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NATURAL SELECTION AND GENETIC CONSTRAINTS ON FLOWERING PHENOLOGY IN AN INVASIVE PLANT

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Theory suggests that the contemporary evolution of local adaptation may increase the rate of biological invasion, yet natural selection has rarely been measured in invasive species. A recently published model predicted that latitudinal variation in the strength of stabilizing selection on two correlated traits—flowering time and size—can result in local adaptation during plant invasion but implicitly assumed that population × environment interactions are weak. We tested for stabilizing selection and the effect of growing environment on 13 populations of the invasive plant *Lythrum salicaria* sampled along a latitudinal gradient. Plants were grown under uniform glasshouse conditions and in a 3-year common garden field experiment at midlatitude. Consistent with model assumptions, the ranking of population means was highly correlated across the 3 years, and with glasshouse measurements, despite significant interannual differences in flowering time and vegetative size. As predicted, the first principal component (PC1) of flowering time and size was under stabilizing selection, and directional selection was detected on PC2, thus favoring plants that flowered earlier at a larger size. Our results demonstrate the value of using a priori models of natural selection to guide hypothesis testing and support the prediction that fitness trade-offs can constrain adaptive evolution in invasive species.

Keywords: biological invasion, *Lythrum salicaria*, field experiment, rapid evolution, fitness trade-off, principal components analysis.

Introduction

The spread of invasive species often occurs along latitudinal gradients in climate, and this may result in natural selection for local adaptation. In plants, reproductive timing is an important adaptation to latitudinal variation in environmental conditions, enabling populations to optimize flowering and seed production to the most favorable periods of the growing season. Indeed, genetic differentiation in time to first flower has often been identified in common garden studies of native plant populations (e.g., Kittelson and Maron 2001; Eckhart et al. 2004; Lempe et al. 2005; Franke et al. 2006), and reciprocal transplant experiments have demonstrated natural selection on flowering phenology (e.g., Schemske 1984; Fox 1989; Bennington and McGraw 1995; Nagy 1997; Nagy and Rice 1997; Etterson and Shaw 2001; Etterson 2004; Griffith and Watson 2005; Hall and Willis 2006; Franks et al. 2007). In contrast, the extent to which the contemporary evolution of flowering phenology contributes to local adaptation in invasive populations is not well understood.

Common garden experiments of introduced plant species have demonstrated latitudinal and altitudinal clines in flowering phenology and several other life-history traits (reviewed in Alexander et al. 2009; Colautti et al. 2009). In some cases, geographical clines observed among introduced populations match those documented in the native range (e.g., Weber and Schmid 1998; Maron et al. 2004; Leger and Rice 2007; Montague et al. 2008; Alexander et al. 2009). Parallel clines are consistent with a scenario of local adaptation in response to environmental gradients (e.g., length of growing season) that are similar between the native and introduced range. However, measurements of natural selection on ecologically relevant traits that form latitudinal clines have rarely been made in introduced plants (but see Maron et al. 2007). Other mechanisms such as climate matching could account for phenotype-environment correlations (Maron et al. 2004; Keller et al. 2009). For example, a latitudinal cline in the native range could be reproduced in the introduced range through parallel introductions. Therefore, the link between natural selection and clinal variation in invasive plants remains unclear.

The dearth of direct measurements of natural selection in invasive plant populations is surprising because several hypotheses make clear predictions about natural selection during biological invasion. For example, introduced plant species may experience weaker selection for defenses against specialist herbivores (Blossey and Nötzold 1995; Keane and Crawley 2002; Müller-Schärer et al. 2004), stronger selection for plasticity due to environmental heterogeneity (Baker 1965; Parker et al. 2003; Richards et al. 2006; Hulme 2008), or selection for increased self-fertilization when mating opportunities are limited (Baker 1955; van Kleunen et al. 2008; Barrett 2010). Furthermore, statistical methods have been employed for almost 3 decades to measure selection in wild populations (Lande and Arnold 1983; reviewed in Endler 1986), and measurements of the strength and form of selection on diverse traits

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have now been made in many organisms (Kingsolver et al. 2001), including dozens of plant species (reviewed in Fenster et al. 2004; Herrera et al. 2006; Harder and Johnson 2009). Direct measurements of natural selection on phenotypic traits provide a powerful test of adaptive hypotheses and are therefore crucial for understanding evolutionary processes in invasive species.

Here we report on field and glasshouse experiments that investigate genetic variation and natural selection on flowering phenology and components of reproductive fitness in the invasive wetland perennial *Lythrum salicaria* L. (purple loosestrife, Lythraceae). Our previous work on this species involved glasshouse comparisons and controlled crosses of multiple populations sampled along a 1200-km transect from Maryland to central Ontario, eastern North America (Montague et al. 2008; Colautti et al. 2010*a*). Population comparisons under uniform growing conditions revealed latitudinal clines in time to first flower and plant size at flowering (Montague et al. 2008; Colautti et al. 2010*a*) and a strong genetic correlation between these traits (see fig. 2 and supplementary material in Colautti et al. 2010*a*). Reciprocal crosses confirmed the presence of this genetic correlation and also failed to detect significant maternal or paternal effects on time to first flower and plant size at flowering (Colautti et al. 2010*a*).

To explain the latitudinal clines observed among introduced populations, Colautti et al. (2010*a*; fig. 1) developed and applied a Lande and Arnold (1983) model of stabilizing selection with a genetic constraint on time to first flower versus vegetative size at flowering owing to a trade-off between these traits (hereafter, "time to first flower versus size" or "FTS" model). Because these traits were highly correlated, a principal components analysis (PCA) was used to identify the direction of greatest genetic variance-covariance, with larger values of the first principal component of these traits (PC1) representing larger plants that flower later; larger values of PC2 represent larger plants that flower earlier (fig. 1). Population means and patterns of genetic variation in PC1 were consistent with model predictions, but genetic variation for PC2 of time to first flower and vegetative size was not tested directly. Moreover, this study (i.e., Colautti et al. 2010*a*) did not test the predictions of (i) stabilizing selection on PC1 or (ii) directional selection on PC2 (fig. 1) by directly measuring natural selection. Stabilizing selection on PC1 would be predicted because plants flowering early are constrained to be smaller (i.e., small PC1 values) and therefore have fewer resources to mature seeds, whereas larger plants flower later (i.e., large PC1 values) and do not have sufficient time to mature seeds before the end of the growing season. Directional selection on PC2 is predicted because large, early-flowering plants (i.e., large PC2 values) should have the highest fitness at all latitudes.

The major objective of this study is to empirically validate the FTS model using field and glasshouse comparisons of populations of *L. salicaria* using PCA of measurements of time to first flower and vegetative size at flowering (see fig. 1). We included multiple populations sampled along a latitudinal cline to expand the phenotypic distribution of seed families upon which to measure natural selection. We estimated selection primarily through reproductive fitness because in an expanding population, fecundity is likely to be under strong selection relative to intraspecific competitive interactions and long-term survival (e.g., see Stearns 1976; Lankau et al. 2010). However, we also measured fitness through survival and reproduction using ASTER models (Geyer et al. 2007; Shaw et al. 2008) for an alternative assessment of selection on phenological and size traits.

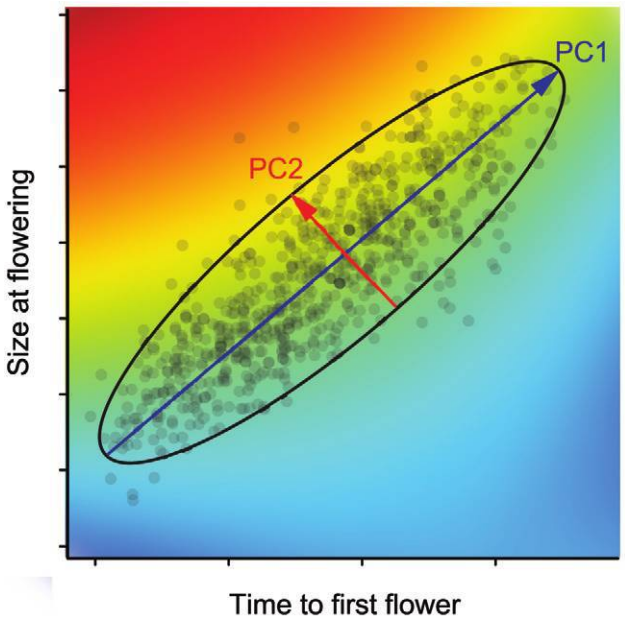

Fig. 1 Lande and Arnold (1983) model of natural selection on time to first flower and size at flowering with a genetic constraint. The fitness "landscape" is represented by a color gradient from late-flowering, small phenotypes with low fitness (blue) to early-flowering, large phenotypes with higher fitness (red). Genotypes (gray dots) are constrained by a positive genetic correlation between time to first flower and size, defined by the first principal component of these traits (PC1, blue vector), with larger values representing larger plants that flower later. The second principal component (PC2) defines genetic variation orthogonal to the genetic constraint, with larger values representing genotypes with higher fitness (i.e., large, early-flowering plants).

In our study of *L. salicaria* populations we specifically addressed three main questions: (i) Does the mean time to first flower and vegetative size at flowering differ between field and glasshouse environments and among 3 years in the field? Large changes in the rank-order of population mean flowering time and size, resulting from interyear differences in growing conditions, would be contrary to our prediction that clinal variation in population means represents evidence of local adaptation to latitude. (ii) What are the levels of standing genetic variation within and among populations for PC1 and PC2 under field conditions? The FTS model assumed, and glasshouse data (Colautti et al. 2010*a*) demonstrated, ample genetic variation for PC1, but genetic variation for PC2 was not investigated. Genetic variation for PC2 should be limited, according to the FTS model, because natural selection should efficiently eliminate genotypes that flower late at a small size. (iii) Is there evidence for stabilizing selection on the PC1 of time to first flower and size and directional selection on PC2, as predicted by the FTS model?

Material and Methods

Study Species

Lythrum salicaria is an insect-pollinated, outcrossing, autotetraploid, perennial herb that is native to Eurasia and was introduced to North America at the end of the eighteenth century (Thompson et al. 1987). Herbarium records indicate a progression of colonization fronts, beginning in the early twentieth century, north and south along the eastern seaboard and northwest into central Ontario, Canada (Thompson et al. 1987). Studies using molecular markers suggest multiple introductions to North America (Houghton-Thompson et al. 2005; Chun et al. 2009), but the spread of *L. salicaria* into central Ontario is relatively recent, occurring over the past 50 years. Colonization of new sites by *L. salicaria* occurs exclusively by seed dispersal because clonal growth is limited to ramet production from a common rhizomatous genet (Mal et al. 1992; Yakimowski et al. 2005).

Experimental Design

We chose 13 of the 25 populations investigated by Montague et al. (2008), where collection details and field data are presented. Populations were chosen to represent a latitudinal gradient from Timmins, Ontario (48.48°N, 81.30°W) to Easton, Maryland (38.75°N, 75.99°W). Eight seeds from each of 20 families from each of the 13 populations were sown into individual 2 × 2-cm plug trays at the University of Toronto glasshouse facility (43.66°N, 79.40°W) on May 27, 2005. Seedlings were grown in the glasshouse for 40 d and then moved to the Koffler Scientific Reserve at Jokers Hill (hereafter, "field site"; 44.03°N, 79.54°W), where they were placed in the shade for 2 d prior to transplant. This field site lies at the center of the latitudinal cline sampled by Montague et al. (2008) and studied in Colautti et al. (2010*a*). On July 8 we transplanted three seedlings from each seed family into each of two experimental blocks (six seedlings total) in a tilled marsh.

Plants were left to establish over the summer of 2005, and each subsequent year (2006–2008) we monitored plants for the date of first flowering. We sprayed plants with Dursban 2E by DowElanco (active ingredient Chlorpyrifos 240 g/L), administered at 4 mL/L once each year in mid-June to reduce the incidence of herbivorous insects introduced to eastern North America for biological control of *L. salicaria* (Malecki et al. 1993). On the day of first flowering we recorded the height of the vegetative portion of the primary stem, measured from the soil surface to the base of the inflorescence (hereafter, "vegetative size"). At the end of the growing season in September 2007 and 2008, all aboveground growth was harvested and dried to constant weight before the biomass of vegetative and reproductive structures (hereafter, "vegetative biomass" and "reproductive biomass," respectively) was measured. Reproductive biomass correlates strongly with fruit set in natural populations (ln-ln regression: $N = 682$, $r = +0.93$, $P < 0.001$; Montague et al. 2008). In 2007 we measured stem length of the five longest stems on each plant to assess whether height of the primary stem was a reliable predictor of total vegetative growth. We compared population means from our earlier glasshouse experiment (Colautti et al. 2010*a*) with those measured in our field experiment to test for differences in vegetative size and time to first flower due to the contrasting growing conditions.

Interyear and Field-Glasshouse Comparisons

We used Pearson product-moment correlations of seed family and population means to test for correlations of time to first flower and vegetative size among years in the field experiment (2006–2008) and linear regression to test the ability of seed family and population means in the glasshouse experiment to predict time to first flower and size at flowering under field conditions.

In the field experiment, we statistically tested for differences in time to first flower and size at flowering among years, seed families and populations, and for family × year and population × year interactions among seed families and population means as follows:

$$\text{trait} = \text{block} + \text{year} + \text{pop} + \text{fam(pop)} + (\text{pop} \times \text{year}) + [\text{fam(pop)} \times \text{year}] + \varepsilon,$$

where trait was either days to flower or size at flowering (i.e., two separate statistical models), measured in an experimental block, in a particular year, on an individual plant from a sampled seed family (fam) nested within a sample population (pop), with error ε. Seed family and family × year interactions were treated as random effects, with all other factors fixed. We used SAS 9.1 (SAS Institute, Cary, NC) for all statistical analyses unless otherwise noted. We did not transform days to first flower or vegetative size at flowering because log transformations failed to improve the normality of these traits within populations. For this analysis we used the MIXED procedure, with a Satterthwaite approximation for the degrees of freedom for fixed effects.

Quantifying Standing Genetic Variation

We tested assumptions and predictions of the flowering time versus size (FTS) model as follows. We used principal components analysis (PCA) to recharacterize these traits as two orthogonal principal component "traits" for each year. Thus, the first principal component (PC1) represents the direction of greatest variance-covariance and was predicted to be under stabilizing selection with larger values representing larger plants that begin flowering later. Larger values of PC2 represent plants that flower earlier at a larger size and therefore should be under strong directional selection. Factor loadings in a PCA of any two traits are always the same magnitude (i.e., 0.707) when variances are standardized, and therefore they did not differ among years. In addition, we averaged PC1 and PC2 estimated from each year to combine measurements across years (hereafter, $PC1_{avg}$ and $PC2_{avg}$).

We investigated the occurrence of significant standing genetic variation for PC1 and PC2 in the field experiment using two statistical tests. First, we used a mixed model of either $PC1_{avg}$ or $PC2_{avg}$, with experimental block as a fixed effect

and population and family effects as random factors. In contrast to the previous statistical model in which population was a fixed factor, we were interested in testing whether significant genetic variation is maintained within populations in eastern North America and thus it is appropriate to treat population as a random factor. It is important to note that we calculated PC1 and PC2 for this study differently from PC1 and PC2 in Colautti et al. (2010*a*), in which inflorescence length was added as an additional trait in the PCA to test a null model of population divergence. PC1 should be very similar between the two calculations, but PC2 will be different because inflorescence length loaded heavily on PC2 in the earlier study.

As a second test for genetic variation in PC1 and PC2, we used a single statistical mixed model to test the dimensionality of the variance-covariance matrix among populations and seed families using factor-analytic models in the MIXED procedure. Since each variance-covariance matrix contains two traits, a significant second component of the factor-analytical structures (i.e., population and seed family) identifies significant variation in PC2 among populations or among seed families.

Measuring Natural Selection on Time to First Flower and Size

We estimated relative fitness by dividing each individual's reproductive biomass by the overall mean and calculated standardized selection gradients using least squares linear regression following Lande and Arnold (1983). Because our measure of relative fitness (i.e., reproductive biomass) was log normally distributed, we used the following generalized linear model (GENMOD) procedure with a Poisson distribution and log-link function to test the significance of linear and nonlinear natural selection and to determine whether it differed between 2007 and 2008:

$$\text{relative fitness} = \text{PC} + \text{year} + \text{PC}^2 + (\text{year} \times \text{PC}) + (\text{year} \times \text{PC}^2).$$

The same model was used on all individuals (hereafter, "phenotypic selection") and family means (hereafter, "genotypic selection"). As an alternative estimate of the general form of natural selection on the principal components averaged across years (i.e., $PC1_{avg}$ and $PC2_{avg}$), we summed reproductive biomass measured over both years and fitted cubic splines separately to each trait using a general additive model in R 2.8.1 (R Foundation, Vienna, Austria) following Schluter (1988).

Measuring fitness through reproduction alone can be problematic because it ignores plants that do not survive or flower. To estimate total fitness through survival in 2006–2008 and reproduction in 2007–2008, we used the ASTER package in R (Geyer et al. 2007; Shaw et al. 2008). ASTER uses maximum likelihood and accounts for dependencies among different components of fitness (i.e., survival and reproduction), which may have different probability distributions (i.e., Bernoulli and Poisson, respectively). Following model descriptions in Shaw et al. (2008), survival in 2006 was dependent on the model's "root" node, and survival to 2007–2008 was dependent on survival in previous years, with Bernoulli probability distributions. Survival to first flowering within growing season acted as predecessor "nodes" for the probability that a plant flowered in that year (2006–2008), also with Bernoulli probabilities. These in turn formed predecessor nodes for seed production in 2007 and 2008, treated as a truncated Poisson distribution by rounding reproductive biomass to the nearest gram. The ASTER model used was as follows:

$$\text{fitness} = \text{block} + \text{PC1}_{\text{avg}} + \text{PC2}_{\text{avg}} + \text{PC1}_{\text{avg}}^2 + \text{PC2}_{\text{avg}}^2 + \frac{1}{2}(\text{PC1}_{\text{avg}} \times \text{PC2}_{\text{avg}}),$$

where fitness was a function of experimental block and the linear and nonlinear coefficients for each of $PC1_{avg}$ and $PC2_{avg}$. To compare with multiple regression and cubic spline models, we used ASTER to test for directional, stabilizing, and disruptive selection separately for PC1 and PC2.

Results

Trait Comparisons among Years in the Field

There was considerable variation in time to first flower and size at flowering among populations in the field experiment. Flowering over all years began as early as June 25 and as late as September 6 at sizes ranging from 48.5 cm to 192 cm. Experimental blocks did not differ significantly in mean time to first flower or size, but there were significant differences in both traits among years, populations, and seed families (table 1).

Population × year interactions were highly significant for both traits, indicating that year affected time to first flower and size differently in each population. However, interannual correlations for time to first flower were also highly significant

Table 1

Results of a Statistical Mixed Model of Measurements of *Lythrum salicaria* Growing in a Field Experiment, 2006–2008, Koffler Scientific Reserve, with Numerator (ndf) and Denominator (ddf) Degrees of Freedom

Effect	ndf[a]	ddf	F (or χ^2)	P
Time to first flower:				
Block	1	2170	.35	.56
Year	2	2055	63.49	<.001
Population	12	255	86.47	<.001
Season × population	24	2052	3.62	<.001
Seed family	1	...	(136.2)	<.001
Vegetative size:				
Block	1	2139	.17	.68
Year	2	2042	49.47	<.001
Population	12	226	110.03	<.001
Year × population	24	2038	3.03	<.001
Seed family	1	...	(208)	<.001

Note. Parentheses indicate χ^2 values of LRTs. Seed family × year interactions were nonsignificant (LRT: $P > 0.9$) and were therefore not included in the final models.

[a] Difference in number of model parameters for likelihood ratio test (LRT) of seed family, and Satterthwaite approximation for the degrees of freedom for F-tests of all other factors.

($P < 0.001$) and ranged from $r = +0.94$ to $r = +0.99$ among population means and $r = +0.82$ to $r = +0.88$ among seed families (fig. 2). Likewise, interannual correlations for vegetative size were also highly significant ($P < 0.001$) and ranged from $r = +0.98$ to $r = +0.99$ among population means and $r = +0.89$ to $r = +0.92$ among seed families. Spearman's rank correlations were similarly high among populations (days to flower: $\rho = +0.92$; vegetative size: $\rho = +0.98$) and family means (days to flower: $\rho = +0.79$; vegetative size: $\rho = +0.89$), indicating a general consistency in the ranking of time to first flower and size of flowering among years. Most of the changes in ranking occurred among early-flowering populations (fig. 2).

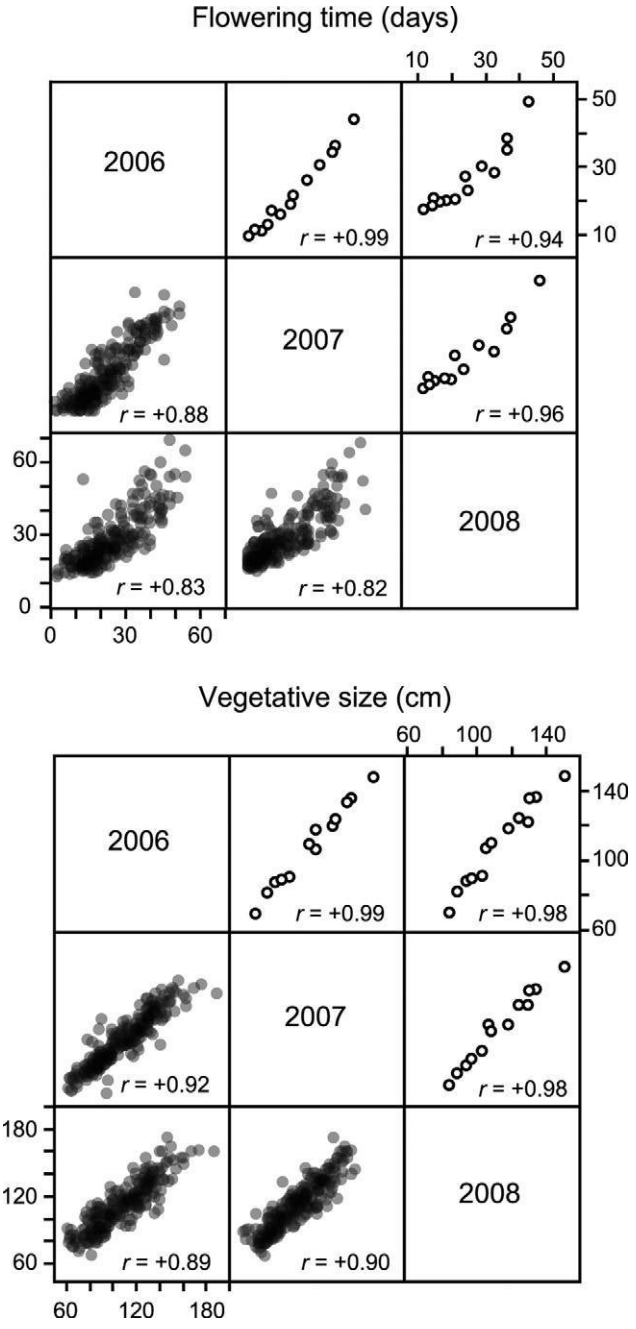

Fig. 2 Interyear correlations of time to first flower (top panel) and size at flowering (bottom panel) in 13 populations (above diagonal) and 240 seed families (below diagonal) of *Lythrum salicaria* grown at the Koffler Scientific Reserve. Populations were sampled along a latitudinal gradient in eastern North America.

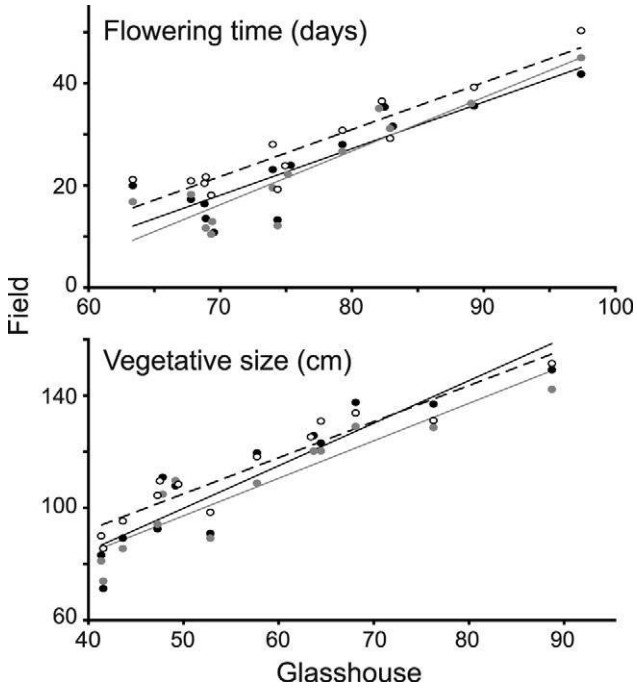

Fig. 3 Comparison of population mean time to first flower (top panel) and vegetative size (bottom panel) measured on plants from 13 *Lythrum salicaria* populations sampled along a latitudinal gradient in eastern North America and grown in a glasshouse (*X*-axes) and a field experiment at the Koffler Scientific Reserve (*Y*-axes). Separate regressions are shown for field measurements taken in 2006 (black dots and solid black line; days: $R_2 = 0.790$, $P < 0.001$; size: $R_2 = 0.847$, $P < 0.001$), 2007 (gray dots and solid gray line; days: $R_2 = 0.845$, $P < 0.001$; size: $R_2 = 0.857$, $P < 0.001$), and 2008 (white dots and dashed black line; days: $R_2 = 0.873$, $P < 0.001$; size: $R_2 = 0.900$, $P < 0.001$). We measured time to first flower as the number of days from sowing in the glasshouse (June 2, 2004) and from the earliest date of flowering in the field (June 25).

Trait Comparisons between the Field and Glasshouse

The mean vegetative size at flowering of populations in the glasshouse ranged from 41.3 cm to 91.5 cm, a decrease of 30.0–59.9 cm compared with the same populations grown under field conditions (fig. 3). Additionally, growth in the glasshouse experiment was largely restricted to vertical extension of the primary stem, while plants grown in the field experiment added large secondary stems in addition to growing taller (R. I. Colautti, unpublished data). Instead of direct comparisons of the average time to first flower between experiments, correlations that contrast growth from seed sowing in 2004 in the glasshouse, with regrowth from rootstock after a year of establishment in the field in 2006, are more informa-

tive. However, there were strong correlations ($P < 0.001$) among population mean time to first flower in the glasshouse with each of the 3 years at the field site (2006: $r = +0.89$; 2007: $r = +0.92$; 2008: $r = +0.93$), and the rank order of population means, although weaker (2006: $\rho = +0.78$; 2007: $\rho = +0.80$; 2008: $\rho = +0.79$), was still highly significant ($P < 0.001$). Similar to interyear differences in trait expression, field-glasshouse differences in the ranking of time to first flower occurred mostly among early-flowering populations (fig. 3). Also consistent with interyear variation, the ranking of population mean vegetative size at flowering was significantly ($P < 0.001$) correlated across the two experiments (2006: $r = +0.92$; 2007: $r = +0.93$; 2008: $r = +0.95$), and despite the large variation in average size, the rank order was stronger for vegetative size (2006: $\rho = +0.95$; 2007: $\rho = +0.96$; 2008: $\rho = +0.95$) than for time to first flower.

Standing Genetic Variation for Time to First Flower and Size

We detected significant genetic variation for both principal components ($PC1_{avg}$ and $PC2_{avg}$) in both field and glasshouse environments (table 2). The proportion of total phenotypic variance explained by differences among populations (V_{pop}) was also higher for $PC1_{avg}$ than for $PC2_{avg}$, while estimates of among-family variance (V_{fam}) were similar (table 2). Estimates of genetic variance among populations from the glasshouse experiment were also highly significant and comparable to field estimates, except that there was a much larger difference in standing genetic variation among families within populations. Factor-analytical tests of genetic variance for PC2 were significant for seed families (2006: $\chi^2 = 72.2.$, df = 1, $P < 0.001$; 2007: $\chi^2 = 80.0$, df = 1, $P < 0.001$; 2008: $\chi^2 = 73.1$, df = 1, $P < 0.001$) but not for populations (2006: $\chi^2 = 3.5$, df = 1, $P = 0.06$; 2007: $\chi^2 = 2.79$, df = 1, $P = 0.09$; 2008: $\chi^2 = 0.6$, df = 1, $P = 0.43$). Therefore, there was evidence for significant genetic variation for both PC1 and PC2 within populations in both the glasshouse and the field, and genetic variation among population means was higher for PC1 than for PC2.

Table 2

Proportion of Phenotypic Variation of Two Principal Component "Traits" Within and Among 13 Populations of *Lythrum salicaria* Field Grown, Koffler Scientific Reserve, and 20 Populations Glasshouse Grown, University of Toronto

Trait	V_{pop}	V_{fam}	V_{res}
Field:			
$PC1_{avg}$	.706	.040	.253
$PC2_{avg}$	.258	.069	.673
Glasshouse:			
$PC1_{avg}$	.521	.136	.387
$PC2_{avg}$	.214	.084	.620

Note. Variance components are calculated from a mixed model and describe divergence among populations (V_{pop}), variation among seed families within populations (V_{fam}), and residual variation (V_{res}). V_{pop} and V_{fam} were both highly significant effects in likelihood ratio tests (df = 1, $P < 0.001$) for each principal component in each experiment.

Table 3

Selection Gradients for the First Principal Component (PC1) of Time to First Flower and Vegetative Size in *Lythrum salicaria*, 2-yr Field Experiment, Koffler Scientific Reserve

Parameter	Estimate	χ^2	P
Phenotypes:			
PC1	.251	125.4	<.001
PC1 × PC1	−.391	111.5	<.001
Year	−.524	58.09	<.001
Year × PC1	−.091	3.63	.057
Year × PC1 × PC1	.170	1.29	.257
Seed family means:			
PC1	.334	48.2	<.001
PC1 × PC1	−.415	37.4	<.001
Year	−.484	11.06	<.001
Year × PC1	−.160	1.1	.294
Year × PC1 × PC1	−.162	.03	.854

Note. We calculated estimates of linear and nonlinear selection coefficients from standard linear and nonlinear selection models following Lande and Arnold (1983), with significance tested by a generalized linear model (χ^2). Estimates of year effects show the difference in the 2007 growing season relative to 2008.

Selection on Time to First Flower and Size

We detected significant stabilizing selection on the first principal component of time to first flower and vegetative size at flowering (PC1) in the generalized linear models of selection on phenotypes and seed family means (table 3). Although there was also a significant linear selection gradient (table 3), relative fitness reached a maximum at intermediate values of PC1, indicating that plants flowering at an intermediate time and size had the highest fitness (fig. 4). This result was confirmed by cubic spline analysis (fig. 4) and by using the ASTER model (fig. 5). On average, plants in the 2008 growing season had higher reproductive fitness relative to the 2007 season, resulting in a significant "year" effect. However, the shape of natural selection on PC1 did not change significantly between years (table 3).

In contrast to PC1, the second principal component of time to first flower and vegetative size (PC2) represents plants flowering later at a smaller size (smaller values of PC2) or earlier at a larger size (larger values of PC2). Results of the generalized linear model of PC2 indicated that plants flowering earlier at a larger size had the highest fitness, with no evidence for stabilizing selection (table 4). Similar to PC1, average fitness was higher in 2008, and the shape of natural selection on PC2 did not change significantly between years (table 4).

Because selection on PC1 and PC2 did not differ significantly among years, we summed reproductive biomass to measure selection on $PC1_{avg}$ and $PC2_{avg}$ using cubic spline analysis. The fitness function for $PC1_{avg}$ resembled a bell-shaped curve characteristic of stabilizing selection (fig. 4), while the shape of selection on $PC2_{avg}$ was more consistent with directional selection in which phenotypes that flowered earlier at a larger size have exponentially greater fitness (fig. 4). Note that the nonsignificant quadratic term for PC2 in table 3 is consistent with the shape of the best-fit curve in figure 4. This is because a linear fit on a Poisson scale of relative fitness (the generalized linear model results in table 3) translates

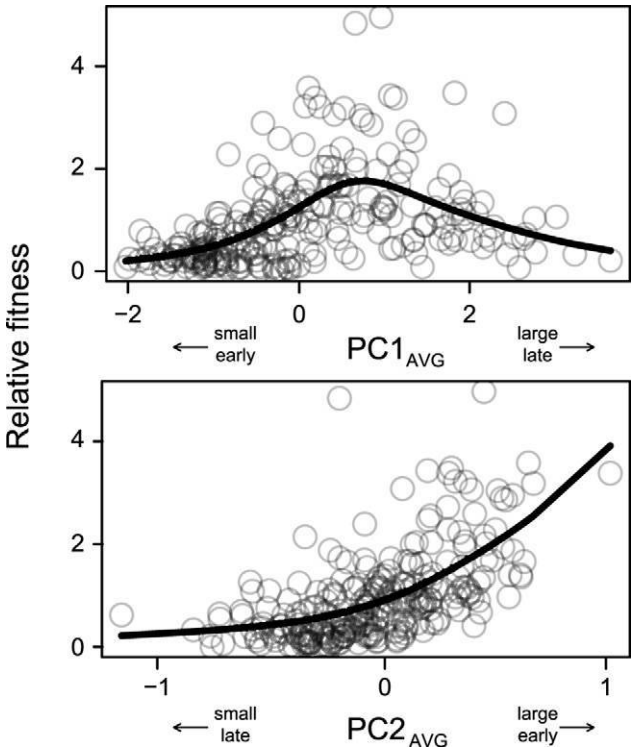

Fig. 4 Estimates from the Koffler Scientific Reserve field experiment on *Lythrum salicaria* of natural selection on $PC1_{avg}$, a composite measure of the genetic constraint on time to first flower and size at flowering across 3 years (top panel), or $PC2_{avg}$, the orthogonal vector to $PC1_{avg}$ representing variation against the genetic constraint on time to first flower and size at flowering (bottom panel). We measured total dry reproductive biomass summed in 2007 and 2008 in 242 seed-family means from 13 populations. Fitness curves were estimated by cubic spline. Larger values of $PC1_{avg}$ represent plants that flowered later at a larger size.

to a curvilinear fit on the untransformed scale of relative fitness (*Y*-axis in fig. 4).

Estimates of natural selection using ASTER were qualitatively similar to estimates from multiple regression and cubic spline and indicated stabilizing selection on PC1 ($z = 290$, $df = 1$, $P < 0.001$) and nonlinear directional selection on PC2 ($z = 159$, $df = 1$, $P < 0.001$) as well as a significant interaction term ($z = 21$, $df = 1$, $P < 0.001$). The similarity is probably the result of the relatively high survival of plants among years: of the 940 seedlings planted in 2005, 93% survived to 2006, 99% to 2007, and 98% to 2008. Of the plants that survived to each year, 99%, 83%, and 78% flowered in 2006, 2007, and 2008, respectively. Thus, most fitness variation among individual plants resulted from variation in reproductive output rather than survival to flowering.

Discussion

In a previous study involving a glasshouse experiment on *Lythrum salicaria*, we demonstrated clinal patterns of genetic variation among invasive populations in mean time to first flower and size at flowering and a genetic correlation between these traits (Colautti et al. 2010*a*). These findings were consistent with the predictions of a model of local adaptation and genetic constraint on the evolution of time to first flower (fig. 1). However, differences in phenotypic plasticity of these traits among populations could complicate the interpretation of our previous results. For example, strong population × environment interactions between field and glasshouse conditions, or among growing years, could significantly alter model predictions of local adaptation. Moreover, a key prediction of the FTS model—that selection for earlier time to first flower and larger size results from stabilizing selection on the first principal component (PC1) of these two traits—was not tested by Colautti et al. (2010*a*).

In this study we measured selection on plants collected from 13 populations of *L. salicaria* representing a latitudinal gradient in eastern North America. We found significant differences in time to first flower and size between glasshouse and field environments and among years, indicating significant phenotypic plasticity for these traits. However, the ranking of population means were consistent among years (fig. 2) and environments (fig. 3), indicating relatively low population × environment interactions. This result supports the FTS model assumption that genetic differences among populations in time to first flower and vegetative size are maintained despite plasticity in growth and phenology. We also found evidence for stabilizing selection on the first principal component of time to first flower and size (PC1), as predicted by the FTS model. However, contrary to our expectations, we found significant genetic variation for PC2, despite evidence for directional selection for plants that flowered earlier at a large size

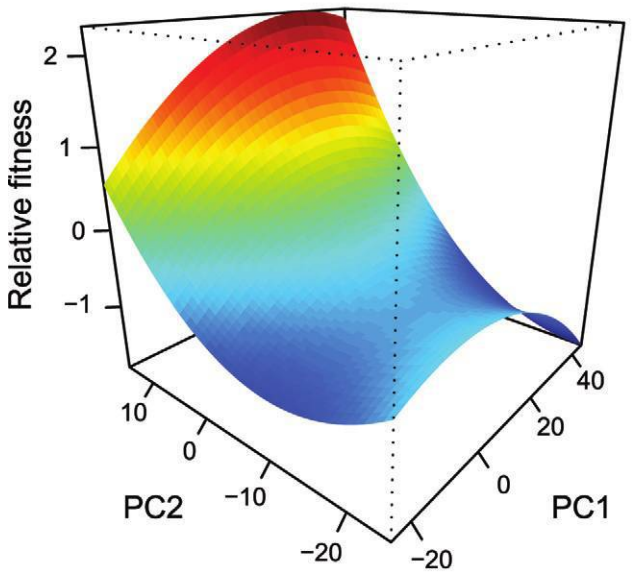

Fig. 5 Fitness surface estimated by ASTER from the Koffler Scientific Reserve field experiment on *Lythrum salicaria*. Relative fitness (*Z*-axis) is a composite measure of survival over 3 years and reproduction over 2 years and was modeled with respect to $PC1_{avg}$, a composite measure of the genetic constraint on time to first flower and size at flowering across 3 years, or $PC2_{avg}$, the orthogonal vector to $PC1_{avg}$ representing variation against the genetic constraint on time to first flower and size at flowering.

Table 4

Selection Gradients for the Second Principal Component (PC2) of Time to First Flower and Vegetative Size in *Lythrum salicaria*, 2-yr Field Experiment, Koffler Scientific Reserve

Parameter	Estimate	χ^2	P
Phenotypes:			
PC2	.765	203.5	<.001
PC2 × PC2	.183	2.61	.106
Year	−.539	46.69	<0.001
Year × PC2	−.339	1.79	0181
Year × PC2 × PC2	−.077	0.56	.455
Seed family means:			
PC2	.712	67.9	<.001
PC2 × PC2	.164	1.14	.285
Year	−.465	13.6	<.001
Year × PC2	−.367	3.03	.082
Year × PC2 × PC2	−.046	1.78	.182

Note. We calculated estimates of linear and nonlinear selection coefficients from standard linear and nonlinear selection models following Lande and Arnold (1983), with significance tested by generalized linear models. Estimates of season effects show the difference in the 2007 growing season relative to 2008.

(i.e., large PC2 values). This result was unexpected because the FTS model predicts that selection should favor these genotypes regardless of growing-season length. Below we discuss our findings and their relevance for understanding the evolution of reproductive phenology in invasive species.

Phenotypic Plasticity and Population Divergence

Phenotypic plasticity may play an important role in biological invasions by allowing species to survive and reproduce under diverse environmental conditions (Baker 1965; Williams et al. 1995; Parker et al. 2003; Ross et al. 2009). Previous studies of *L. salicaria* identified phenotypic plasticity in growth and reproduction in response to different levels of soil nutrients and moisture (Mal et al. 1992; Mal and Lovett-Doust 2005; Chun et al. 2007). Consistent with the results of these studies, we found large differences in plant growth between field and glasshouse environments and among years. First, population mean vegetative size in the field ranged from 71.3 cm to 151.4 cm, an increase of 30.0–59.9 cm compared with the same populations grown under glasshouse conditions (fig. 3). Mean vegetative size also differed significantly among years (table 1), with plants generally growing larger with age (fig. 2). Second, growth in the glasshouse experiment was largely restricted to vertical extension of the primary stem, while plants grown in the field experiment produced large secondary branches in addition to growing taller. These contrasting patterns of development probably result from different growing conditions and age: plants in the glasshouse experiment were grown in 10-cm-diameter pots with minimal fertilizer for a single growing season, whereas plants in the field experiment were grown over 4 years in a freshwater marsh.

Local adaptation is predicted to evolve when phenotypic plasticity is too costly or when it poorly tracks environmental conditions (Via and Lande 1985; Levin 1988; Scheiner 1993; Tufto 2000; Sultan and Spencer 2002). Plasticity and the concept of the "general purpose genotype" (sensu Baker 1965; see also Parker et al. 2003; Richards et al. 2006; Maron et al. 2007; Hulme 2008; Ross et al. 2009) are often contrasted with local adaptation as competing explanations for the success of invasive species. However, some aspects of plant growth exhibit plasticity to environmental factors that vary over small spatial and temporal scales (e.g., changes in moisture or nutrients), whereas other features of life history become locally adapted to climatic factors that vary in a predictable manner over much larger spatial scales (e.g., photoperiod, season length). We found evidence for both of these responses. Significant plasticity in vegetative size was evident among growing conditions, but there was also maintenance of the rank order of mean time to first flower and size at the population level, as predicted by local adaptation to latitudinal gradients in climate. Thus, *L. salicaria* has likely benefited from rapid evolution in the timing of flowering in response to climatic differences, while maintaining plastic growth in response to nutrient and moisture levels that vary over more restricted spatial scales.

Natural Selection on Time to First Flower and Vegetative Size

We measured selection on PC1 and PC2 using the FTS model to generate a predicted fitness surface for time to first flower and size (fig. 1). We found significant stabilizing selection on PC1 (table 3; fig. 4) and directional selection with an accelerating fitness function for PC2 (table 4; fig. 4). Our analysis differs from previous studies of selection in *L. salicaria* (O'Neil 1997, 1999), and this could lead to different conclusions about the strength and form of selection on vegetative size. First, these earlier studies used only a single population, while we included 13 populations sampled along a latitudinal gradient. Our sampling undoubtedly increased the genetic variance for time to first flower and vegetative size, improving statistical power to measure selection gradients. Second, we used PCA to estimate selection separately on two uncorrelated principal component vectors rather than estimating selection simultaneously on two highly correlated traits in a single model. We used this approach because we were interested in testing the FTS model, which made a priori predictions about the form of selection on PC1 and PC2. Thus, we avoided estimating partial correlation coefficients on highly correlated traits and included fewer parameters in our selection models, thereby reducing statistical error of the estimated linear and quadratic selection coefficients for each trait.

The comparison of our analysis with previous estimates of selection in introduced populations of *L. salicaria* reveals how differences in experimental design and analysis of natural selection can alter the significance and slope of selection coefficients, resulting in different conclusions (reviewed in Lande and Arnold 1983; Mitchell-Olds and Shaw 1987; Schluter 1988). This reinforces the value of having a theoretical framework that provides testable a priori predictions about the form of selection, such as those arising from our FTS model. Conclusions about the shape of the fitness function may have been quite different if we had analyzed a number of life-history traits and inferred selection post hoc.

Standing Genetic Variance for Correlated Traits

The extent to which invasive species evolve in response to selection in novel environments will depend partly on the availability of standing genetic variation within populations for ecologically relevant traits and constraints imposed by genetic correlations among them (Fisher 1930; Lande 1979; Maynard Smith et al. 1985; Conner 2002; Lee 2002; Roff and Fairbairn 2007; Gomulkiewicz et al. 2010). Standing genetic variation within populations of *L. salicaria* for PC1 declines significantly with latitude in both glasshouse and field experiments, as predicted by increasing stabilizing selection with latitude (Colautti et al. 2010*a*). Population means for PC1 also correlate negatively with latitude, as northern populations flower earlier at a smaller size compared with southern populations, consistent with an adaptive response to latitudinal changes in growing-season length. Thus, significant population divergence and standing genetic variation for PC1 (table 3) within the 13 populations examined in this study was not unexpected.

In contrast to PC1, the FTS model does not predict significant population divergence and genetic variation for PC2 within the populations investigated here. Instead, the model predicts (i) that higher values of PC2 (i.e., plants that flower early at a large size) are limited by the strong positive genetic correlation between time to first flower and size (fig. 1) and (ii) that selection will favor genotypes with high PC2 values at all sites, regardless of season length. Below we consider a number of potential explanations for our finding of standing genetic variation for PC2 in this study.

The existence of standing genetic variation for traits under strong directional selection is a long-standing paradox in evolutionary biology (e.g., Bulmer 1971; Barton and Turelli 1987; Rowe and Houle 1996; Turelli and Barton 2004; Zhang et al. 2004) that has yet to be clearly resolved (Johnson and Barton 2005). Theoretical models suggest two possibilities. First, selection from unmeasured factors may vary over time and equalize the fitness surface. For example, if herbivores of *L. salicaria* preferentially attacked larger plants that flower earlier, then the genotypes with the highest PC2 values could potentially have lowest fitness in the presence of herbivores. Only direct measurements of selection in the presence of herbivory could assess this possibility. Selection on components of reproductive fitness did not change significantly on PC2 between the 2007 and 2008 growing seasons, suggesting a relatively constant fitness surface over these years.

Another explanation for the presence of standing genetic variation for PC2 is that selection has not been strong enough to eliminate alleles causing smaller plants that flower later. This seems plausible because seed families with relatively low fitness dominated the genetic variance for PC2. Indeed, only 10.2% of seed families had relative fitness >2 (fig. 4), and most of the variance in fitness was among seed families with intermediate PC1 values (fig. 4). Studies of *L. salicaria* populations have consistently identified strong self-incompatibility, and in eastern North America this trait does not appear to change with latitude (reviewed in Colautti et al. 2010*b*). As a result of obligate outbreeding, it is likely that *L. salicaria* populations harbor significant genetic loads composed of detrimental alleles. Indeed, a study of inbreeding depression in *L. salicaria* provides some support for this (O'Neil 1994). Most mutations are detrimental with respect to fitness (e.g., Caballero et al. 1991; Mackay et al. 1994; Lynch et al. 1999; Eyre-Walker and Keightley 2007), and selection against detrimental alleles may be weaker in invasive species because detrimental mutations arising early in the invasion process have a higher probability of fixation (Edmonds et al. 2004; Miller 2010) and because large expanding populations limit opportunities to purge detrimental alleles (Lynch et al. 1999; Byers and Waller 1999). Weak selection against detrimental alleles should produce a distribution of genotypes that is skewed with a disproportionate number of genotypes of relatively low fitness, as we observed (fig. 4).

In our study the majority of seed families had moderate to low values of PC2 and relatively low fitness (fig. 4). This asymmetry in genetic variation for PC2 is similar to the high level of genetic variation identified in low-quality mates in a recent study of sexual selection in *Drosophila* (McGuigan and Blows 2010) and is consistent with theoretical predictions based on mutation bias toward detrimental effects in sexual populations (Hill and Caballero 1992; Falconer and Mackay 1996). An asymmetry in patterns of genetic variation for fitness could have important implications for predicting the direction and speed of evolution during biological invasion. For example, our results indicate that natural selection strongly favors large plants that flower early (i.e., high PC2 values). According to the multivariate breeder's equation, an evolutionary response to selection should occur, albeit slower than in the direction of PC1 because significant genetic variance exists for PC2 (Falconer and Mackay 1996; Lynch and Walsh 1998). However, the FTS model of constraint predicts no response to selection because the trade-off prevents plants from simultaneously flowering earlier and also growing larger. This apparent discrepancy can be explained because most of the genetic variation for PC2 we detected is in the direction of lower fitness. Thus, a response to selection could occur quickly for smaller plants that flower later but not for large plants that flower earlier.

Understanding the causes of genetic variation in PC2 despite directional selection requires further study. Because PC2 had a strong influence on reproductive fitness, it could have important implications for the evolution and spread of invasive populations of *L. salicaria*. For example, the mean fitness of the top 5% of seed families was three to five times greater than the overall mean. Thus, the 95% of seed families that flowered later at a smaller size had relatively low fitness and would be expected to reduce the per capita reproductive output of a population below what it would be in the absence of these genotypes. An analysis of additional life-history traits could help to determine whether other traits constrain the evolution of PC2.

Conclusions

Biological invaders may evolve rapidly in response to local environmental conditions, but this depends on the strength of natural selection, the amount of standing genetic variation in populations, and the extent of genetic correlations among fitness traits. Our results demonstrate that time to first flower and size at reproduction are under stabilizing selection in *L.*

salicaria and that populations respond similarly to variation in growing conditions, maintaining similar rankings of time to first flower and size despite plasticity in growth. This result supports the assumptions of a previously published model (Colautti et al. 2010*a*) concerning genetic constraints on the evolution of local adaptation in *L. salicaria*. Our results also suggest that selection has not been sufficiently strong to eliminate genotypes that flower later at a smaller size. The presence of "low-quality genotypes" in an outbreeding species such as *L. salicaria* is likely to reduce the per capita reproductive output of populations, with consequences for future invasive spread.

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