Running Head: Determinants of ecotype distribution

Allocation patterns contribute to the distributional limits of a flood tolerant ecotype of

Chamaecrista fasciculata

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Abstract

To determine factors limiting the distribution of two ecotypes of the annual legume *Chamaecrista fasciculata* along an abrupt flooding gradient, we quantified the relative importance of tolerance, intra-specific competition, and allocation patterns to vegetative and reproductive functions and their genetic basis. The ecotypes grow in dry upland and freshwater tidal marsh habitat, respectively. Intolerance to flooding limited the upland ecotype from the marsh site, whereas the marsh ecotype had high survival in the upland site. Competition experiments revealed no competitive hierarchy between the two ecotypes under upland conditions. However, the upland ecotype allocated double the proportion of total above ground biomass to reproductive structures compared to the marsh ecotype. In addition, the marsh ecotype produced larger but fewer seeds per fruit than upland ecotype plants. These allocation patterns resulted in the upland ecotype having much greater seed production in the upland habitat. Using a crossing experiment, we quantified the genetic correlation between seed mass and seed number. A negative genetic correlation between seed mass and seed number, as well as a positive genetic correlation with seed mass and shoot elongation of flooded plants suggests that the trade-off in allocation patterns between these two components of reproductive output represents a pleiotropic genetic constraint associated with adaptation to a flooded environment. Thus resource allocation patterns leading to striking trade-offs between above ground vegetative and reproductive allocation appear to limit the marsh ecotype from invading upland habitat.

Keywords: competition; ecotype; flood tolerance; reproductive allocation; trade-offs

1. Introduction

Marsh habitats are useful model systems to examine the relative importance of edaphic and biological factors influencing plant species distributions. Plant tolerance to flooding is often related to plant morphology and metabolic adaptations (Crawford, 1966; Blom and Voesenek 1996; Voesenek et al., 2006; van Veen et al. 2014). Flooded soils can limit the amount of oxygen available to roots for cell division and regular metabolic processes (Crawford, 1992; Perata et al., 2011). Because of the relatively few species that comprise flooded communities, they are also ideal for studies of inter-specific competition as a factor in marsh zonation (Pennings and Callaway, 1992). A number of studies have documented correlations between species distributions, flooding and competitive ability (Linhard, 1973; Shida and Nakamura 2011). For example, competitive ability and flooding tolerance of three related species of *Polygonum* were negatively correlated when studied under greenhouse conditions suggesting that species boundaries are more influenced by competitive ability as flooding stress decreases (Carter and Grace, 1990). Competition has also been found to limit species to particular zones in salt marsh habitats (Pennings and Callaway, 1992).

Adaptations that allow a plant to thrive under flooded conditions may also confer lower fitness in non-flooded environments. For example, roots adapted to flooding are often easily desiccated in dry conditions due to increased porosity (Laan et al., 1989; Blom, 1990). Growth patterns that are adaptive in flooded areas, such as increased shoot growth, may be selected against in drier habitat where other factors limit growth (Touchette et al., 2010). In several cases, the distribution of ecotypes across flooded and upland habitats have been studied to quantify trade-offs between

adaptations to alternate environments, e.g., *Nyssa sylvatica* (Keeley, 1979), *Salicornia* spp. (Teege et al., 2011). Van der Sman et al. (1993) examined life history traits with regard to flood tolerance and found trade-offs between seed size and seed number, as well as differing allocation strategies dependent upon their placement in flooded or upland habitats. In these studies, plants were examined under greenhouse or common garden conditions and the role of genetic constraints underlying trade-offs in allocation patterns were not quantified. One important genetic mechanism underlying genetic constraints and trade-offs is negative pleiotropy, which refers to an allele or alleles (if polygenic) associated with the stronger expression of one trait and the weaker expression of another trait. Negative pleiotropy is commonly found to underlay the expression of yield components in crops (Falconer and McKay, 1996). Thus crossing studies using ecotypes provides a method to quantify the contribution of genetic trade-offs to distribution limits.

Investigations using ecotypes of a single species occupying distinct environments is ideal for examination of both ecological (e.g., competition) and genetic effects on species distribution. This type of population approach has the advantage that the ecotypes share a common and recent evolutionary history such that relatively recent evolution underlying the delimitation of their respective ranges may be easier to ascertain. We can use competition and transplant studies to determine whether one ecotype is eliminated from the other's environment by competition or environmental filtering. Ecotype studies, if the ecotypes are cross-compatible, allow one to also directly examine the genetic basis of distribution patterns in terms of levels of genetic variation (Bradshaw, 1984) and negative pleiotropy (Falconer and McKay, 1996),

Here we examine the factors delimiting the distribution of two ecotypes of *Chamaecrista fasciculata* associated with flooded and upland environments. Our study differs from many others in that it was conducted in the field, and in that we quantify the contribution of genetic constraints.. Because the flood tolerant ecotype strikingly differs from the upland ecotype by its larger seed mass and greater internode distances (Fenster 1997), we focus on how these traits are correlated with one another and performance under flooded conditions, in addition to how seed mass may be correlated with seed number. Our objectives were four-fold. We examined the two ecotypes and quantified the role of 1) environmental tolerance, 2) intra- and inter-ecotypic competitive interactions, 3) resource allocation patterns to vegetative and reproductive structures and 4) the genetic basis underlying the observed allocation patterns as potential factors influencing ecotype distributions.

2. Materials and Methods

2.1 Study organism

Chamaecrista fasciculata (Michx.) Greene (Fabaceae) is an annual herbaceous species native throughout eastern North America from the Atlantic coast to as far west as eastern Kansas. It is common in open, sandy habitats (Irwin and Barneby, 1982), including open woodlands, old fields, roadsides, dunes, and tall- and short-grass prairies. Plants are self-compatible but are primarily outcrossing (Fenster 1991, 1995; Fenster et al., 2003; Williams and Fenster, 1998).

Chamaecrista fasciculata var. *macrosperma* is endemic to tidally influenced freshwater marshes along the tributaries of the James River, Virginia, USA. These marshes are characterized by

water inundation for 0-4 hours twice daily with typical maximum flooding of 30 cm. Soils are predominately silts and clay with high organic content. The populations found in marsh habitat are designated as variety *macrosperma* based on their 4-10 fold greater seed mass compared to upland *C. fasciculata*, 21-58 mg vs 4 - 16 mg (Irwin and Barneby, 1982; Fenster, 1997). The marsh ecotype is fully cross-compatible with upland *C. fasciculata* and thus we consider it an ecotype of *C. fasciculata*. Under greenhouse conditions both ecotypes survive in the relatively dry upland conditions, but only variety *macrosperma* can survive in flooded conditions (Fenster, 1997). In addition to having larger seed mass, the marsh ecotype produces fewer seeds per pod, a two-fold greater rate of shoot elongation, higher germination rates, and increased shallow lateral root growth compared to the upland ecotype (Fenster, 1997). The marsh ecotype does not have aerenchyma. Both produce root nodules in their respective environments and the marsh ecotype also produces root nodules in the upland sites. Because we are unable to grow the upland ecotype in the marsh environment, we do not know if the upland ecotype has the ability to become nodulated in the marsh environment.

2.2 Study sites

The field study was conducted at Point of Rocks County Park, Chesterfield County, Virginia, US, approximately 15 miles south and slightly east of Richmond, Virginia. Two representative sites were chosen for the field experiments. The first site, indicative of the dry upland type habitat of *C. fasciculata* (hereafter the upland site), was located in a clearing within a sandy forest (mixture of red oak, tulip poplars, and American holly), an herbaceous community of selfing congener *C. nictitans*, *Solidago* spp., and other flora typical of early succession in the

south-eastern US. The second site, an open freshwater marsh (hereafter the marsh site), was located in the freshwater tidal marsh of Aston Creek, a tributary of the Appomattox River, which in turn is a tributary of the James River. Common plants found at the marsh site include perennials, such as pickerelweed (*Pontederia cordata*) and cattails (*Typha latifolia*), and annual species, including native N. American wild rice (*Zizania aquatica*) (Fig. 1 supplement). The marsh site was located approximately 300 m from the upland site, and the difference in elevation between the sites is approximately 15 m.

2.3 Tolerance to upland and flooded conditions using reciprocal transplant experiments

We conducted reciprocal transplants into the upland and marsh habitats to verify the ecological factors that may be important in delimiting each ecotype's distribution. *Chamaecrista fasciculata* seeds were collected in mid-October (when fruit are naturally mature) and pooled from approximately 50 plants along a 50 m transect through a disturbed site at the edge of the deciduous forest separating the two habitats. Variety *macrosperma* seeds were collected on the same day and also pooled from approximately 50 plants along a 100 m transect in the tidal marsh. To induce germination, seeds were manually scarified by removing a portion of the seed coat with a metal file, opposite the root radical while leaving the underlying cotyledons intact (Galloway and Fenster, 2000) (overwintering is typically required for seed germination, but there is high overwinter mortality of seeds). Scarified seeds were planted into flats of 2.54 cm square cubits containing a standard soil mix of sphagnum peat moss, fine perlite, gypsum, dolomitic limestone, and low fertilizer designed for seedlings. Seeds were sown into the flats in mid April to correspond with the timing of germination of natural populations in this area, which is about 1 week later. At the 2-3 true leaf stage (late April), seedlings were transplanted into the upland

and marsh sites. It was necessary to plant seedlings, as seeds could have been transported by tidal movement in the marsh site. The developmental stage of the experimental plants exactly corresponded with the developmental stage of native plants. A completely randomized design was used in the upland and marsh sites, with 33 individuals of each ecotype planted into the upland site (total = 66 individuals) and 33 individuals of each ecotype planted into the marsh site (total = 66 individuals). Plants were placed in four rows of 16 or 17 individual plants. Rows and individuals within a row were separated by 15 cm, equal to the low inter plant distance of the competition experiment (see below), and reflecting the lower end of inter plant distance of either ecotype in their natural environments. Plants dying in the first week following transplant were removed from the analysis and considered to have died of transplant shock. Survival was monitored through the reproductive stage for the remaining surviving plants. At the end of the growing season (early October), all plants were harvested, corresponding to when native plants were ripening their fruit, but prior to fruit dehiscence. Plants were dried in an oven to constant mass at 50 degrees C for 4 weeks. From harvested plants, we quantified above ground vegetative mass (stem and leaf mass), reproductive mass (seed, fruit and peduncle mass), and number of seeds and fruits and the proportion of biomass allocated to reproductive vs. vegetative function. In addition, 30 individuals from the wild populations of each ecotype were simultaneously collected (early October) to compare performance and allocation patterns of the ecotypes growing in the experimental plots to unmanipulated populations, by haphazardly collecting plants from the marsh and upland habitats and dried in drying ovens as above.

2.4 Competition

The effects of distance to nearest neighbor and competitive interaction on both ecotypes for vegetative and reproductive traits were quantified by conducting a factorial designed experiment in the upland site. This competition study was restricted to the upland site because we had previous evidence that upland plants cannot tolerate marsh conditions (Fenster, 1997 greenhouse study, confirmed by results of the field reciprocal transplant experiment below). The experimental unit was two interacting plants separated by one of two distances. Each experimental unit was randomized in a completely randomized design with 15 cm separating experimental units in a single row and 0.5 m separating rows of plants. The factors examined were 1) ecotype of the target plant (upland or marsh), 2) ecotype of the nearest neighbor (upland or marsh), and 3) distance separating the two ecotypes (5 cm or 15 cm). The distances selected, 5 and 15 cm, correspond to the range of interplant distances commonly observed between *C. fasciculata* plants at both sites. We assumed no competition between individuals that were spaced at a distance of 15 cm apart, given that seedlings were unlikely to directly interact over this distance. The short distance treatment served as control of non-interacting individuals. Therefore, we assumed that replicates spaced 15 cm apart also did not interact or affect one another. Seeds were sown into the site in mid April to correspond with the timing of germination of natural populations in this area, which is about 1 week later. We planted seeds, rather than seedlings as we did in the reciprocal transplant experiment, because the constraint of water potentially moving our seeds was not relevant at the upland site. Four-foot high poultry fencing was erected around the experimental plot to exclude deer. Plants were watered once in late April to encourage germination and establishment of the seeds and seedlings. The site was weeded throughout the experiment to limit competitive interactions to those between the two ecotypes. Final survivorship data was calculated by determining which plants of those that initially

germinated survived to harvest date. Initially 200 seed were planted for each treatment combination of upland ecotype grown with an upland ecotype neighbor and marsh ecotype grown with a marsh ecotype neighbor and 100 seed of the upland ecotype grown with the marsh neighbor and vice versa. However, due to incomplete germination and plant mortality, final sample sizes ranged from 32 to 97 for each of the eight treatments (2 ecotypes x 2 neighbors x 2 interplant distances). All plants were harvested in the fall after fruit had matured but prior to fruit dehiscence, thus all seed were on the plants at time of harvest. Plants were uprooted so that all above ground parts were collected. To estimate seed number per plant, three fruits were haphazardly collected from each plant and, from these, seeds were counted and weighed. Total seed number per plant was then extrapolated by multiplying the number of fruit per plant x the average number of seed estimated from the sampled fruit. Plants were then dried, as above, prior to measurements of above ground vegetative mass (stem and leaf mass), reproductive mass (seed, fruit and peduncle mass), and number of seeds and fruits.

2.5 Genetic constraints

Reciprocal crosses were made between approximately 20 upland *C. fasciculata* and 20 *C. fasciculata* var. *macrosperma* plants from seed haphazardly collected the previous fall from plants from the two sites. Thus F_1 seed were produced on both parental ecotypes. We did not keep track of the specific sires, only that they were from the alternative ecotype to the dam. Plants were grown and all crosses were conducted in the UMD greenhouse. Approximately 40 F_1 individuals (not keeping track of the specific sire, only that the cross was to another individual) were reciprocally crossed to generate F_2 seed of which approximately 100 seedlings were then grown, roughly equally representative of the 40 F_1 parents. These 100 F_2 plants were then

crossed reciprocally to generate F_3 seeds (not keeping track of the specific sire, only that the cross was made to another individual). Thus the crossing program represented an initial sample of 40 parents, 40 F_1 and 100 F_2 , with attempts to maintain as many of the original parental lines as possible. Although some inbreeding is expected with a finite sized crossing program by maintaining as many maternal lines as possible we were able to reduce the amount of inbreeding in the experiment. Because all of the crosses were made under upland conditions (in the greenhouse), we may have contributed unknown epigenetic factors to the performance or phenotypes of the eventually tested genotypes. However, the ecotypes largely maintain their phenotypic differences under upland conditions (large seeds and much more biomass for the marsh ecotype relative to the upland ecotype), so we are confident that the results reported below reflect genetic and not transient epigenetic effects.

We quantified genetic correlations among the focal traits by growing five plants from each of 28 F_2 dams (equally representing the original maternal parental origins), resulting in 28 sets of full-sib F_3 progeny. Crosses are described in Figure 1. These 28 F_3 full sib families were grown in a common garden outside of the UMD greenhouse in a randomized design from spring to fall. Plants were harvested at the end of the growing season, after the first frost, and placed in drying ovens until completely dry (see above). We quantified above ground vegetative biomass including all stems and fruits. For each plant reproductive biomass measurements included total seed mass, total fruit mass (excluding seeds), biomass of 60 randomly chosen fruits (excluding seeds), and biomass of 80 randomly chosen seeds. We also measured the first three internodes to determine if average internode length, an important feature of flooding tolerance (Fenster, 1997),

is genetically correlated with seed mass. To estimate total seed number on a given plant the total seed mass was divided by the individual seed mass for that plant.

2.6 Statistical analyses

All statistical analyses were conducted using SAS. For presentation purposes, all results are presented in terms of the original units. Traits which required transformation for the analyses are presented as back-transformed means ± 2 SE. For the competition experiment, seed number, seed mass, reproductive mass, fruit number, vegetative mass, total plant mass and percent reproductive mass were analyzed using a three-way ANOVA model (PROC GLM) with fixed effects (target plant, neighbor plant and interplant distance) and associated interactions. Testing for interaction effects allows one to quantify the uniformity of the response across the factors. To meet the assumptions of ANOVA, seed number, seed mass and fruit number were log₁₀ transformed and vegetative mass and reproductive mass were square root transformed. Germination and survival data were tested with using a logistic regression model (SAS CATMOD). Data from the reciprocal transplant experiment and from the native plants were analyzed with t-tests on untransformed or log₁₀ transformed data to meet assumptions of homoscedasticity etc. We protected against Type I error associated with many t-tests by correcting the P levels with sequential Bonferroni correction. Here and below, we consider a P value < 0.05 as significant.

The genetic correlation between seed mass and seed number was determined for plants grown under dry conditions, independent of plant size by regressing each variable on total plant vegetative biomass. Residuals from the regression for both variables were used to determine the

correlation between seed mass and seed size for the 28 full sib families for all comparisons. For the 16 full sib families grown under flooded conditions we also used the residuals from the regression of the response variables on plant size to determine whether seed mass and seed number are both correlated to performance under wet conditions for the 16 full sib families. All variables were log10 transformed to meet assumptions of parametric correlation analyses. To control for Type I error, the significance levels of all correlations was corrected by sequential Bonferroni correction.

3. Results

3.1 *Field collected ecotypes*

When native plants were collected from their respective environments, marsh ecotypes produced much more vegetative mass ($t_{[58]} = 6.618$, $P = <0.001$, $P < 0.05$ after sequential Bonferroni correction; Table 1, Supplementary Figure 2A) and reproductive mass than the upland ecotype plants ($t_{[58]} = 4.764$, $P < 0.001$, $P < 0.05$ after sequential Bonferroni correction; Table 1, Supplementary Figure 2B). Fruit production was greater in marsh ecotype plants than upland ecotype plants ($t_{[58]} = 2.957$, $P = <0.005$, $P < 0.05$ after sequential Bonferroni correction; Table 1, Supplementary Figure 2C), and total seed mass was greater for the marsh ecotype ($t_{[58]} = 2.058$, $P < 0.05$, but not after sequential Bonferoni ($P < 0.10$; Table 1, Supplementary Figure 2D). Seed production or total seed number was similar ($t_{[58]} = 0.968$, $P = 0.337$; Table 1, Supplementary Figure 2E). Upland ecotype plants invested more in reproductive mass (1.7 X) relative to vegetative mass than marsh ecotype plants ($t_{[58]} = 6.885$, $P = <0.0001$; $P < 0.05$ after sequential Bonferroni correction; Table 1, Supplementary Figure 2F).

3.2 *Tolerance to upland and flooded conditions*

In the marsh, marsh ecotype plants had significantly higher survival than upland ecotype plants (58% vs. 3%, respectively, $\chi^2_{[1]} = 31.58$, $P < 0.0001$). In the marsh site, only one upland ecotype plant survived to the harvest date and its vegetative mass was 0.03 g and it did not produce any fruit. Of the marsh ecotype plants that survived to harvest, mean vegetative mass was 4.18 g (SE = 0.14) and 72% (of $n=18$) produced fruit, with a mean of 6.2 fruit per plant (SE = 0.4). In contrast, in the upland site, there was no significant difference in survival between the two ecotypes at the upland site. Both upland (93%) and marsh (100%) transplants had high survival.

For individuals surviving to the season's end in the upland site, the marsh ecotype produced significantly more above ground vegetative mass than the upland ecotype ($t_{[62]} = 9.615$, $P < 0.0001$, $P < 0.05$ after sequential Bonferroni correction; Table 1, Supplementary Figure 3A). Reproductive biomass (including seed and pericarp) was significantly greater in marsh than upland ecotypes ($t_{[62]} = 2.383$, $P < 0.05$, but not after sequential Bonferroni, $P < 0.07$; Table 1, Supplementary Figure 3B), although the number of fruits produced did not significantly differ between the ecotypes ($t_{[62]} = 0.437$, $P = 0.664$; Table 1, Supplementary Figure 3C), and there was no significant difference in total seed mass of the two ecotypes ($t_{[62]} = 1.422$, $P = 0.161$; Table 1, Supplementary Figure 3D). However, total seed number of upland ecotype plants was nearly two-fold greater than that of marsh ecotype plants ($t_{[62]} = 2.609$, $P < 0.012$, $P < 0.05$ after sequential Bonferroni correction; Table 1, Supplementary Figure 3E). Upland ecotype plants allocated approximately two-fold more to reproductive structures than marsh ecotype plants ($t_{[62]}$

= 3.349, $P < 0.0001$, $P < 0.05$ after sequential Bonferroni correction; Table 1, Supplementary Figure 3F).

3.3 Competition effects

Germination and survival rates of upland and marsh ecotypes were uniformly high and unaffected by neighbor distance or by intra- or inter-ecotype competitive interactions. The marsh ecotype produced slightly greater above ground biomass than the upland ecotype ($F[1,432] = 4.75$, $P = 0.03$; Fig. 2A) and both ecotypes in the upland site accumulated less biomass at lower neighbor distance, independent of neighbor ecotype ($F[1,432] = 17.09$, $P = 0.0001$). The upland ecotype produced significantly more reproductive biomass than the marsh ecotype under all competitive conditions ($F[1,420] = 85.33$, $P < 0.0001$; Fig. 2B). The interaction for reproductive biomass between ecotype and interplant effects was significant ($F[1,420] = 6.43$, $P = 0.012$). Orthogonal contrasts indicated that interplant effects only had a negative influence on the upland ecotype ($F[1,420] = 22.16$, $P < 0.0001$). The effect of the neighbor ecotype was not significant for either upland or marsh ecotypes (Fig. 2B).

Marsh ecotype plants produced significantly fewer fruit than upland ecotype plants under all competitive conditions ($F[1,423] = 194.56$, $P < 0.0001$; Fig. 2C). The low interplant treatment reduced fruit production for both ecotypes independent of neighbor ($F[1,432] = 13.98$, $P = 0.0002$). Marsh ecotype plants also produced significantly lower total seed mass than the upland ecotype plants ($F[1,432] = 104.47$, $P = 0.0001$; Fig. 2D). The interaction between ecotype and interplant distance effects was significant ($F[1,432] = 6.51$, $P = 0.011$) but orthogonal contrasts indicated that interplant distance only had a negative effect on the upland ecotype ($F[1,432] =$

21.14, $P < 0.0001$) as marsh ecotype plants had lower total seed mass independent of neighbor distance.

Total number of seeds produced was significantly less in the marsh ecotype compared to the upland ecotype with upland plants producing 2.5 – 10 fold more seed than the marsh ecotype ($F[1,377] = 322.10$, $P < 0.0001$; Fig. 2E). The three-way interaction of ecotype by neighbor by interplant distance was significant ($F[1,377] = 5.33$, $P = 0.022$) and orthogonal contrasts of marsh ecotype plants with marsh neighbors at high and low interplant distances and upland plants with high and low interplant distances of upland neighbors indicated a significant negative effect of these intra-ecotype competition treatments ($F[1, 377] = 4.11$, $P = 0.04$, and $F[1,377] = 21.97$, $P < 0.0001$), but only for the upland ecotype. The orthogonal contrast of marsh ecotype plants with upland neighbors showed no significant effect of neighbor distance, nor did the analogous contrast of upland ecotype plants with marsh neighbors. The ratio of reproductive biomass to total above ground plant biomass was significantly different between the upland and marsh ecotypes ($F[1,377] = 322.57$, $P < 0.0001$; Fig 2F) with upland plants investing relatively more in reproductive biomass than marsh plants independent of neighbor or neighbor distance, with no effect of the latter two factors on proportion allocation to reproduction.

3.4 Genetic correlations among allocation traits

Average seed mass was negatively genetically correlated with the total number of seed a plant produces ($r = -0.531$, $P = 0.0037$, $n = 28$ full sib families) and remained so following sequential Bonferroni correction. Seed mass was positively correlated with inter-node length ($r = 0.523$, $P = 0.004$, $n = 28$ full sib families), and remained so after sequential Bonferroni correction. Seed

number was only marginally significantly negatively correlated with above ground flooded stem mass ($r = -0.4723$, $P = 0.065$, $n = 16$ full sib families).

4. Discussion

The zero fitness of the upland ecotype under flooded marsh conditions is consistent with other reciprocal intraspecific transplant studies between upland and marsh sites (Linhart, 1973; Davy, et al., 1990) and is commonly found with interspecific transplant studies in salt marshes (e.g., Pennings et al., 2005). Thus, evolution to edaphic stresses associated with flooding determines range expansion into these areas. Trade-offs between adaptation to alternate environments have been observed in other studies of flood-tolerant species where flood tolerance is associated with decreased tolerance to dry conditions through decreased survivorship (Keeley, 1979) and growth (Davey et al., 1990; Carter and Grace, 1990). However the ability of *C. fasciculata* var. *macrosperma* to thrive in the marsh is not associated with decreased survivorship or growth reduction in upland habitat. Thus we conclude that lack of tolerance to the drier upland conditions is not a factor limiting var. *macrosperma*'s range to flooded conditions.

Our results indicate that intra-specific competition plays no significant role in the distribution of the marsh ecotype in the upland site and is consistent with those studies finding physical environmental factors to be more important than competition in delimiting range (Pennings and Callaway, 1992). Competitive interactions often affected both ecotypes in a similar fashion, in that both upland and marsh ecotypes responded to increased proximity to neighbor by an equal decrease in vegetative above ground biomass. In a direction opposite of that expected from

competition limiting var. *macrosperma* from the upland site, increased proximity to neighbor only resulted in decreased reproductive biomass in the upland ecotype. In addition, inter-ecotype competitive interactions did not result in significant decreases in seed production although intra-ecotype competition did result in decreased seed production, especially for the upland ecotype. Allocation patterns to reproduction versus vegetative biomass were unchanged by neighbor distance for both ecotypes.

Upland plants allocate proportionally more to reproductive structures than vegetative structures relative to the marsh ecotype. These results are consistent across experimental and field collected plants. Native marsh ecotype plants produced about seven times more vegetative mass than native upland plants in their respective habitats. However, both ecotypes produced about the same number of seed in their respective habitats (Table 1). Although the marsh ecotype outperformed the upland ecotype in terms of vegetative biomass in the upland site, it suffered in terms of growth relative to its native marsh environment. The cumulative effect of the marsh ecotype's reduced growth in the upland site, the greater allocation to vegetative biomass versus reproductive biomass, and the allocation of resources to fewer larger seed, resulted in the marsh ecotype producing many fewer seed, about half as many as the upland ecotype in the upland habitat. Since both ecotypes are annual plants, total seed production should be a critical factor in determining the invasiveness of large seeded var. *macrosperma* into the upland habitat. This is an important caveat. We do not demonstrate with long-term demographic data that seed number is more important than seed size but there is increasing evidence that seed number is an important contributor to propagule pressure and invasive success (Holle and Simberloff, 2005; Jelbert et al., 2015).

Both large seed mass and increased allocation to vegetative structures in *C. fasciculata* var. *macrosperma* are likely adaptations to the marsh environment. The large seeds of var. *macrosperma* may be analogous to food reserve organs, such as rhizomes and tubers, found in many flood-tolerant species (Crawford, 1992). The two-fold greater shoot elongation rate observed in marsh versus upland ecotypes (Fenster, 1997) may be associated with increased seed mass and cotyledon size which provide greater carbohydrate reserves and is observed across many species (Houghton et al., 2013). Photosynthetic carbon gain can be significantly reduced following submergence (Ridge, 1987) and many flood-tolerant species respond to flooding with shoot elongation (Aston and Bradshaw, 1966; Blom, 1990; Laan and Blom, 1990). Thus both large seed mass and increased allocation to vegetative structures in the marsh ecotype are likely evolutionary adaptations to minimize shoot submergence in the flooded marsh habitat. It is likely that trade-offs play a major role in determining plant distributions in the face of flooded versus non-flooded habitats (Huber et al., 2009) as well as many other environmental challenges (Reich et al., 2003).

In order for the alternative allocation strategies to delimit the ranges of the two ecotypes, they must be associated with genetic trade-offs due to negative pleiotropy. Thus if negative genetic correlations exist between energy invested into seed mass versus seed number, or between performance in the marsh versus performance in the upland environment, then the allocation patterns documented here will reflect genetic constraints to the evolution of traits favorable in both upland and marsh habitats. Despite our limited sample size of maternal F₃ full-sib families, we found a clear pattern of negative genetic correlations that both explain the allocation patterns

we observed and the delimitation of the two ecotypes. Greater seed mass, a likely candidate for an adaptation to the marsh environment, is positively genetically correlated to performance under flooded conditions in terms stem elongation, the latter trait being important for getting the shoot above standing water at high tide.

However, seed mass is negatively genetically correlated to seed number. Thus one can envision that the invasion of the marsh habitat by the upland ecotype may have been associated with positive directional selection for seed mass, because of the reasons listed above. The evolution of increased seed mass is associated with the reduction of seed number, because of the negative pleiotropy underlying the two traits. In a best of all possible worlds, seed characteristics of the marsh ecotype would include both large and numerous seed, but the genetics of the organism constrains the path to adaptation to large and fewer seeds. Furthermore, the reduced relative allocation to reproduction may result in shunting of resources to root expansion to combat low oxygen and nutrient uptake in saturated soils. Trade-offs between seed size and number are common and have been observed for both natural populations (Robertson et al., 1994;) and domestic crops (Gambin and Borras, 2010).

This study demonstrates two factors may play an important role in the distribution of the two ecotypes. First the upland ecotype is intolerant to flooded conditions. Second there is an underlying genetic basis of negative pleiotropy that results in the very same adaptations to the marsh habitat, large seeds and increased allocation to vegetative growth through rapid stem elongation, results in poor reproductive performance of the marsh ecotype in the upland environment. This system, with two fully cross-compatible ecotypes occupying extremes of

flooding conditions, may be a model system, in general, for quantifying the genetic mechanisms of range expansion and limitation and, in particular, for the study of the evolution of flood tolerance.

Acknowledgements

We thank S. Baughman, J. Berg, D. Campbell, L. Douglas, M. Dudash, I. Forseth, the editors J. Vermaat and E. Gross and several anonymous reviewers for providing valuable comments on this manuscript, J. Brown A. Gearhard and A. Klinowski for field assistance, and D. Foster and the staff of the Chesterfield County Park, VA for use of field sites. Funding was provided by NSF to C. Fenster.

TEF planned and carried out the experiments, conducted the analyses and contributed to the writing of the manuscript. KBG contributed to the literature review, edited the manuscript, and produced figures. CBF help plan the experiments, provided advice for the analyses and contributed to the writing of the manuscript.

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Figure 1. Crossing regime between upland *Chamaecrista fasciculata* and flood tolerant *C. fasciculata* var. *macrosperma*. Forty parents were randomly and reciprocally crossed to produce F_1 that were in turn randomly and reciprocally crossed to produce 40 F_2 plants that were also randomly and reciprocally crossed to create 100 F_3 that were crossed to construct 28 F_3 maternal families. From these F_3 families, progeny were grown under upland (from 28 families) and flooded conditions (progeny from a subset of 16 of the 28 families), respectively.

Figure 2. Results of the competition experiment with upland and marsh ecotypes of *Chamaecrista fasciculata* in the upland site (mean + 2SE). Dark bars indicate the marsh ecotype and light bars indicate the upland ecotype, grown paired with a given neighbor ecotype (vs. marsh or vs. upland) at high or low interplant distances.

Figure 1.

Figure 2.

Table 1. Growth and reproductive measures (mean + 2SE) of plants in the reciprocal transplant experiment and upland and marsh ecotypes from native populations of *Chamaecrista fasciculata*. The reciprocal transplant experiment represents both ecotypes grown from seed in the upland site. All biomass measurements are in g. Means followed by different letters for a given experiment are significantly different from each other at the $P < 0.05$ level following sequential Bonferroni correction. Note that reproductive biomass includes seed, fruit and peduncle mass.

Trait	Transplant experiment (Upland Site)		Native populations	
	Marsh Ecotype	Upland Ecotype	Marsh	Upland
Vegetative biomass	10.73 _A (± 1.49)	1.74 _B (± 0.75)	9.95 _A (2.00)	1.15 _B (1.83)
Reproductive biomass	1.75 _A (± 0.40)	1.07 _B (± 0.40)	5.39 _A (1.31)	1.19 _B (1.24)
Fruit number	11.50 _A (± 2.05)	10.50 _A (± 4.05)	16.25 _A (3.59)	8.69 _B (3.70)
Seed biomass	0.40 _A (± 0.14)	0.28 _A (± 0.11)	2.37 _A (0.56)	0.45 _A (1.77)
Seed number	16.16 _A (± 5.26)	31.54 _B (± 11.41)	66.71 _A (15.68)	55.71 _A (15.46)
Reproductive:total biomass	0.13 _A (± 0.03)	0.31 _B (± 0.06)	0.34 (0.03) _A	0.50 _B (0.03)