings represent novel combinations of genes that have not yet been exposed to flood/drought cycles in nature. Below, I detail the phenotypic measurements made in both experiments.

Foliar traits from the reciprocal transplant experiment—At the end of two growing seasons (October 2006 and 2007), I harvested green sun and shade leaves from cuttings and seedlings included in the reciprocal transplant experiment (mean ± S.D.: 9 ± 5 leaves/plant). In 2006, I measured leaf area using a leaf area meter (LI-3100, Li-Cor); in 2007, I quantified leaf area through digital photography and the use of Adobe Photoshop. I photographed fresh leaves on a white sheet of standard-sized paper (8.5 × 11 inches^2). In Photoshop, I converted these images to black and white, determined the percentage of black (leaf) pixels on the page and then calculated the area of leaf pixels. These two methods produce very similar results ($F_{1,28}$=70090.5, $p<0.0001$, parameter estimate = 1.01 ± 0.004, n=29). After photographing the leaves, I placed leaf samples in drying ovens at 50°C for 3-4 days. I weighed leaves on a Mettler AE 200 balance (± 0.0001 g) to determine specific leaf area (leaf area per unit

biomass, cm^2/g). In the field, I also noticed habitat-based differences in the extent of leaf herbivory; therefore, in 2007, I calculated the proportion of leaves per individual within each leaf collection with evidence of chewing damage by herbivores.

For all foliar analyses, I tested the effects of habitat of origin, population proximity (ecotone vs. remote), transplant environment, life history stage (2006 transplants because only one life history stage was included in 2005), their interactions, and transplant site nested within transplant environment on the response variable. These models all contained a random statement for family nested within population of origin to account for correlations between relatives and individuals from the same population. I used repeated measures ANOVA to assess the effects of these predictors and interactions on the first and second year SLA from the 2005 transplants (Proc Mixed, repeated statement for year with autoregressive correlation structure). I conducted a mixed model ANOVA to assess the effects of predictors on SLA from the 2006 transplants (measured in 2007 only). A final analysis tested the hypothesis that foliar herbivory was greater in bottomland than upland transplant sites; for this analysis, I pooled data for both years of transplanting (2005 and 2006) and included a fixed effect for the year of planting. Residuals were assessed for normality and homoskedasticity for all analyses.

To assess differences in WUE and photosynthetic capacity (%N), I used a subset of these leaves for stable carbon isotope discrimination and to determine foliar nitrogen (%N) content. Since these leaves were collected in early October, the carbon isotope ratios ($\delta^{13}C$) represent an integration of C fixed during the entire growing season (Farquhar *et al.*, 1989). In both 2006 and 2007, I pooled leaves within each transplant environment by population of origin (n=17 populations in 2006 and 29 populations in 2007, replicated in both upland and bottomland transplant environment); additionally, in 2007, I created additional pools for life history stage

and year of planting. Leaves of each pooled sample were dried at 50ºC and ground to a fine powder. Isotopic composition was determined by mass spectrometry in the Cornell University Stable Isotope Laboratory and expressed relative to the Vienna Pee Dee Belemnite standard. Statistical analyses were done on carbon isotope ratios ($\delta^{13}C$), which can easily be converted to carbon isotopic discrimination (Δ) (Farquhar *et al.*, 1989). I used a multivariate ANOVA (Proc Mixed) to assess whether $\delta^{13}C$ and %N varied as a function of habitat of origin, proximity, transplant environment, life history stage, and their interactions. Since the MANOVA was significant, I present results from the univariate ANOVAs. I included a repeated effect to account for multiple observations on the same population (cuttings vs. seedlings, 2006 vs. 2007).

Foliar and root traits from the greenhouse experiment—In May 2007, at the end of the experiment, I harvested leaves, stems and belowground biomass of all living plants. Aboveground biomass was dried at 50 °C for 4 days and then weighed. I calculated specific leaf area from a subset of leaves produced during the experiment (mean ± S.E.: 13.7 ± 0.4 leaves per plant) using digital images imported into Adobe Photoshop to quantify leaf area. Photographs of leaves were taken before drying.

To assess allocation to roots at varying depths, I divided roots into three sections: within the top 1cm of soil; 1.1 – 5 cm deep; and > 5cm deep. I separated roots by depth, washed soil from these samples, dried the roots at 50-60 °C for 5-7 days, and weighed them. A common response to flooding is the proliferation of roots at the air-water interface (e.g. Fenster, 1997; Johnston *et al.*, 2004); however, shallow roots could be more prone to desiccation under drought conditions. I predicted that the proportion of roots in the top 1 cm of the soil (relative to total root biomass) would be significantly greater for plants in the flooded relative to drought treatments. I also calculated the root:shoot ratio from overall below- and aboveground biomass. I predicted that plants in the drought treatment will allocate more resources to root

production (higher root:shoot ratio) than those in the flooded treatment.

I used a multivariate analysis of variance (MANOVA) to assess the effects of treatment, life history stage, habitat of origin, population proximity and their interactions on several response variables: SLA, leaf retention (leaf biomass to total aboveground biomass), leaf area, root:shoot (natural log transformed), and root architecture (proportion of roots in top 1cm of soil). Root:shoot data were transformed to improve the normality and homoskedasticity of the residuals. Many individuals in the flooded treatment shed their leaves during the experiment; in contrast, drought-stressed plants continually produced new leaves. I included leaf retention to demonstrate statistically this loss of leaves in the flooded treatment. Since the MANOVA was highly significant (results not shown), I used a series of univariate ANOVAs for each response variable. In all analyses, I controlled for correlated data structures by including random statements for family nested within population and block nested within treatment. Due to the large number of predictors and interaction terms, I present the results of reduced models, which were achieved by sequentially eliminating interaction terms and main effects with $p>0.1$. Results from the full models are also available (Appendices 10 and 11).

Root porosity and tissue density—I used a subset of seedlings and cuttings to quantify root porosity and root tissue density (n=26 seedlings, 85 cuttings; 51 of which were from the drought treatment and 60 from the flooded treatment). Root porosity reflects the extent of gas-filled root tissue (aerenchyma), which can be produced either constitutively in wetland plants, or induced by flooding (Evans, 2003; Visser and Bögemann, 2003). The porosity measurements require fresh (not desiccated) root tissue; therefore, I was unable to assess porosity on plants that died during the course of the experiment. I selected young, fine roots from the top 5 cm of the soil and determined root porosity using the microbalance method of Visser and Bögemann

(2003). Because root porosity can increase with distance from the root apex (Visser and Bogemann, 2006), I used samples 30-50 mm from the apex. After completing porosity measurements, I dried the root samples at 60 °C for 5-7 days, weighed them, and then calculated the ratio of dry root to fresh root weight (prior to vacuum infiltration using the microbalance method). This ratio provides an estimate of root tissue density (e.g. Zobel *et al.*, 2006), which should be lower under flooded than drought conditions if flooding induces aerenchyma production. Since root porosity and tissue density are likely correlated, I used a MANOVA to test the effects of treatment, life history stage, habitat of origin and population proximity on root porosity and root tissue density. The MANOVA detected a significant treatment effect (results not shown); therefore, I ran univariate mixed model ANOVAs (Proc Mixed) for the two response variables. For each analysis, I included random statements for family nested within population and treatment nested within block. The residuals of the root porosity analysis were normally distributed, but slightly heteroskedastic. An arcsine(square root) transformation improved the residuals, but did not substantially alter the statistical outcome. I present the statistical results from both analyses.

Leaf physiology from the greenhouse experiment—Both flooding and drought can reduce photosynthesis through diminished stomatal conductance (Li *et al.*, 2007; Mielke *et al.*, 2003). Species from upland systems often show steep declines in stomatal conductance under flooded conditions (Jones *et al.*, 2006). Therefore, I predicted that individuals from upland populations would have significantly lower photosynthetic rates and conductance in the flooded than the treatment. Prior to the initiation of the experimental treatments (September 2006), I gathered baseline data on photosynthesis, stomatal conductance and instantaneous water-use efficiency (WUE = photosynthesis divided by transpiration, Donovan and Ehleringer, 1994) from a subset

of the experimental plants using an infrared gas analysis system (LiCor 6400 portable photosynthetic system, Li-Cor, Lincoln, NE). Instantaneous WUE was positively correlated with photosynthesis/stomatal conductance (A/g_s ; $F_{1,230}$=599.2, $p<0.0001$) and inversely correlated with internal CO_2 concentration (c_i ;$F_{1,230}$=412.1, $p<0.0001$); therefore, I did not consider these alternative metrics of WUE (Campbell *et al.*, 2005).

I selected 2-3 individuals/genotype of cutting distributed between the drought and flooded treatments for these measurements (n=123 total plants; 44 genotypes). Seedlings were not included in these measurements. I accidentally included only 1 individual for two genotypes; these individuals do not alter the results and I retained them in the analyses. The genotypes were distributed among remote bottomland (n=12 genotypes from 4 populations), ecotonal bottomland (n=10 genotypes from 4 populations), remote upland (n=12 genotypes from 5 populations) and ecotonal upland populations (n=10 genotypes from 4 populations). I made spot measurements twice during the experiment to assess how experimental conditions affected physiology through time: in December 2006 after experimental conditions had been imposed for one month and in late April 2007 at the end of the experiment. All measurements were made at 1500 µmol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR), 25°C block temperature, 400 µmol/mol reference CO_2, 56.0 ± 0.66 % relative humidity (mean ± S.E.), 1.75 ± 0.027 kPA leaf to air vapor pressure deficit, and 27.4 ± 0.06 °C leaf temperature. Light response curves of four individuals suggested that maximum photosynthesis occurred at 1000-1500 µmol $m^{-2}s^{-1}$. I recorded measurements for each leaf only when ecophysiological parameters stabilized, which generally occurred within 5 minutes. I averaged over >6 measurements per leaf and used multiple leaves per plant when possible. When leaves were smaller than the area of the cuvette, I traced the leaves after completing the measurements, determined leaf area using a Leaf Area Meter (LI-3100, Li-Cor) and corrected the gas exchange values. I conducted all

physiological measurements between 9 AM and 5 PM. Each round of measurements lasted 3-4 days, during which time I randomly sampled the plants. In December and April, I began the measurements on a day when plants were drained (flooded treatment) and watered (drought treatment). For every subsequent day, I drained the flooded plants that were to be measured and provided a small amount of water to all plants in the drought treatment.

To determine whether time or day influenced ecophysiological parameters during each round of measurements, I ran three separate analyses with baseline, December and April data separately. In these analyses, I correlated photosynthesis, conductance and water-use efficiency (response variables) with day of measurement, time, day by time interaction, as well as treatment, habitat, proximity and their interactions. In these models, I included an R-sided random statement for genotype nested within population and a G-sided random statement for block nested within treatment. Day, time and their interaction were not significant in any of these models and are not included as covariates in other analyses of these data. I also assessed baseline differences in physiological parameters based on the origin of the plants and their treatments. Although I did not impose treatments prior to the initial measurements, I had already allocated plants to their eventual treatments.

I conducted repeated measures ANOVAs in Proc Glimmix, with an R-sided random statement (=repeated statement) for month (baseline, December, April), which was modeled using an autoregressive correlation matrix (AR(1)). I accounted for family-level correlation through a random statement for genotype nested within population of origin. I also included a random statement for block nested within treatment. Individuals that died over the course of the experiment were included in the analysis, but were missing values for time periods after their mortality. One common response to flooding was for leaves to turn red and then senesce. Individuals were

given 0 values for time periods during which they had no leaves. Plants under water stress can reduce water loss by closing their stomata (McDowell *et al.*, 2008; Pezeshki, 2001). Several individuals in the study exhibited negative stomatal conductance values, which are biologically meaningless and can be reported by the LiCor 6400 when the stomata are closed and conductance is very low; I adjusted negative values to 0. Separate analyses were conducted for photosynthesis, stomatal conductance and WUE. I used a natural log transformation to improve the residuals for stomatal conductance (+0.01 because of 0 values) as well as photosynthesis (+2 because of negative and 0 values).

Stable isotope analysis—At the end of the experiment, I used stable carbon isotopes to assess physiological responses of plants to drought and flooding. I collected leaves that had been produced over the course of the experiment and pooled leaves for each population of origin within each treatment. I had no problem collecting leaves from the drought treatment; however, leaves of many plants in the flooded treatment turned red and senseced. Thus, at the end of the experiment, plants that retained leaves in the flooded treatment had primarily red leaves with only very few green leaves. These green leaves, however, were clearly produced during the experiment. I created separate collections for red and green leaves in the flooded treatment, which resulted in 3 distinct population-level pools for each life history stage: drought treatment leaves, red leaves from the flooded treatment, and green leaves from the flooded treatment (n=95 pools of leaves). I assessed stable carbon isotope ratios and foliar N content (%N) as a function of treatment (drought, flooded red leaves, flooded green leaves), habitat of origin, population proximity, life history stage and interaction terms in mixed model ANOVAs that included a repeated statement for population (Proc Mixed). Additionally, I pooled leaves from plants that died over the course of the experiment to quantify selection on ecophysiological

parameters (see below).

Selection analyses—I used selection analyses to determine whether: 1) divergent selection favors different phenotypic optima under contrasting environmental conditions, 2) phenotypic plasticity in foliar and root traits is adaptive, and 3) phenotypic plasticity is costly. In the first analysis, I regressed fitness on family-mean trait values (averaged within a treatment or transplant habitat). I also included transplant environment (field experiment) or treatment (greenhouse experiment) in the model. Divergent selection can be detected by a significant interaction between trait and transplant environment (field experiment) or treatment (greenhouse experiment). Selection analyses included the following traits: SLA (field experiment); root:shoot ratio, allocation to shallow roots, SLA, leaf retention (leaf biomass to total aboveground biomass), and leaf size (greenhouse experiment).

Logistic regression in Proc Glimmix was used to model survivorship of each family (number of surviving individuals/ number of individuals included in the study), as a function of traits, with a random statement for family nested within population of origin. Relative growth rate was also used as a fitness component in separate analyses; for those analyses, I calculated mean RGR for each family within each treatment or transplant environment (Proc Mixed, random statement for family nested within population of origin). I conducted analyses separately for the greenhouse experiment and each transplant year. I standardized phenotypic traits to a mean of zero and a standard deviation of one to facilitate comparison of traits measured on different scales. I evaluated quadratic terms, but removed them because they were not signfiicant. For the cuttings included in the reciprocal transplant experiment in 2005, I calculated family-level means across years for foliar traits and growth rate (RGR) measured in October 2006 and 2007.

In the greenhouse, I quantified trait values on plants that died over the course

of the experiment; however, in the field experiment, many individuals died before foliar traits were measured. These individuals could have died due, at least in part, to limited phenotypic plasticity and trait values that were inappropriate for the transplant environment. I took two approaches to the viability selection analyses in the field experiment. Phenotypic traits of dead individuals can be estimated based on trait values of their surviving relatives (Hadfield, 2008). For each family in the field experiment, I calculated family-level mean trait values from individuals that survived until traits were measured. I assessed survivorship as the number of family members that survived until April 2008 over the number of family members that were initially included in the study. The analyses on traits and plasticity in the field study are likely conservative and underestimate viability selection. In the second approach, I included only the subset of the 2005 transplants that survived until October 2006, when the first set of foliar traits were measured. I regressed individual-level survivorship from October 2006 to April 2008 on foliar trait values from the 2006 leaf collections. This approach eliminated all individuals that died before October 2006 (for which I have no trait data) and assessed whether survivorship from that point forward was influenced by foliar trait values. Both logistic regressions were implemented in Proc Glimmix and included an R-sided random statement for family nested within population of origin. The individual-level analysis of the second approach also included a fixed effect for transplant site nested within transplant habitat, which was not possible in the first analysis because families were averaged across sites.

Finally, I assessed selection on carbon isotope ratios and elemental composition of leaves from the greenhouse study. In addition to the pools of leaves from living plants used to quantify differences in carbon isotope ratios between treatments, I also pooled leaves from dead plants of each population and life history stage. I used logistic regression to determine whether stable carbon isotope ratios, C

and N content differed between plants that survived and those that did not; such differences would indicate that viability selection operated on these ecophysiological traits. For this analysis, I excluded the samples from the flooded treatment that consisted of red leaves because these leaves were produced before the experiment began and reddened over the course of the experiment. Therefore, they may not have trait values entirely representative of the experimental conditions. The model in Proc Glimmix included an R-sided random statement to account for correlation between samples from the same population.

Selection on plasticity—I conducted an across-environment genotypic selection analysis to assess whether plasticity is adaptive in this system (Stinchcombe *et al.*, 2004; Van Kleunen and Fischer, 2001). In multiple regression analyses, I determined the effect of family-mean traits (averaged across environments) and family-level plasticities on mean family fitness. For each family, I quantified plasticity as the difference between average trait values in environment one and average trait values in environment two, where environment refers to transplant environment (reciprocal transplant experiment) or treatment (greenhouse experiment). I calculated plasticity so as to maintain positive expected values. For example, in the field, SLA was consistently greater in bottomland than upland transplants, so I subtracted average upland from average bottomland values for each family. I calculated separate plasticity values for seedlings and cuttings. In the reciprocal transplant experiment, I assessed fitness (survivorship and relative growth rate) as a function of plasticity in SLA; in the greenhouse experiment, I included plasticity in SLA, leaf size, root:shoot ratio, and allocation to shallow root biomass.

Cost of plasticity—To assess the cost of plasticity, I determined whether plastic genotypes were at a fitness disadvantage in each of the transplant environments (field experiment) or treatments (greenhouse experiments). I regressed family-level

fitness within each of the transplant habitats and treatments on family-level plasticities (calculated as above) and average family-level trait values within an environment (Caruso *et al.*, 2006; DeWitt, 1998a; Scheiner and Berrigan, 1998; Stinchcombe *et al.*, 2004; van Kleunen *et al.*, 2000; van Tienderen, 1991). Again, fitness components were: survivorship (number of surviving family members over initial number of family members, binomial distribution in Proc Glimmix) and average RGR of surviving individuals (Proc Mixed). Significant negative correlations between plasticity and fitness indicate that plasticity is costly, whereas positive correlations suggest that plastic genotypes have a fitness advantage, even within a habitat.

Results

Foliar traits from the reciprocal transplant experiment—Cuttings and seedlings showed consistent phenotypic plasticity for specific leaf area; across years, SLA was significantly higher in bottomland than upland forests (Tables 3.1 and 3.2, Figure 3.1). For the 2005 transplants, there was a significant effect of the year of measurement, but the difference in values between the two years was not large (LSMEANS ± S.E.: SLA measured in 2006: 256.7 ± 3.2 cm^2/g; SLA measured in 2007 on the same plants: 266.5 ± 3.2). Over those 2 years, SLA rose significantly in the bottomland transplant environment (LSMEANS ± S.E.: 2006 SLA in bottomlands: 335.2 ± 5.6 cm^2/g; 2007 SLA in bottomlands: 351.5 ± 5.8, $t_{646}=4.9$, adjusted $p<0.0001$), but there was no difference in uplands (adjusted $p=0.19$). For the 2006 transplants (leaves collected in October 2007 only), life history stage and population proximity were also significant predictors of SLA (Table 2, Fig. 1b). As in the analysis of the 2005 transplants, SLA was significantly greater for individuals planted in bottomland sites than their relatives in transplant sites. Cuttings had significantly lower SLA than seedlings (LSMEANS ± S.E. Cuttings: 240.5 ± 2.2; seedlings: 277.4 ± 3.1). Additionally, plants from remote populations had

significantly lower SLA than plants from ecotonal populations (LSMEANS ± S.E. remote populations: 255.2 ± 2.3; ecotonal populations: 262.7 ± 2.8).

Table 3.1: Results of repeated measures ANOVA on specific leaf area (SLA) of leaves collected in October 2006 and 2007 from the 2005 transplants. Here, I present the reduced model; all other effects and interactions were not significant ($p>0.2$).

	F	p-value
Transplant environment	$F_{1,1166}=725.6$	<0.0001
Year of measurement	$F_{1,1166}=28.1$	<0.0001
Year × transplant environment	$F_{1,1166}=12.1$	0.0005
Site(transplant environment)	$F_{2,1166}=28.5$	<0.0001

Table 3.2: Mixed model ANOVA of SLA of leaves collected in October 2007 from the 2006 transplants. All other main effects and interaction terms were nonsignificant and were removed from the model ($p>0.15$). Proximity refers to ecotonal vs. remote populations.

	SLA	
	F	p-value
Transplant environment	$F_{1,480}=997.7$	<0.0001
Life history stage	$F_{1,480}=75.2$	<0.0001
Proximity	$F_{1,480}=4.01$	0.046
Site(transplant environment)	$F_{2,480}=10.32$	<0.0001

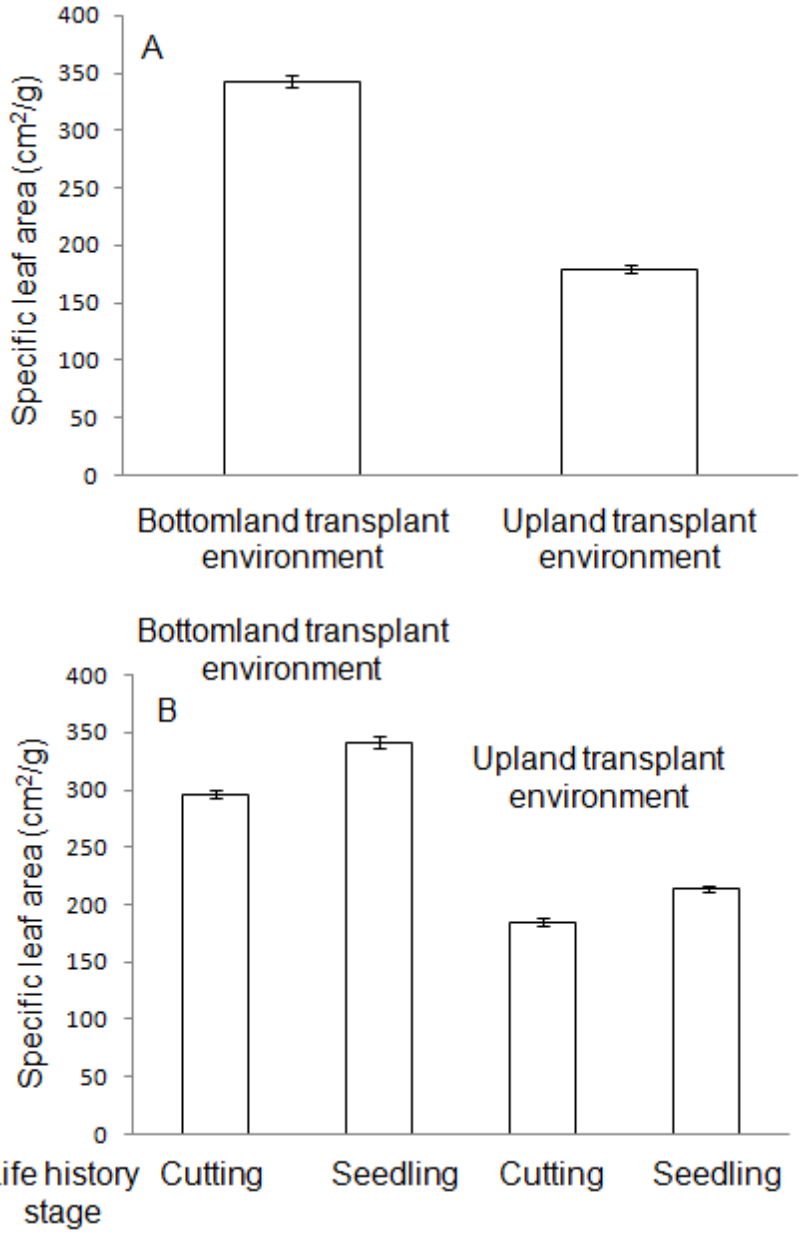

Figure 3.1: Specific leaf area as a function of transplant environment for (a) the 2005 and (b) the 2006 transplants. Habitat of origin and the interaction between habitat of origin and transplant environment were not significant.

The frequency of leaf herbivory was significantly greater in bottomland than upland transplant sites (Table 3.3, Figure 3.2). Additionally, cuttings sustained more herbivore damage than seedlings and there was a significant life history by transplant environment interaction. This interaction term was driven by significantly greater

herbivore damage on leaves of cuttings relative to seedlings in upland transplant sites (comparison of cuttings and seedlings within the uplands: t_5=4.1, p-value adjusted for multiple tests = 0.034).

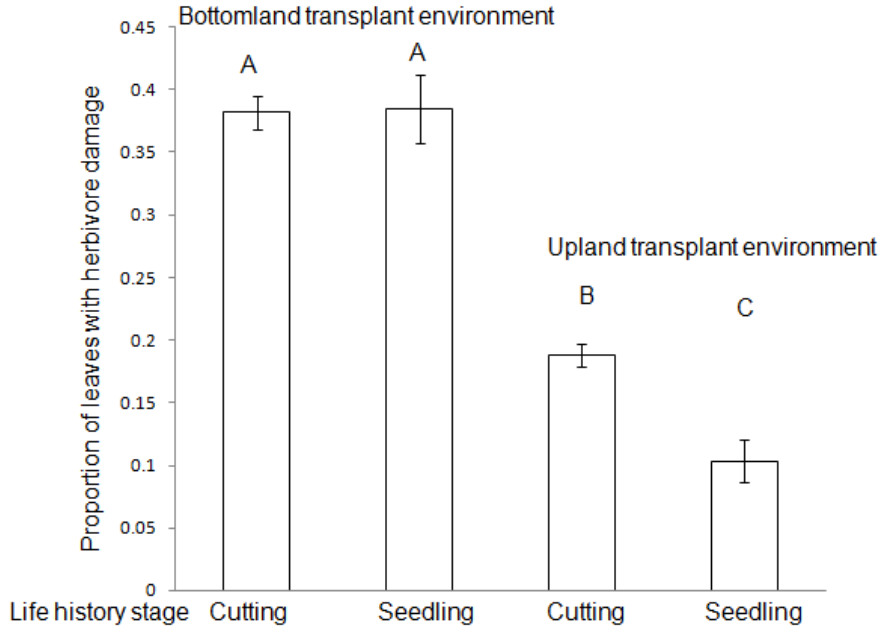

Figure 3.2: The extent of foliar herbivory on leaves from cuttings and seedlings included in the reciprocal transplant experiment. Herbivory was measured as the proportion of leaves with clear evidence of chewing damage. Letters represent significant differences per Tukey's HSD tests.

Table 3.3: Mixed model ANOVA on foliar herbivory. All other main effects and interactions were excluded from the model because they were not significant.

	F	p-value
Transplant environment	$F_{1,960}$=166.8	<0.0001
Life history stage	$F_{1,960}$=4.99	0.026
Life history stage × Transplant environment	$F_{1,960}$=8.07	0.0046
Site(transplant environment)	$F_{2,960}$=23.4	<0.0001
year of transplanting	$F_{1,960}$=8.7	0.0032

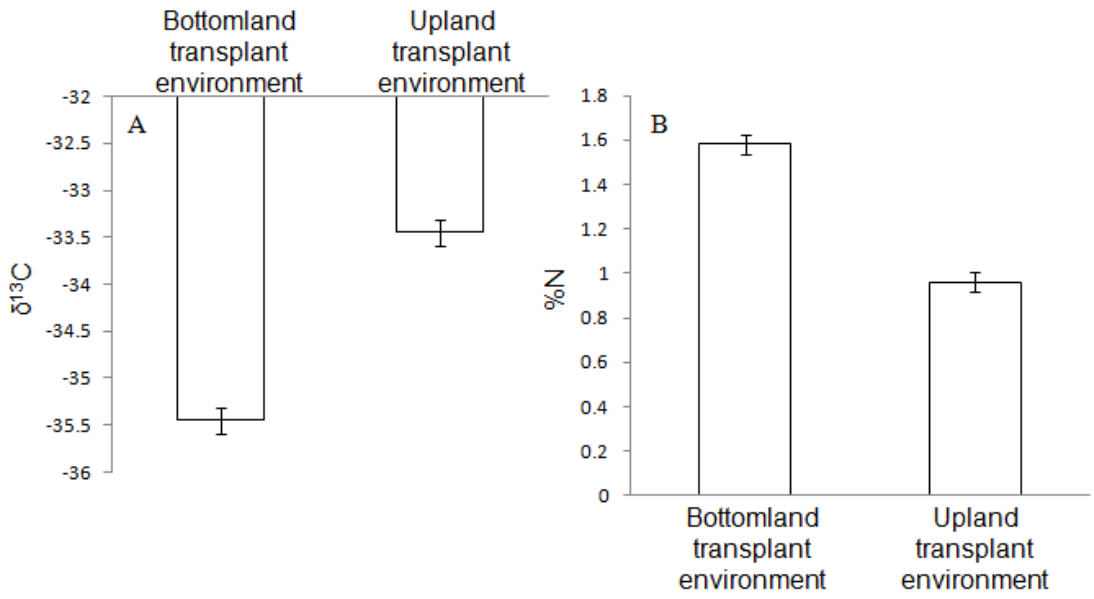

Figure 3.3: Analyses of stable carbon isotope ratios (a) and foliar N content (b) in leaf collections from the reciprocal transplant experiment.

Stable carbon isotope ratios varied by transplant environment ($F_{1,25}$=119, p<0.0001, Fig. 3.3a) as well as the year of sampling ($F_{1,16}$=5.13, p=0.038) and the year of planting ($F_{1,11}$=6.1, p=0.03). Similarly, %N varied by transplant environment ($F_{1,25}$=117.1, p<0.0001, Fig. 3.3b). No other main effects or interactions were significant. Foliar C:N was significantly greater in upland than bottomland forests ($F_{1,25}$=253.7, p<0.0001), similar to differences in soil chemistry (Chapter 2). I also detected a significantly negative correlation between carbon isotope ratios and foliar %N ($F_{1,96}$=6.8, p=0.011, Fig. 3.4) in a model that included transplant environment ($F_{1,28}$=17.7, p=0.0002), year of planting ($F_{1,11}$=16.4, p=0.002) and an interaction between transplant environment and %N ($F_{1,96}$=5.5, p=0.02). The year of sampling was removed from this model because it was not significant.

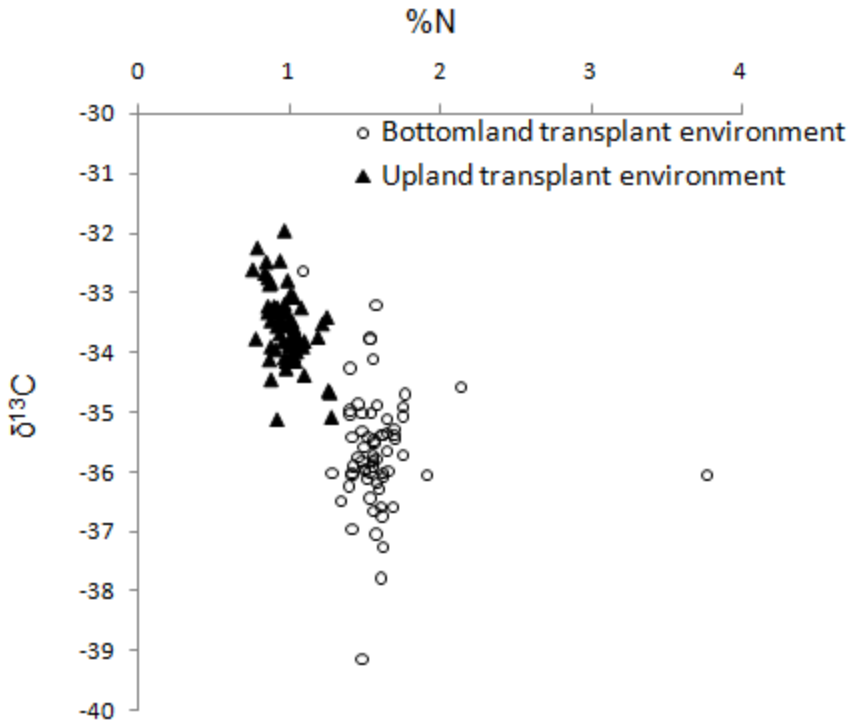

Figure 3.4: Correlation between stable carbon isotope ratio and foliar N content in the reciprocal transplant experiment.

Foliar and root traits from the greenhouse experiment—Plants in the flooded treatment had significantly greater allocation to shallow roots (within the top 1 cm of the soil surface) than plants in the drought treatment ($F_{1,52}$=64, p<0.0001, Figure 3.5a). In both treatments, seedlings exhibited a greater allocation to shallow roots than cuttings; this difference was accentuated in the flooded treatment (life history stage: $F_{1,205}$=69, p<0.0001; life history stage × treatment: $F_{1,205}$=11, p=0.001). The full model suggested that individuals from bottomland populations had a significantly greater allocation to shallow roots than those from upland populations (Appendix 10); the reduced model suggested a similar trend that did not quite reach statistical significance ($F_{1,205}$=2.9, p=0.09). The analysis of root:shoot ratio showed that drought-stressed plants had significantly greater overall allocation to root production than flooded plants ($F_{1,52}$=120, p<0.0001, Figure 3.5b). Additionally, plants from

ecotonal populations had significantly lower root:shoot than plants from remote populations (LSMEANS ± S.E.: ecotonal genotypes: 0.52 ± 0.04; remote genotypes: 0.65 ± 0.06 g/g).

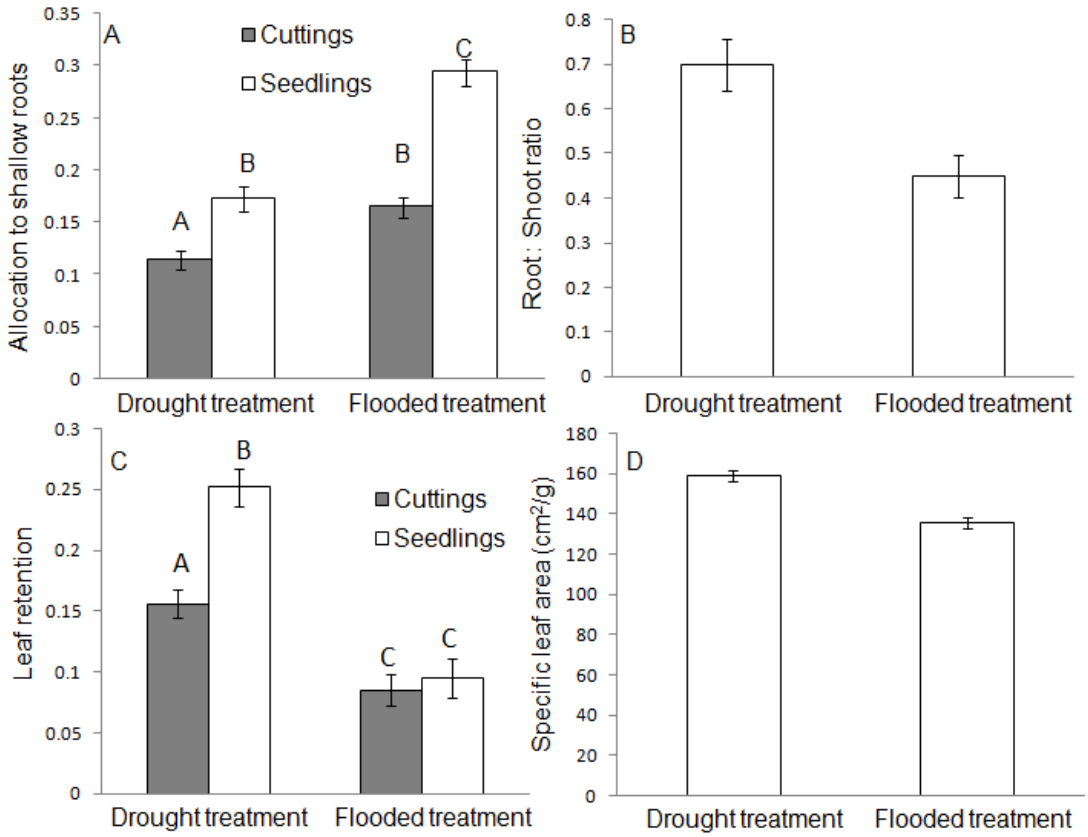

Figure 3.5: Phenotypic responses of cuttings and seedlings to flood and drought stress in the greenhouse experiment: (a) rooting architecture (proportion of roots in top 1 cm of soil), (b) root : shoot ratio, (c) leaf retention (leaf biomass to total aboveground biomass), and (d) specific leaf area. Letters represent significant differences according to Tukey's HSD tests.

Foliar traits also responded to treatment. Given the observation that flooded plants shed their leaves during the experiment, it was not surprising that drought-stressed plants had significantly greater leaf biomass (to total aboveground biomass) than flooded plants ($F_{1,52}$=83, p<0.0001, Figure 3.5c). Seedlings had significantly greater leaf biomass: aboveground biomass than cuttings in the drought treatment;

however, there was no difference between seedlings and cuttings in the flooded treatment because both life history stages lost leaves in that treatment (life history stage: $F_{1,205}$=12.5, p=0.0005; life history stage × treatment: $F_{1,205}$= 14, p=0.0002). Specific leaf area was significantly lower in flooded than drought treatments ($F_{1,52}$=46, p<0.0001, Figure 3.5d). Additionally, seedlings had higher SLA than cuttings (LSMEANS ± S.E.: seedlings: 159.0 ± 3.3; cuttings: 135.9 ± 2.4 cm^2/g; $F_{1,185}$ =36, p<0.0001) and families from ecotonal populations had higher SLA than those from remote populations (ecotonal genotypes: 153.9 ± 3.1; remote genotypes: 141.0 ± 2.7 cm^2/g; $F_{1,185}$ =11, p=0.0011). Although the interaction between treatment and habitat had a significant effect on SLA in the full model (Appendix 11), it was not significant in the reduced model ($F_{1,336}$=2.79, p=0.096 in a model that included the nonsignificant

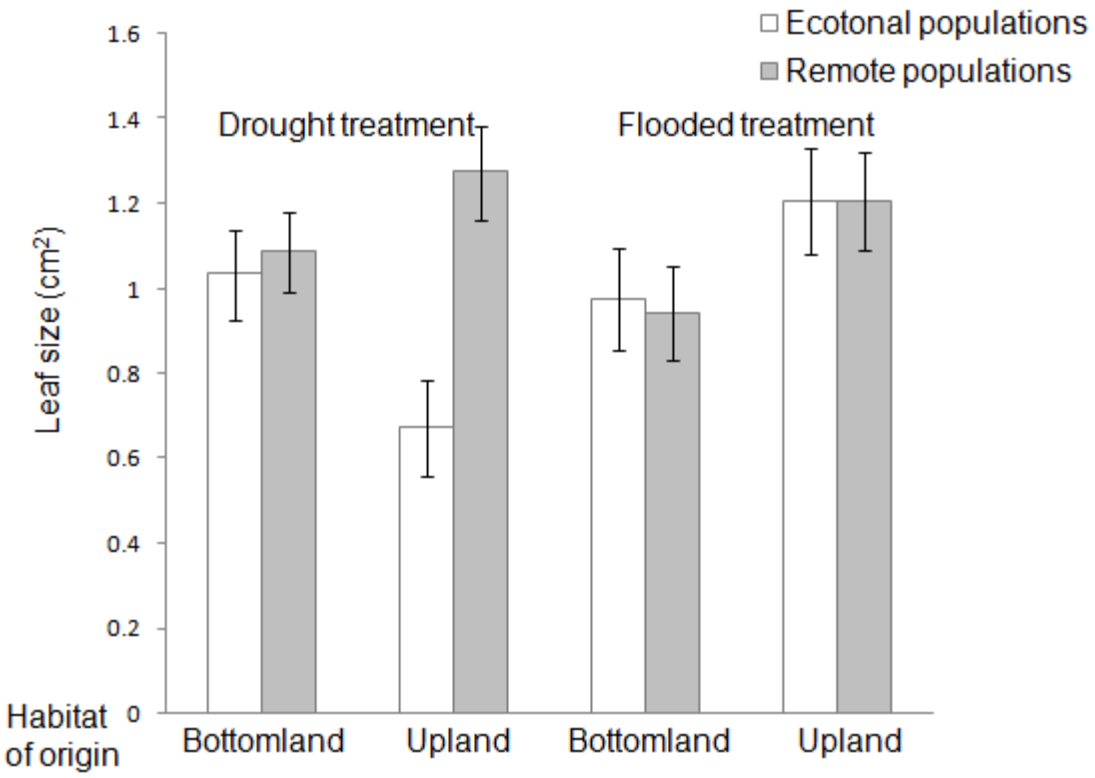

Figure 3.6: Leaf size of plants included in the greenhouse experiment. Habitat of origin is noted on the X-axis.

effect of habitat; $F_{2,336}$=1.4, p=0.24 in a model that excluded habitat). Finally, leaf size did not change with treatment; however, I found significant effects of life history stage ($F_{1,168}$=17, p<0.0001), treatment by habitat ($F_{1,168}$=5.8, p=0.017) and the three way interaction of habitat of origin by population proximity by treatment ($F_{3,168}$=4, p=0.008). Overall, seedlings had significantly smaller leaves than cuttings. The three-way interaction was driven by the very small leaves of drought-stressed plants from upland ecotonal populations (Fig. 3.6).

Root porosity and tissue density—Root porosity was significantly greater in flooded than drought-stressed plants (untransformed data: $F_{1,36}$=6.98, p=0.012; arcsine(square root) transformed data: $F_{1,36}$=6.67, p=0.014, Fig. 3.7a). Tissue density (dry root mass: fresh root mass), in contrast, was significantly higher in the drought than flooded treatment ($F_{1,32}$=7.3, p=0.011, Fig. 3.7b). Both of these results conform with predictions. Habitat of origin, proximity, life history stage, and all interaction terms were not significant in either analysis.

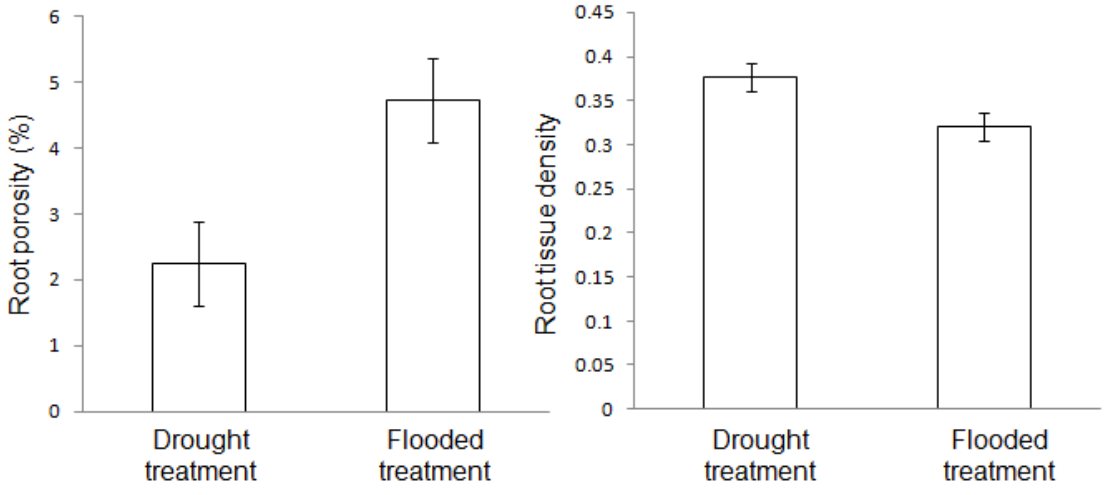

Figure 3.7: a) Root porosity and b) root tissue density (dry mass : fresh mass) as a function of treatment in the greenhouse experiment.

Physiology—Ecophysiological traits varied by time of measurement, treatment, population proximity, and a time by treatment interaction (Fig. 3.8; reduced models: Table 3.4; full models: Appendix 12). In all cases, physiological parameters were higher in drought than flooded plants. In an analysis of the pre-treatment baseline data, I found no effect of treatment on photosynthesis, stomatal conductance, or instantaneous water-use efficiency, but individuals from remote populations had significantly greater photosynthetic rates and stomatal conductance values than individuals from ecotonal populations (Appendix 13).

Photosynthesis and stomatal conductance continuously declined through time in the flooded treatment. In the drought treatment, however, these physiological parameters declined between September (baseline) and December, but started to rebound by the end of the experiment (late April). Genotypes from remote populations had significantly greater values for both of these traits than those from ecotonal populations. Water use efficiency decreased through time in both treatments, which is contrary to predictions of increased WUE in drought conditions.

Table 3.4: Repeated measures analyses of ecophysiological traits. All other main effects and interaction terms were not significant ($p>0.1$). Time refers to baseline, month 1 and final month measurements. WUE is instantaneous water use efficiency.

	Photosynthesis		Stomatal conductance		WUE	
	F	p	F	p	F	p
Time	$F_{2,205}=29.6$	**<0.0001**	$F_{2,205}=35.8$	**<0.0001**	$F_{2,207}=24.9$	**<0.0001**
Geography	$F_{1,205}=6.91$	**0.0092**	$F_{1,205}=6.1$	**0.015**		n.s
Treatment	$F_{1,46}=8.71$	**0.005**	$F_{1,46}=4.9$	0.055	$F_{1,46}=5.3$	**0.0254**
Time × Treatment	$F_{2,205}=5.4$	**0.0051**	$F_{2,205}=10.1$	**<0.0001**		**n.s.**

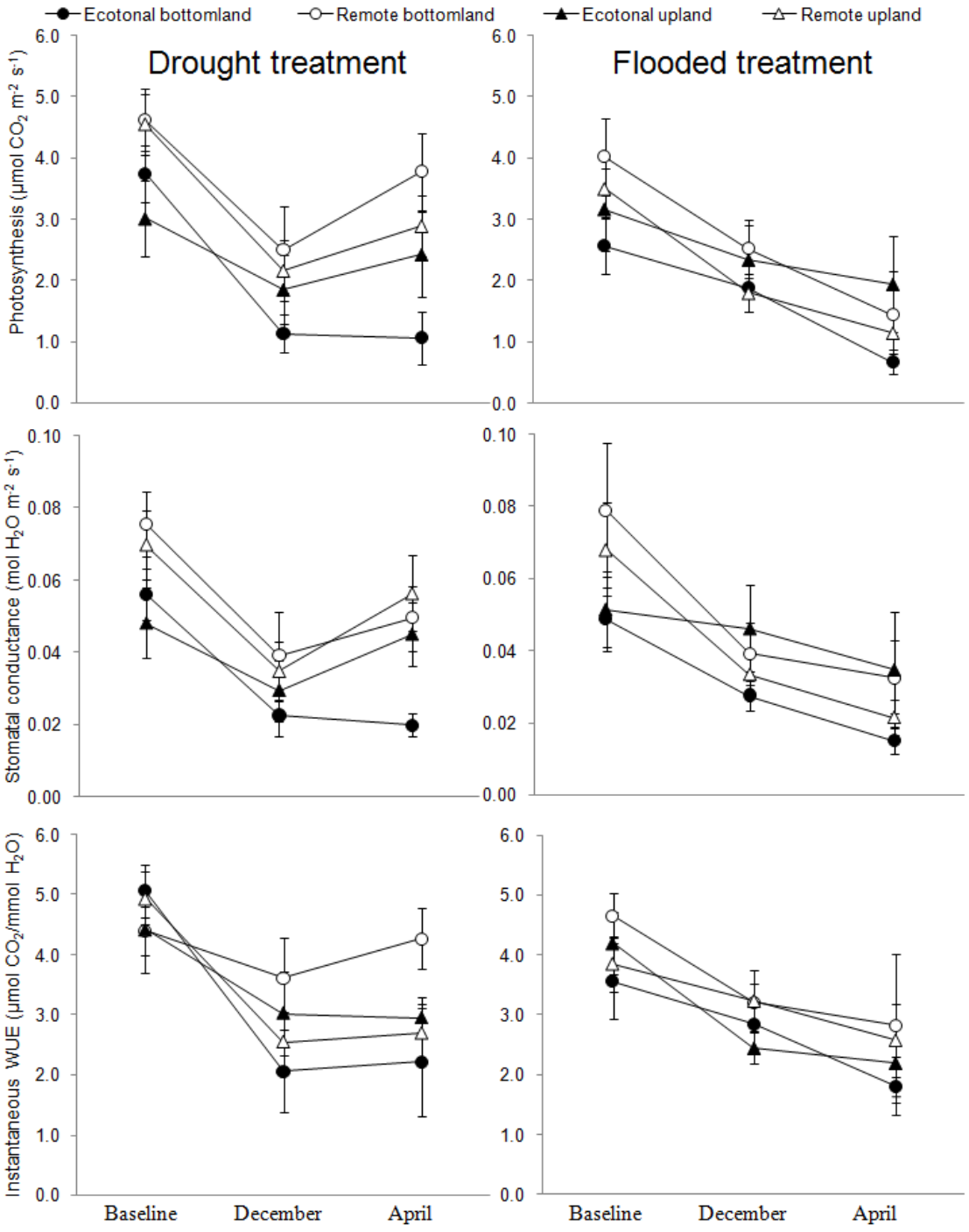

Figure 3.8: Photosynthesis, stomatal conductance, and instantaneous water-use efficiency measured on cuttings in the drought and flooded treatments in the greenhouse experiment.

Stable isotope analysis—Carbon isotope ratios ($\delta^{13}C$) were significantly lower (more negative) for red leaves from the flooded treatment (LSMEANS ± S.E.: -29.03 ± 0.23) than for either green flooded leaves (-27.4 ± 0.24) or green leaves from the drought treatment (-28.0 ± 0.23; $F_{2,42}$=10.9, p=0.0002). I found no difference between green leaves from the flooded and drought treatments. No other main effects or interaction terms were significant. Red leaves were produced before the initiation of the experiment and turned red over the course of the experiment. In contrast, green leaves were produced during the experiment and are therefore younger.

Not surprisingly, red leaves from the flooded treatment had significantly lower N content (LSMEANS ± S.E. 1.2 ± 0.06 %N) than green leaves from both the flooded treatment (1.52 ± 0.06) and the drought treatment (1.52 ± 0.06; $F_{2,42}$=10.9, p=0.0002). Additionally, cuttings had greater N content than seedlings (LSMEANS ± S.E.: cuttings: 1.5 ± 0.04 %N; seedlings: 1.3 ± 0.05 %N; $F_{1,10}$=9.3, p=0.012). No other main effects or interactions terms influenced N content.

Divergent selection—In the greenhouse, there was an overall negative relationship between specific leaf area (SLA) and survivorship and a negative relationship between leaf size and survivorship (Table 3.5), indicating viability selection for smaller thicker leaves in both treatments. Viability selection on leaf retention signifies that families with individuals that retained more leaves had greater survivorship, regardless of treatment. Additionally, there was divergent selection on root:shoot ratio and this divergent selection was concordant with the direction of phenotypic plasticity (Figure 3.9). That is, selection favored decreased root:shoot in the flooded treatment, and increased root:shoot ratio in the drought treatment. Finally, the significant interaction between leaf retention and treatment showed that leaf retention had a higher positive effect on survivorship in the drought treatment.

Table 3.5: Selection analyses on phenotypic traits measured under flood and drought stress in the greenhouse. In these analyses, the flooded treatment is the reference treatment and treatment by trait interactions are given for the drought relative to the flooded treatment. Parameter estimates and odds ratios are provided for significant effects; p-values of marginally significant effects are italicized. Odds ratios (OR) > 1 indicate a positive relationship between a trait and survivorship. Survivorship was assessed for all families, but relative growth rate was analyzed only for families in which at least one individual per treatment survived until the end of the experiment.

	Survivorship				RGR			
	OR	95% CI	$F_{1,119}$	p	β	SE	$F_{1,65}$	p
Root:Shoot (R:S)				0.54	-0.057	0.01	**13.3**	**0.0005**
Shallow root allocation (SR)				0.79				0.32
Specific leaf area (SLA)	0.57	(0.4, 0.8)	11.3	**0.001**				0.32
Leaf retention (LT)	1.79	(1.2, 2.8)	6.7	**0.011**	0.017	0.03	21.6	**<0.0001**
Leaf size (LS)	0.73	(0.6, 0.9)	8.9	**0.0035**				0.36
Treatment	3.6	(2.3, 5.6)	30.7	**<0.0001**	0.084	0.03	10.0	**0.0024**
R:S × Treatment	2.2	(1.2, 3,8)	7.5	**0.007**				0.22
SR × Treatment				0.77				0.12
SLA × Treatment				0.71				0.65
LT × Treatment	2.5	(1.0, 6.0)	4.1	**0.046**	0.11	0.03	13.0	**0.0006**
LS × Treatment				0.8				0.89

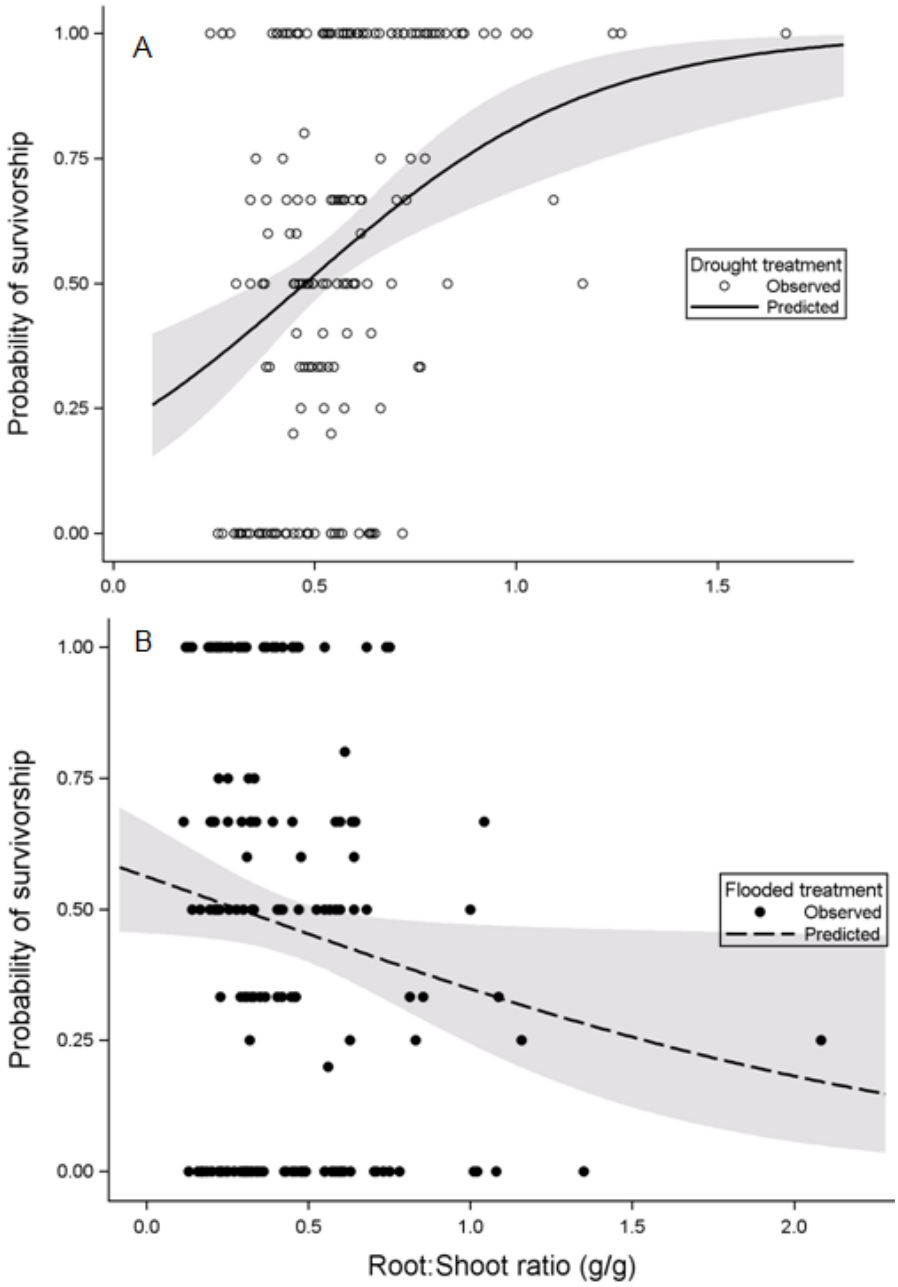

Figure 3.9: Viability analysis on root : shoot ratio in (a) the drought and (b) the flooded treatments. Family-level survivorship is plotted as a function of mean family traits within treatments. The predicted relationship (and 95% confidence interval) is from logistic regression.

Among the families with individuals that survived until the end of the experiment, RGR was actually negatively correlated with the root:shoot ratio (Table 3.5, Figure 3.10). This analysis also showed that RGR increased with leaf retention, which is not surprising because plants that retained more leaves had greater potential for carbon fixation (Figure 3.10). The significant interaction between treatment and leaf retention was due to the smaller range of leaf retention values within the flooded treatment (0-0.5 g/g) relative to the drought treatment (0-2.0 g/g). Individuals within the flooded treatment lost the majority of their leaves during the experiment.

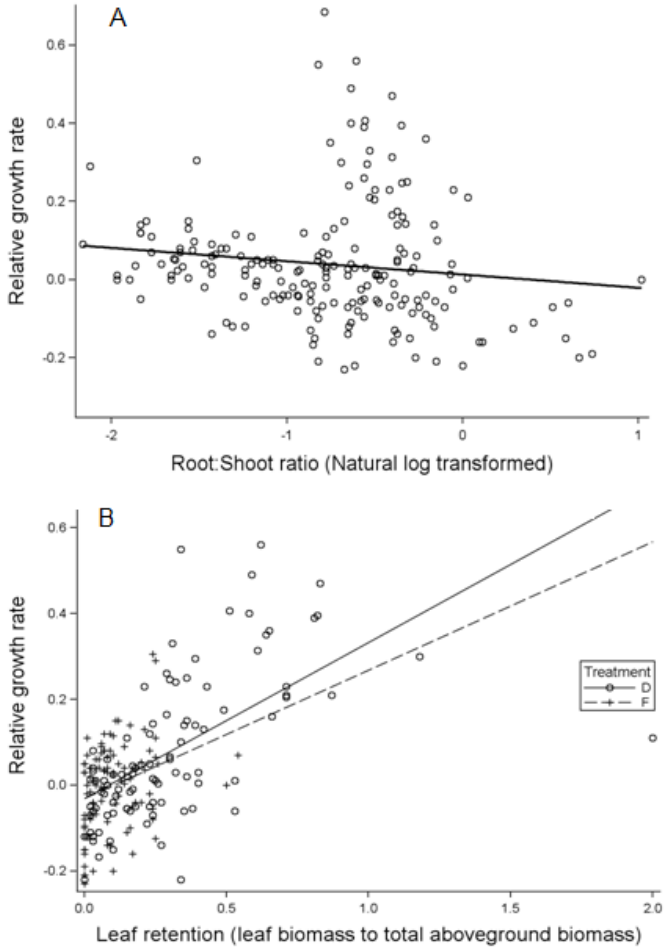

Figure 3.10: Effect of family mean phenotypic traits on family mean relative growth rate (RGR) in the greenhouse experiment: (a) root : shoot ratio, (b) leaf retention.

In the greenhouse, I also found selection for increased carbon isotope ratios (i.e. less negative values), as well as decreased foliar N content (Table 3.6). The interaction between treatment and foliar N content was driven by strong selection for lower %N in the drought treatment and virtually no selection on %N in the flooded treatment.

Table 3.6: Selection on stable carbon isotope rates, and elemental leaf N and C content. Logistic regression compared trait values of samples of leaves from individuals from the same population that survived the experiment vs. those that did not. Odds ratios (OR) are shown for significant predictors only.

	OR	95% CI	$F_{1,87}$	p
Carbon isotope ratio (δ‰)	1.98	(1.15, 3.42)	6.2	**0.0149**
C content (%C)				0.43
N content (%N)	0.41	(0.23, 0.71)	10.2	**0.002**
Treatment				0.4
δ‰× Treatment				0.93
%C × Treatment				0.49
%N × Treatment	0.25	(0.082, 0.77)	6.0	**0.016**

Field results indicate viability selection for lower SLA (2006 transplants) (Table 3.7). An interaction between SLA and transplant environment in 2006 was the result of a steeper negative regression line in upland transplants sites (Figure 3.11). However, even in bottomland sites, viability selection favored reduced SLA, which is not concordant with the plasticity in this trait. Interestingly, the interaction between SLA and transplant environment in the analysis of relative growth rate showed a negative correlation between SLA and RGR in the bottomlands, but no pattern in the uplands. The second approach to viability selection analyses in the field experiment

showed the same pattern (Table 3.8).

Selection on plasticity—In the greenhouse, I found significant viability and growth rate selection for plasticity in root:shoot ratio and viability selection for plasticity in specific leaf area (Table 3.9, Figures 3.12 and 3.13). However, there was a negative relationship between relative growth rate and plasticity in SLA.

Table 3.7: Selection analyses on specific leaf area (SLA) in the field experiment. Parameter estimates (± S.E.) or odds (OR) ratios (95% confidence intervals) are provided for significant effects. No predictors were significant in the analysis of relative growth rate (RGR) from 2005 transplants; therefore, RGR is excluded for that year from this table.

	2005 transplants			2006 transplants					
	Survivorship			Survivorship			RGR		
	OR	$F_{1,63}$	p	OR	$F_{1, 104}$	p	β	$F_{1,75}$	p
Specific leaf area (SLA)			0.9	0.47 (0.4, 0.6)	26.9	**<0.0001**			0.4
Transplant environment	0.3 (0.1, 1)	3.9	**0.05**	2.2 (1.3, 3.7)	$F_{1,98}$ = 8.6	**0.004**			0.2
SLA × Transplant environment			0.89	2.1 (1.2, 3.8)	6.7	**0.01**	-0.008 ± 0.004	4.7	**0.05**

Despite consistent plasticity in foliar traits in the field, there was no selection for plasticity in specific leaf area in the reciprocal transplant experiment. Instead, survivorship and growth rate of the 2005 transplants decreased with increasing plasticity in SLA [survivorship: odds ratio (95% CI): 0.83 (0.71, 0.98), $F_{1,74}$ = 5.2,

p=0.03; relative growth rate: slope (± S.E.): -0.002 ± 0.001, $F_{1,74}$ = 4.1, p=0.045]. The 2006 transplants showed no relationship between fitness and plasticity in SLA.

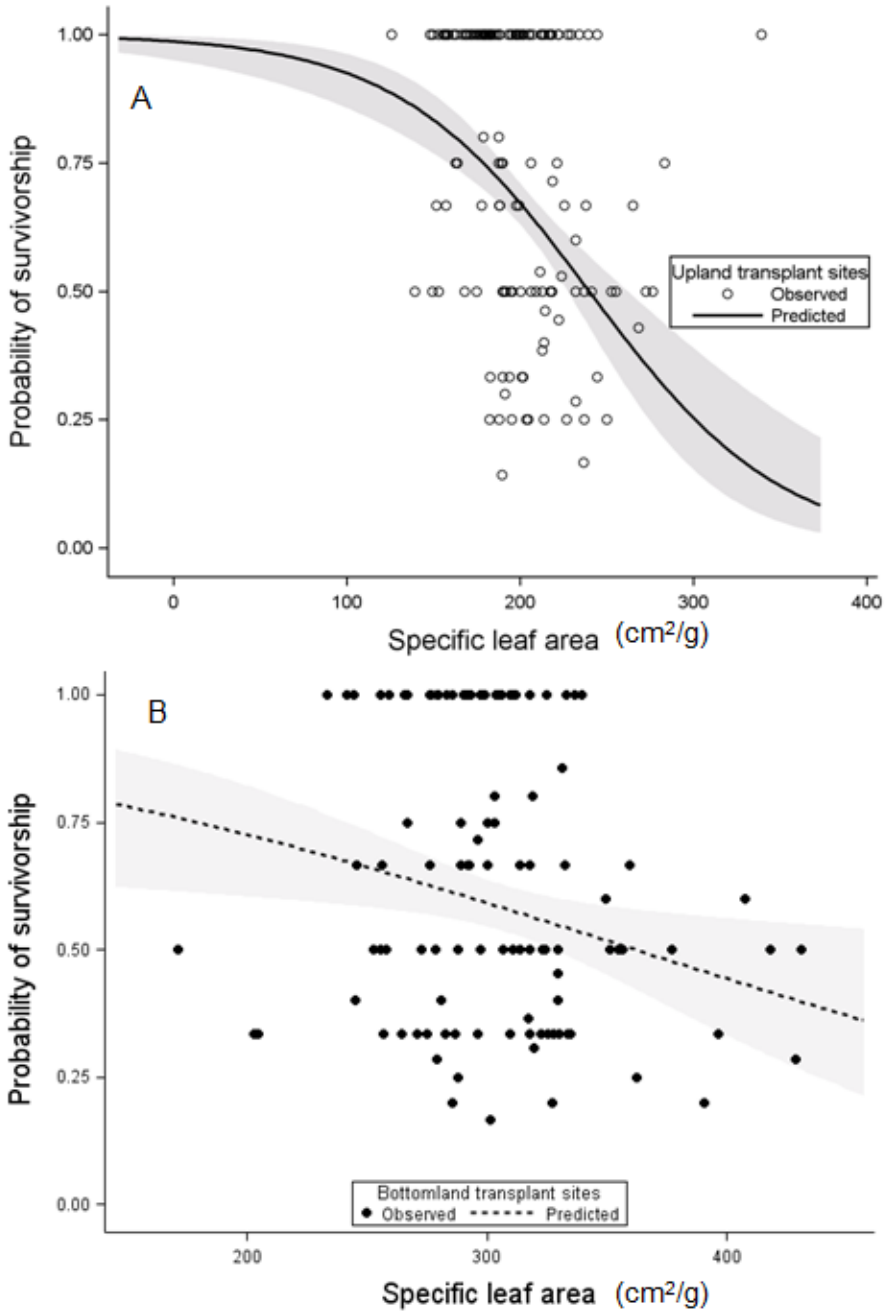

Figure 3.11: Viability selection on specific leaf area in (a) upland and (b) bottomland transplant sites for the 2006 transplant experiment.

Table 3.8: Selection analysis: Approach 2: Survivorship of the 2005 transplants from October 2006 to April 2008 was regressed on specific leaf area measured in October 2006. This analysis only included individuals with both survivorship and phenotypic data. Odds ratios (OR) are shown for significant effects.

	OR	95% CI	F	p
Specific leaf area (SLA)	0.55	(0.35,0.87)	$F_{1,408}$=6.7	**0.01**
Transplant environment			$F_{1,92}$=1.9	0.17
SLA × Transplant environment			$F_{1,403}$=0	1
Site(Transplant environment)	10.0	(2.7, 36.5)	$F_{2,5}$= 10.5	**0.016**

Table 3.9: Selection on plasticity in the greenhouse experiment for both survivorship and relative growth rate (RGR). Odds ratios (OR) and parameter estimates are given for significant effects.

	Survivorship				RGR			
	OR	95% CI	$F_{1,88}$	p	β	SE	$F_{1,83}$	p
Root:Shoot (R:S)				0.59	-0.04	0.015	7.2	**0.009**
Shallow root allocation (SR)				0.81				0.57
Specific leaf area (SLA)				0.19				0.55
R:S plasticity	1.2	(1.0,1.5)	7.2	**0.046**	0.032	0.014	5.6	**0.02**
SR plasticity				0.78				0.39
SLA plasticity	1.5	(1.1, 2.0)	3.1	**0.01**	-0.05	0.02	4.35	**0.04**

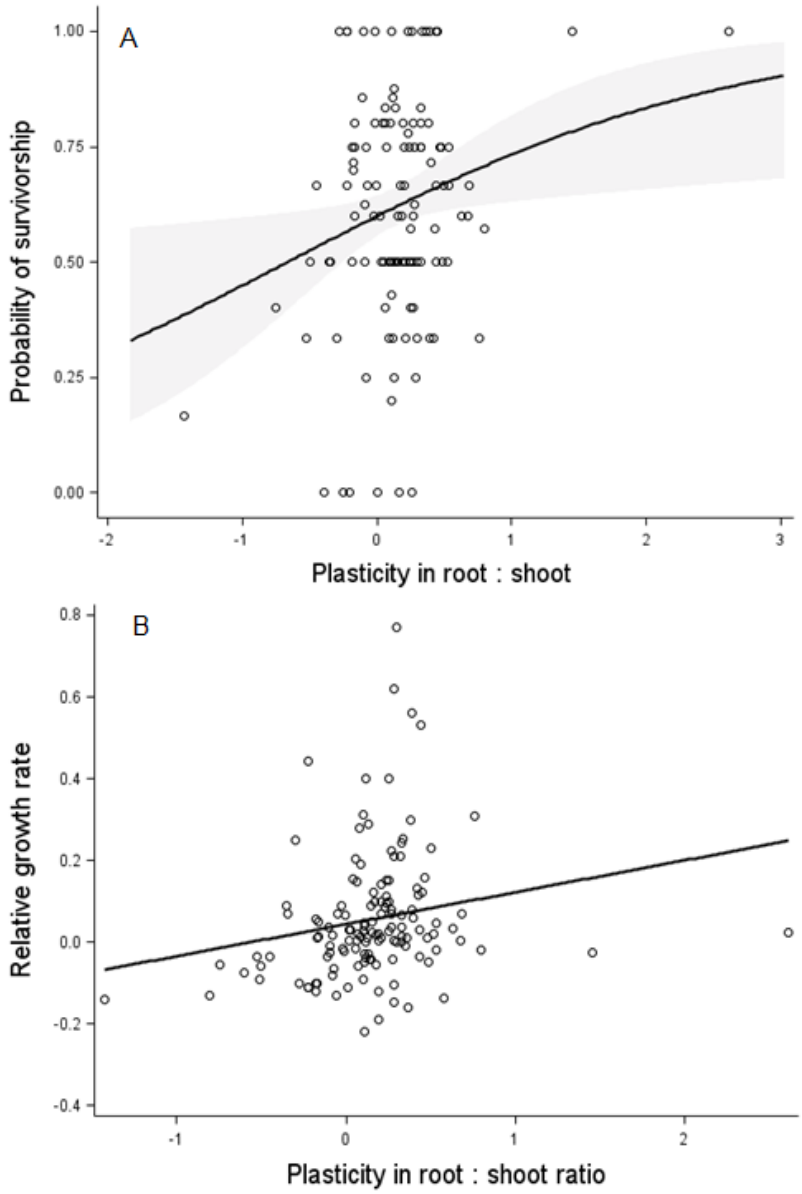

Figure 3.12: Selection on plasticity in root : shoot ratio in the greenhouse experiment for two fitness components: (a) survivorship, and (b) growth rate.

Cost of plasticity—I found very limited evidence for a cost to plasticity either in the greenhouse or in the field experiment. In the flooded treatment in the greenhouse, there were no relationships (negative or positive) between fitness (survivorship or growth rate) and plasticity in any trait. In contrast, in the drought treatment, there was a negative relationship between survivorship and plasticity in leaf

size [odds ratio (95% confidence interval): 0.59 (0.37, 0.94), $F_{1,80} = 5.0$, p=0.028]. This cost of plasticity did not hold for the other traits. Instead, there was a positive relationship between survivorship and plasticity in root : shoot ratio [odds ratio (95% confidence interval): 2.2 (1.2, 3.8), $F_{1,80} = 5.8$, p=0.018]. Additionally, in the drought treatment, I uncovered no cost of plasticity when relative growth rate was the fitness component. Rather, there were positive correlations between growth rate and plasticity in root : shoot ratio (parameter estimate ± S.E.: 0.075 ± 0.035, $F_{1,68} = 4.5$, p=0.038) and plasticity in allocation to shallow roots (0.04 ± 0.02, $F_{1,68} = 3.8$, p=0.057).

In the field, plasticity in specific leaf area appears costly in the bottomland habitat: both the 2005 and the 2006 transplants showed a significant negative relationship between RGR and plasticity in SLA (2005 transplants parameter estimate ± S.E.: -0.0085 ± 0.003, $F_{1,70} = 6.3$, p=0.035; 2006 transplants: -0.004 ± 0.002, $F_{1,97} = 4.6$, p=0.014). In the upland sites, there were no survivorship or growth rate costs of plasticity in SLA.

Discussion

Vaccinium elliottii individuals exhibited phenotypic plasticity (E) for almost all of the traits measured in the reciprocal transplant and greenhouse experiments. I found virtually no evidence, however, that trait values varied by habitat of origin (G) or that reaction norms changed as a function of habitat of origin (G×E interaction). Survivorship and growth data from these experiments indicate that *V. elliottii* populations are not locally adapted to upland vs. bottomland habitats (i.e., there is no G×E interaction in fitness components, Chapter 2). Additionally, analysis with six polymorphic microsatellite loci revealed virtually no neutral population divergence (Chapter 2). The lack of adaptive and neutral population differentiation in a spatially and temporally heterogeneous landscape accords well with the high degree of

phenotypic plasticity because plasticity is a strategy used to maximize fitness across environments (van Tienderen, 1997). Interhabitat gene flow, in conjunction with reliable cues of changing environmental conditions, can promote the evolution of phenotypic plasticity (Sultan and Spencer, 2002; van Tienderen, 1997).

Interestingly, this species also shows habitat-based fitness differences (Chapter 2). Survivorship and growth rate were significantly greater in upland relative to bottomland transplant environments; individuals also exhibited enhanced performance in the drought relative to the flooded treatment in the greenhouse (Chapter 2). These fitness differences could be a response to a variety of abiotic and biotic stresses in bottomland systems (Chapter 2), including foliar herbivory, which was significantly greater in bottomland transplant sites than upland sites for both life history stages. Ecophysiological traits from this study substantiate previous results that *V. elliottii* populations are better adapted to long-term drought than flooding. Whereas photosynthesis and stomatal conductance began to rebound in the drought treatment by the end of the greenhouse experiment, these ecophysiological traits continued to decline in flooded individuals. Indeed, *V. elliottii* showed symptoms of flood intolerance, such as leaf reddening, senescence and necrosis (Pereira and Kozlowski, 1977). Furthermore, asymmetrical gene flow is likely from upland to bottomland populations because of greater population sizes and genetic diversity in upland forests (Chapter 2). These results are consistent with source-sink dynamics. Models of source-sink dynamics predict that selection is biased toward the source habitat because more individuals encounter that habitat (Holt and Gaines, 1992; Kawecki, 1995). Indeed, asymmetrical gene flow from high to low quality habitats or central to range edge populations can inhibit local adaptation to marginal habitats (García-Ramos and Kirkpatrick, 1997; Kawecki, 2000; Kawecki and Holt, 2002). Thus, according to source-sink theory, *V. elliottii* should exhibit locally adapted phenotypic traits that

enhance fitness in upland forests at the expense of fitness in the bottomland. The phenotypic plasticity documented in this study contradicts this theoretical expectation (Dias, 1996).

There are several possible explanations for the existence of phenotypic plasticity in a system where habitats vary in quality. For one, phenotypic plasticity could actually be favored in the source habitat. For *V. elliottii*, phenotypic plasticity could be advantageous in upland forests due to subtle spatial variation in light level that alters foliar phenotypes. In this case, I would expect limited plasticity in traits related to flooding tolerance because upland systems do not experience floods. Another explanation is that habitat-based differences in fitness do not reflect long-term source-sink dynamics (e.g. Holt, 1997), but rather incipient expansion in niche breadth. Phenotypic plasticity is thought to enhance range and niche expansion (e.g. Schlichting and Smith, 2002); in the absence of plasticity, *V. elliottii* might not be able to establish in the stressful bottomland systems. Phenotypic plasticity would maximize fitness across the landscape and genotypes with higher levels of phenotypic plasticity would spread faster in both habitat types. Finally, it is possible that phenotypic plasticity is a phylogenetic constraint and is not adaptive in this system. The genus *Vaccinium* occupies a wide range of habitats, with broadly varying moisture regimes (e.g Hill and Vander Kloet, 2005; Sandler *et al.*, 2007; Yang *et al.*, 2008) and many species occur in both wetland and upland systems (U.S. Fish and Wildlife Service, 1996). Plasticity that was advantageous in an ancestor of *V. elliottii* could still exist, especially if the costs of plasticity are low. Examination of the phenotypic traits from the greenhouse and the field as well as the selection analyses can address some of these issues.

Foliar traits—In the field and the greenhouse, I quantified several key foliar traits: leaf size, specific leaf area, N content, and stable carbon isotope ratios. I found

substantial phenotypic plasticity in the field, but limited to no plasticity in the greenhouse. In the greenhouse, I only varied water stress, whereas in the field, a suite of abiotic and biotic conditions differ between upland and bottomland forests, including water table depth, light level (greater in uplands) and soil nutrient levels (lower in uplands, Chapter 2). The limited plasticity in the greenhouse suggests that flooding and drought both induce similar foliar characteristics: lower SLA and lower internal leaf CO_2 concentration as seen from the overall higher (less negative) carbon isotope ratios. In the field, habitat-based differences in light levels and edaphic factors could be responsible for plasticity in foliar traits. Thus, plasticity in specific leaf area could be advantageous in upland forests if there is spatial variation in light level. Finally, repeated measures analysis of variance on SLA values from 2005 transplants showed a significant effect of year, indicating temporal plasticity that might accord with yearly fluctuations in abiotic conditions. SLA values in both greenhouse treatments were lower than SLA of leaves from upland transplant sites in the field experiment. This result suggests that prolonged water deprivation and flooding can both alter the morphology of *V. elliottii* leaves. This flexibility in leaf anatomy likely improves plant performance in temporally variable habitats.

Due to the close relationship between SLA, foliar N content and photosynthetic rate (Shipley *et al.*, 2006), it is not surprising that these results also indicate a high degree of phenotypic plasticity in ecophysiological traits in the field. Various stages in the photosynthetic process discriminate against the heavier ^{13}C isotope relative to the lighter, more abundant ^{12}C. Fractionation occurs during CO_2 diffusion through the stomata and boundary layer, internal CO_2 transfer through the mesophyll, carboxylation, and photorespiration (Seibt *et al.*, 2008). The low carbon isotope ratios of bottomland relative to upland transplants reflects a higher ratio of CO_2 concentration within the leaf to CO_2 concentration of the atmosphere (c_i/c_a)

(Farquhar *et al.*, 1989). Similarly, the higher elemental nitrogen content (%N) of bottomland transplants suggests increased photosynthetic capacity because, across species, foliar N is allocated primarily to proteins involved in photosynthesis (e.g. Evans, 1989). The negative correlation between %N and $\delta^{13}C$ suggests that increased photosynthetic capacity corresponds with increased internal leaf CO_2 (Sparks and Ehleringer, 1997). The $\delta^{13}C$ values from the reciprocal transplant experiment are very negative for C_3 plants and indicate high stomatal conductance, especially in the bottomland (Dawson *et al.*, 2002; Farquhar *et al.*, 1989). This result suggests that during carbon fixation, plants are not water-stressed in either habitat, which is surprising because upland forests in this region have sandy soils, and a deep water table relative to bottomland forests (Megonigal *et al.*, 1997). The increased foliar N content in bottomland transplants corresponds well with increased soil nitrogen in that habitat (Chapter 2). Heightened photosynthetic capacity (%N) and c_i might be beneficial in the relatively darker bottomland forests than the sunnier upland forests by allowing bottomland plants to take advantage of sunflecks (Farquhar *et al.*, 1989).

In the greenhouse experiment, I found substantially higher (less negative) $\delta^{13}C$ values than in the field. Furthermore, carbon isotope ratios did not differ between drought-stressed and green leaves from flooded plants. Thus, under water stress in the greenhouse, plants in both treatments had lower c_i and presumably lower stomatal conductance and greater water use efficiency than in the field. Flooded and drought-stressed plants in the greenhouse were exposed to the same light conditions, but very different water stress; therefore, differences in light level and potentially soil nutrient availability between bottomland and upland forests likely alter the physiological function of leaves and their stable carbon isotope ratios in the field. Viability selection for increased $\delta^{13}C$ values in both greenhouse treatments suggests that selection favors similar ecophysiological performance and water use efficiency under disparate water

stresses.

Root and other traits associated with flood tolerance—In the greenhouse, flooding did not induce the enlargement of lenticels or the production of adventitious roots, even though these traits facilitate gas exchange with flooded roots in other wetland plants (e.g. Fenster, 1997; Mielke *et al.*, 2003; Parolin, 2001). Other wetland species exposed to waterlogging or flooding often have root porosities in excess of 20% (Lenssen *et al.*, 2004; Mommer *et al.*, 2006; Purnobasuki and Suzuki, 2004; Visser and Bögemann, 2003), which far surpasses the average porosity of flooded *V. elliottii* from this study (~5%). Nevertheless, *V. elliottii* is not completely flood-intolerant. Individuals from the flooded treatment had a higher proportion of their roots in the top 1 cm of the soil, and the proliferation of shallow roots at the air-water interface can enhance plant performance under flooded conditions (Fenster, 1997). It is unlikely that phylogeny constrains the evolution of flood tolerance in *V. elliottii*. Although few ecological studies have been conducted on the flood tolerance of *Vaccinium* species, the horticultural literature reveals that close relatives of *V. elliottii* are tolerant of flooding. For example, in response to flooding, the closely related *V. corymbosum* (Bruederle and Vorsa, 1994) expresses aerenchyma (porous root tissue) (Abbott and Gough, 1987). Additionally, cranberries (*V. macrocarpon*), are native to North American bogs and are cultivated in flooded conditions (Sandler *et al.*, 2007) and as such are expected to be flood tolerant. Instead, the relative flood intolerance of *V. elliottii* could be a function of asymmetrical gene flow from upland to bottomland populations, or weak selection for flood tolerance in bottomland forests.

Flooding events are highly variable interannually at Beidler forest, ranging from 3-139 total days of flooding (43.6 ± 36 days, mean ± S.D.) and 1-7 flood events per year during the growing season (N. Brunswig, M. Dawson, Beidler forest unpublished river level data, beginning 1977). Each flood event lasts an average of 20

days (±14, S.D), but can be as long as 80 consecutive days. During the lifespan of a *V. elliottii* individual growing in bottomland habitat, it is likely to experience multiple years with extensive growing season floods. The evolution of phenotypic plasticity requires that reliable cues of incipient environmental change exist and that individuals can respond appropriately to these cues (Sultan and Spencer, 2002). In this system, flooding occurs gradually over several days; thus, increasing soil moisture may serve as a reliable cue for the induction of flood-related plastic traits. It is possible, however, that prolonged floods are too infrequent to favor a plastic response (e.g. Benz *et al.*, 2007); indeed, down-regulation of ecophysiological function could be an appropriate response to short term flooding during the growing season. Additionally, the evolution of plasticity in flood tolerant traits could be hindered by the lag time between environmental change and plant response (e.g. DeWitt *et al.*, 1998). That is, it might take longer for individuals to produce flood tolerant phenotypes than the flood actually lasts. It is difficult to determine whether the relative lack of flood-induced traits is a function of gene flow from upland to bottomland forests, or constraints on the evolution of flood tolerance within the bottomland.

Selection and the cost of plasticity—In the greenhouse, divergent selection acted on root:shoot ratio and this selection was concordant with the plasticity documented in this trait; additional selection analyses indicated significant selection for plasticity in both of these traits. In the field experiment, I uncovered a significant interaction between SLA and transplant environment on survivorship of the 2006 transplants; however, this interaction term was due to a difference not of direction, but of slope (steeper in upland than bottomland sites). Despite the very consistent phenotypic plasticity in SLA in the field, there was no evidence of selection for larger SLA values in bottomland sites and smaller values in the uplands, or for selection on plasticity of this trait. It is possible that I underestimated viability selection in the field

(e.g. Hadfield, 2008). A large proportion of bottomland transplants died before foliar traits were measured; in contrast, trait values were quantified on plants that died during the greenhouse experiment. Thus, the viability analyses from the greenhouse likely incorporate individuals with a larger range of trait values, whereas analyses from the field experiment exclude plants with the lowest survivorship and perhaps the most inappropriate trait values.

I found very limited evidence that plasticity was costly in the greenhouse or the field experiments. Therefore, in this system, neutral phenotypic plasticity could persist because it is not costly. These analyses of the cost of plasticity, however, also revealed that plasticity in foliar and root traits could be advantageous under drought conditions. For one, selection favored plasticity in root : shoot ratio in the drought treatment and growth rate correlated positively with allocation to roots in the top 1 cm of the soil. In the analyses of selection on plasticity across environments, I found no evidence that plasticity in allocation to shallow roots was advantageous. Thus, landscape-level plasticity in certain traits can be favored in one environment even if it does not have an advantage across environments.

It is important to consider that the magnitude and direction of selection and the costs of plasticity can change when different fitness components are considered. Often, selection detectable using one fitness component was nonsignificant for the other fitness component. Such differences could occur if the range of trait values is smaller for one fitness component than the other. For example, viability selection could remove individuals with very inappropriate trait values and selection at later life stages could refine trait values.

Conclusions—Despite the high degree of phenotypic plasticity in foliar, ecophysiological, and root traits, *V. elliottii* did not express several traits known to improve flood tolerance like lenticels, adventitious roots or high root porosity.

Additionally, phenotypic plasticity in certain traits, such as leaf size, specific leaf area and stable carbon isotope ratios, could potentially be an adaptation to light conditions that could vary within upland forests. This evidence for phenotypic plasticity might not contradict predictions of source-sink models if the plasticity itself is favored within upland systems (the source). I propose that phenotypic plasticity in foliar and root traits allows *V. elliottii* individuals to colonize and persist in the stressful bottomland forests. Furthermore, migration from upland into bottomland forests likely increases the genetic diversity of bottomland populations and promotes the evolution of phenotypic plasticity. However, this interhabitat gene flow could also reduce the potential for bottomland populations to adapt to local conditions.

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APPENDICES

Appendix 1: Differences in abiotic conditions at the four sites used in the reciprocal transplant experiment.

	Upland site 1	Upland site 2	Bottomland site 1	Bottomland site 2
Fall soil moisture (%)	0.04	0.10	0.10	0.13
Spring soil moisture (%)	0.08	0.09	0.19	0.25
Soil bulk density (g/cm^3)	0.76	0.91	1.22	1.06
Direct solar radiation (Mols m-2 day-1)	12.27	12.25	8.85	8.55
Diffuse solar radiation (Mols m-2 day-1)	11.81	10.10	7.87	8.23
% Organic matter	1.56	0.76	2.03	1.98
Soil C:N	27.75	27.27	15.99	17.15

Appendix 2: Winbugs code for frailty analysis. I implemented this model for the greenhouse experiment. The models for the field experiment included a fixed effect for transplant site, but did not include the random effect of block. Here, gen = family (or genotype); pop = population; env = treatment; hab = habitat of origin; prox= proximity; life= life history stage; biom = initial plant biomass; block = block; z= natural logarithm of time, measured in days.

```
model
        {
        # Set up data
                for(i in 1:N) {
                        for(j in 1:T) {
        # risk set = 1 if obs.t >= t
                                Y[i,j] <- step(obs.t[i] - t[j] + eps)
        # counting process jump = 1 if obs.t in [ t[j], t[j+1] )
        #               i.e. if t[j] <= obs.t < t[j+1]
                                dN[i, j] <- Y[i, j] * step(t[j + 1] - obs.t[i] - eps) * fail[i]
                        }
                }
        # Model
                for(j in 1:T) {
                        for(i in 1:N) {
                                dN[i, j]   ~ dpois(Idt[i, j])            # Likelihood
                                Idt[i, j] <- (b[gen[i]] *d[pop[i]]) * Y[i, j] *
exp(beta[1]*trt[i]+beta[2]*env[i] + beta[3]*life[i]+ beta[4]*prox[i] +
beta[5]*env[i]*prox[i] + beta[6]*trt[i]*env[i]+ beta[7]*trt[i]*prox[i] +beta[8]*biom[i]
+beta[9]*trt[i]*env[i]*prox[i] +beta[10]*trt[i]*life[i] +beta[11]*prox[i]*life[i] +
beta[12]*env[i]*life[i]  +beta[13]*trt[i]*env[i]*prox[i]*life[i]+beta[14]*trt[i]*z[i]+
beta[15]*life[i]*z[i]+  f[tray[i]]) * dL0[j]      # Intensity

                        }
                        dL0[j] ~ dgamma(mu[j], c)
                        mu[j] <- dL0.star[j] * c    # prior mean hazard

                }
        for(k in 1 : Ngen) {
                   b[k] ~ dgamma(tau.gen,tau.gen);}
                        for(l in 1 : Npop) {
                   d[l]~ dgamma(tau.pop,tau.pop);
                                                        }

                #Prior:
                tau.gen ~ dgamma(0.1,0.01);
                sigma.gen<- 1/sqrt(tau.gen); # s.d. of random effects
                        tau.pop ~ dgamma(0.1,0.01);
```

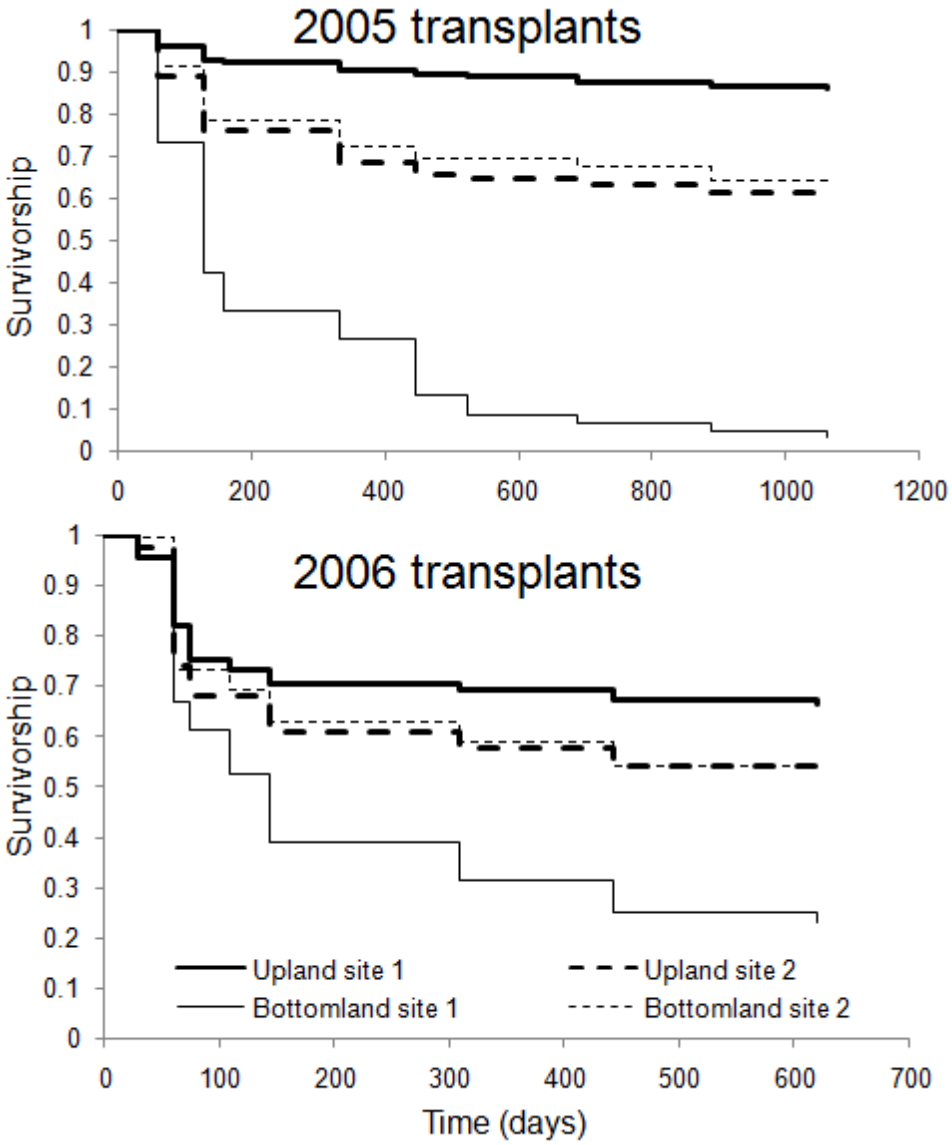
```
sigma.pop<- 1/sqrt(tau.pop); # s.d. of random effects
c <- 0.001
r ~ dgamma(1.0, 0.001)
for (j in 1 : T) {  dL0.star[j] <- r * (t[j + 1] - t[j])  }
        for (o in 1:15) {beta[o]~ dnorm(0.0,0.001)

                }}
```

Appendix 3: Populations used for microsatellite work. Logistical constraints did not allow sampling of seeds at two upland populations: Oakridge and SSP.

Population	Habitat	Proximity	Latitude	Longitude	Sample size (adults)	Sample size (seeds)
Cabin	Upland	remote	33°11'53	080°20'23	28	15
Mizzell	Upland	remote	33°10'00	080°20'09	25	8
Grooms	Upland	remote	33°12'10	080°17'52	19	12
Oakridge	Upland	remote	33°08'25	080°21'20	21	N/A
SSP	Upland	remote	33°32'37	080°29'41	11	N/A
Canoe	Bottomland	remote	33°12'45	080°20'04	26	13
NW15	Bottomland	remote	33°17'56	080°29'27	19	12
NE	Bottomland	remote	33°14'29	080°21'03	20	10
ML	Bottomland	remote	33°12'02	080°19'51	20	6
Uedg	Upland	ecotonal	33°12'49	080°19'35	20	17
UeGN	Upland	ecotonal	33°12'08	080°18'26	14	13
UeNE	Upland	ecotonal	33°13'55	080°20'06	10	14
UeOS	Upland	ecotonal	33°07'25	080°21'33	18	12
Fedg	Bottomland	ecotonal	33°12'48	080°19'36	21	12
FeGN	Bottomland	ecotonal	33°12'07	080°18'27	16	9
FeNe	Bottomland	ecotonal	33°13'56	080°20'07	8	9
FeOS	Bottomland	ecotonal	33°07'25	080°21'32	19	12

Appendix 4: MANOVA (Proc Mixed) examining the effect of habitat (upland vs. bottomland forest), proximity (ecotone vs. remote population), life history stage (seedling vs. adult), and two and three-way interactions on *Vaccinium elliottii* population-level genetic diversity (allelic richness, private allelic richness, expected heterozygosity). I included random statements for locus nested within population and life history stage nested within population.

Explanatory variable	F	P
Habitat	$F_{3,225}$= 0.08	0.43
Proximity	$F_{3,225}$= 0.65	0.58
Life history stage	$F_{3,33}$= 3.37	**0.03**
Life history × Habitat	$F_{3,225}$= 0.09	0.96
Life history × Proximity	$F_{3,225}$= 0.52	0.67
Habitat × Proximity	$F_{3,225}$= 0.40	0.75
Life history stage × Habitat × Proximity	$F_{3,225}$= 2.85	**0.038**



Appendix 5: Kaplan-Meier survivorship curves comparing the transplant sites for the 2005 and 2006 transplants. Floodplain site 1 has a dense understory of dwarf palmetto (*Sabal minor*), which appears to restrict growth and limit survivorship of *V. elliottii*

Appendix 6: Test of selective neutrality in GENEPOP revealed that these loci are likely not under selection (i.e., p>0.05).

Locus	Sample Heterozygosity	Sample Fst	Test statistic	P (simulated Fst < sample Fst)
CA23F	0.11	0.11	2.13	0.98
CA787F	0.11	0.11	2.04	0.97
CA855F	0.6	0.037	-0.54	0.32
NA961	0.53	0.04	-0.2	0.43
CA169F	0.085	0.06	0.85	0.78
CA190R	0.38	0.04	-0.13	0.46
CA94F	0.91	0.034	-1.62	0.063
NA398	0.16	0.061	0.85	0.78

Appendix 7: Genotyping scoring error for mother-offspring pairs.

Locus	Mother-offspring pairs	Polymerase chain reactions	# of mistyped reactions	# of mistyped alleles	Detection probability	Estimated error rate
CA23F	89	178	0	0	0.0051	0
CA787F	87	174	1	1	0.0043	1.346
CA855F	87	174	3	3	0.211	0.0817
NA961	84	168	1	1	0.1379	0.0432
CA169F	89	178	1	1	0.0034	1.6354
CA190R	82	164	0	0	0.0553	0
CA94F	79	158	7	7	0.6721	0.0659
NA398	87	174	0	0	0.0146	0

Appendix 8: *Vaccinium elliottii* mean F-statistics (± S.E., derived from jackknifing over populations) for adults in Four Holes Swamp watershed. Multilocus means follow Weir and Cockerham (1984). N_A refers to the number of alleles.

Locus	Repeat motif	N_A	$H_{observed}$	$H_{expected}$	F_{IS}	F_{IT}	F_{ST}
					0.076	0.099	0.025
CA94F	(AG)7	20	0.79	0.89	(0.033)	(0.033)	(0.007)
					.093	0.135	0.046
CA855F	$(GA)_{14}…(CGA)_5$	14	0.52	0.58	(0.035)	(0.04)	(0.019)
					0.083	0.100	0.018
NA961	$(TAC)_5$	6	0.46	0.51	(0.047)	(0.047)	(0.017)
					-0.076	-0.026	0.046
NA398	$(AAAT)_5$	4	0.11	0.10	(0.020)	(0.026)	(0.023)
					0.096	0.138	0.047
CA190R	$(TGC)_5$	6	0.31	0.35	(0.085)	(0.075)	(0.022)
					-0.022	-0.012	0.009
CA169F	$(GAT)_4$	6	0.044	0.043	(0.011)	(.004)	(0.010)
					0.078	0.108	0.032
Multilocus mean		9.3	0.37	0.41	(0.009)	(0.012)	(0.007)

Appendix 9: Mean F-statistics (± S.E., derived from jackknifing over populations) for seeds in Four Holes Swamp watershed. Repeat motif and other notes as in Appendix 7.

Locus	N_A	$H_{observed}$	$H_{expected}$	F_{IS}	F_{IT}	F_{ST}
				0.055	0.092	0.040
CA94F	18	0.829	0.878	(0.036)	(0.035)	(0.011)
				0.077	0.096	0.020
CA855F	16	0.558	0.608	(0.045)	(0.047)	(0.012)
				0.023	0.078	0.057
NA961	5	0.492	0.509	(0.063)	(0.064)	(0.042)
				-0.155	-0.079	0.067
NA398	5	0.225	0.192	(0.054)	(0.032)	(0.038)
				0.054	0.083	0.031
CA190R	6	0.350	0.378	(0.092)	(0.090)	(0.021)
				0.114	0.138	0.026
CA169F	6	0.073	0.079	(0.137)	(0.139)	(0.014)
Multilocus				0.043	0.080	0.038
mean	9.3	0.421	0.441	(0.019)	(0.014)	(0.007)

Appendix 10: Full models of root traits of plants that survived the entire duration of the greenhouse experiment. Root architecture refers to the proportion of roots that occur in the top 1cm of the soil (relative to total root biomass). Block nested within treatment and family nested within population were treated as random effects. Explanatory effects are as follows: H=habitat of origin; P=proximity (ecotone vs. remote); T=treatment; L=life history stage.

	Root:Shoot		Root architecture	
	F	p	F	p
H	$F_{1,196}$=1.6	0.21	$F_{1,196}$=5	**0.026**
P	$F_{1,196}$=1.7	0.19	$F_{1,196}$=0.9	0.33
H × P	$F_{1,196}$= 0.9	0.34	$F_{1,196}$= 0.2	0.69
T	$F_{1,52}$=75.5	**<0.0001**	$F_{1,52}$=44.5	**<0.0001**
H × T	$F_{1,196}$= 0.3	0.6	$F_{1,196}$=0.4	0.53
P × T	$F_{1,196}$=0	0.98	$F_{1,196}$=0.4	0.55
H × P × T	$F_{1,196}$= 1.4	0.24	$F_{1,196}$= 0.4	0.55
L	$F_{1,196}$=1.4	0.23	$F_{1,196}$=59	**<0.0001**
L × T	$F_{1,196}$=0	0.99	$F_{1,196}$=6.9	**0.0093**
L × H	$F_{1,196}$= 0.1	0.76	$F_{1,196}$=0.9	0.3
L × P	$F_{1,196}$= 1.7	0.2	$F_{1,196}$=0.1	0.8
L × H × P	$F_{1,196}$= 0	0.91	$F_{1,196}$= 2.9	0.09
L × T × H	$F_{1,196}$=0.1	0.82	$F_{1,196}$=1.6	0.2
L × T × P	$F_{1,196}$= 1.1	0.3	$F_{1,196}$= 0.8	0.4
L × T × H × P	$F_{1,196}$=0.17	0.68	$F_{1,196}$=0.7	0.4

Appendix 11: Full models of foliar traits of plants that survived the entire duration of the greenhouse experiment. SLA is specific leaf area. Leaf retention is measured as leaf biomass: total aboveground biomass. Block nested within treatment and family nested within population were treated as random effects. Explanatory effects are as follows: H=habitat of origin; P=proximity (ecotone vs. remote); T=treatment; L=life history stage.

	SLA		Leaf retention		Leaf size	
	F	p	F	p	F	p
H	$F_{1,175}$=0.1	0.8	$F_{1,196}$= 0.8	0.37	$F_{1,161}$=0.1	0.73
P	$F_{1,175}$= 13.4	**0.0003**	$F_{1,196}$=0	0.98	$F_{1,161}$= 5.1	**0.025**
H × P	$F_{1,175}$= 1.3	0.25	$F_{1,196}$=0	0.99	$F_{1,161}$= 2.8	*0.054*
T	$F_{1,52}$= 33.1	<**0.0001**	$F_{1,52}$=53.7	<**0.0001**	$F_{1,52}$=0.2	0.62
H × T	$F_{1,175}$= 3.1	*0.08*	$F_{1,196}$=0.7	0.40	$F_{1,161}$=2.8	0.096
P × T	$F_{1,175}$= 0.7	0.4	$F_{1,196}$=0.2	0.68	$F_{1,161}$= 0.7	0.4
H × P × T	$F_{1,175}$= 3.9	*0.05*	$F_{1,196}$=0.1	0.77	$F_{1,161}$= 1.3	0.25
L	$F_{1,175}$= 36	<**0.0001**	$F_{1,196}$=13.8	**0.0003**	$F_{1,161}$=20.1	<**0.0001**
L × T	$F_{1,175}$= 0.1	0.8	$F_{1,196}$=8.6	**0.0038**	$F_{1,161}$= 4.4	**0.037**
L × H	$F_{1,175}$= 0.4	0.5	$F_{1,196}$=0.1	0.74	$F_{1,161}$= 0.9	0.35
L × P	$F_{1,175}$= 2.6	0.11	$F_{1,196}$=0.6	0.45	$F_{1,161}$= 1.6	0.21
L × H × P	$F_{1,175}$= 0.04	0.85	$F_{1,196}$= 3.6	*0.059*	$F_{1,161}$=0.4	0.53
L × T × H	$F_{1,175}$= 1.8	0.19	$F_{1,196}$= 0.02	0.9	$F_{1,161}$= 0.2	0.69
L × T × P	$F_{1,175}$= 0	0.97	$F_{1,196}$= 0.8	0.38	$F_{1,161}$= 2.9	0.096
L × T × H × P	$F_{1,175}$= 1.2	0.3	$F_{1,196}$=0.2	0.65	$F_{1,161}$=0.7	0.42

Appendix 12: Full model for repeated measures analyses of ecophysiological traits measured in the greenhouse with the Li-Cor 6400. Explanatory effects are as follows: H=habitat of origin; P=proximity (ecotone vs. remote); Trt=treatment; L=life history stage. Time includes measurements made in September 2006 (baseline), December 2006 (1 month) and April 2007 (final).

	Photosynthesis		Stomatal conductance		Instantaneous water-use efficiency	
	F	p	F	p	F	p
Time	$F_{2,190}$=30.6	**<0.0001**	$F_{2,190}$=35.6	**<0.0001**	$F_{2,190}$=23.2	**<0.0001**
H	$F_{1,190}$=0.04	0.85	$F_{1,190}$=0.06	0.80	$F_{1,190}$=0.41	0.52
P	$F_{1,190}$= 6.9	**0.0096**	$F_{1,190}$= 6.2	**0.0136**	$F_{1,190}$= 4.3	**0.039**
H × P	$F_{1,190}$= 3.2	0.076	$F_{1,190}$= 1.6	0.21	$F_{1,190}$= 2.9	0.09
Trt	$F_{1,46}$=7.7	**0.008**	$F_{1,46}$=2.7	0.11	$F_{1,46}$=2.7	0.11
H × Trt	$F_{2,190}$=0.02	0.89	$F_{2,190}$=0.1	0.71	$F_{2,190}$=0.04	0.85
P × Trt	$F_{1,190}$=1.1	0.29	$F_{1,190}$= 0.01	0.92	$F_{1,190}$= 0.3	0.61
H × P × Trt	$F_{1,190}$=0.1	0.75	$F_{1,190}$=0.01	0.92	$F_{1,190}$=0.1	0.74
Time × H	$F_{2,190}$=0.8	0.43	$F_{2,190}$=0.9	0.41	$F_{2,190}$=0.2	0.86
Time × P	$F_{2,190}$=0.5	0.60	$F_{2,190}$=1	0.37	$F_{2,190}$=0.5	0.60
Time × H × P	$F_{2,190}$=2	0.14	$F_{2,190}$=1.3	0.28	$F_{2,190}$=0.6	0.53
Time × Trt	$F_{2,190}$=5.2	**0.0064**	$F_{2,190}$=8.5	**0.0003**	$F_{2,190}$=1.9	0.15
Time × H × Trt	$F_{2,190}$=0.6	0.57	$F_{2,190}$=1.0	0.37	$F_{2,190}$=0.3	0.78
Time × P × Trt	$F_{2,190}$=0.6	0.54	$F_{2,190}$=0.04	0.96	$F_{2,190}$=0.2	0.79
Time × H × P × Trt	$F_{2,190}$=0.9	0.42	$F_{2,190}$=0.01	0.99	$F_{2,190}$=2.7	0.071

Appendix 13: Baseline ecophysiological data (September 2006). Treatments had not yet been imposed, but these individuals were assigned to treatments before measurements were made. Explanatory effects are as follows: H=habitat of origin; P=proximity (ecotone vs. remote); Trt=treatment; L=life history stage.

	Photosynthesis		Stomatal conductance		Instantaneous water-use efficiency	
	F	p	F	p	F	p
Trt	$F_{1,45}$=3.05	0.088	$F_{1,45}$=0.03	0.87	$F_{1,45}$=2.99	0.09
H	$F_{1,28}$= 0.09	0.77	$F_{1,28}$= 0.38	0.54	$F_{1,28}$= 0.04	0.84
P	$F_{1,28}$=6.43	**0.017**	$F_{1,28}$= 8.24	**0.0077**	$F_{1,28}$= 0.17	0.69
Trt × H	$F_{1,28}$= 0.26	0.62	$F_{1,28}$= 0.02	0.89	$F_{1,28}$= 0.01	0.92
Trt × P	$F_{1,28}$= 0.26	0.62	$F_{1,28}$= 0.01	0.92	$F_{1,28}$= 0.6	0.45
H × P	$F_{1,28}$= 0.02	0.9	$F_{1,28}$= 0.09	0.77	$F_{1,28}$= 0.04	0.84
Trt × H × P	$F_{1,28}$= 1.14	0.3	$F_{1,28}$= 0.31	0.58	$F_{1,28}$= 3.54	0.07

Note: Individuals from remote populations had significantly greater baseline photosynthesis values than individuals from ecotonal populations (LSMEANs ± SE: remote populations: 4.18 ± 0.3; ecotonal populations: 3.12 ± 0.3 $\mu CO_2 m^{-2} s^{-1}$). Similarly, stomatal conductance was significantly greater in plants from remote populations (LSMEAN S ± SE: 0.074 ± 0.005 mol H_2O $m^{-2} s^{-1}$) than those from ecotonal populations (0.051 ± 0.0056).