Supplementary Materials for

Rapid Adaptation to Climate Facilitates Range Expansion of an Invasive Plant

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Materials and Methods

Seed origins, invasion history and common garden design

We established common garden locations in eastern North America at three points along a ~1,000km north-south transect covering ~10° of latitude (Figure S1). Sites were chosen to represent the complete range of growing season lengths experienced by populations of *Lythrum salicaria* in this part of its range *(4, 5, 9, 10, 23)*. The 'southern' site was located at the University of Virginia's Blandy Experimental Farm (39.06°N; hereafter 'BEF') near Boyce, Virginia, U.S.A (http://www.virginia.edu/blandy). The 'mid-latitude' site was the University of Toronto's Koffler Scientific Reserve at Jokers Hill (44.03°N; hereafter 'KSR') near Newmarket, Ontario, Canada (http://www.ksr.utoronto.ca). The 'northern' site was located on private property in the city of Timmins, Ontario (48.47°N; hereafter 'Timmins').

North American populations show considerable genetic diversity at both neutral marker loci *(14, 15)* and quantitative traits *(5, 15)*, consistent with multiple introductions from diverse source regions. Genetic differences among introduced populations could have evolved after colonization of N. America, or alternatively could have established initially from parallel introductions of divergent native genotypes into distinct geographic regions of N. America. In the latter case, multilocus genotypes from N. America should be genetically more similar to European source regions than to other regions of N. America, but this is not the case as introduced genotypes form a single genetic cluster (see Figs. 2 in both *14, 15*). Moreover, quantitative trait variation among population means is continuous, with many traits forming latitudinal clines *(4, 5)* whereas multiple parallel introductions of distinct genetic demes would have resulted in geographical clustering of genotypes. Reconstruction of the invasion history of *L. salicaria* from herbarium and historical records shows initial establishment at ports in New York and Maryland during the mid 18th century and spreading northwest into shorter growing seasons of central and northern Ontario after the 1940s (see Figs 5-8 in *6*). Genetic markers, quantitative trait variation, and historical records therefore do not support the establishment and maintenance of pre-adapted lineages introduced to N. America from different climatic regions of Europe. Rather, genetic differentiation of northern populations from southern sources occurred following northward migration within the last half-century.

To test for local adaptation and evaluate its ecological consequence we used three pairs of populations previously sampled along a latitudinal gradient in eastern N. America, as described in *(9)* and illustrated in Figure S1. The two 'northern' populations were sampled near Echo Bay, Ontario toward the eastern tip of Lake Superior (46.43°N, 84.09°W) and Timmins, Ontario (48.48°N, 81.30°W). The two populations selected from 'mid latitude' were sampled near Ellisberg, New York on the eastern edge of Lake Ontario (43.69°N, 76.19°W) and at the southern edge of Algonquin Provincial Park near Whitney, Ontario (45.49°N, 78.24°W). Finally, the two 'southern' populations were sampled near Easton, Maryland (38.75°N, 75.99°W) and Princeton, New Jersey (40.34°N, 74.65°W).

We used seed families collected from the field because our previous studies indicate that maternal effects do not significantly influence long-term growth and phenology in these populations. Seed provisioning is extremely low in *L. salicaria* as seeds are tiny

(~1mm × 2mm) and lack endosperm *(6)*. Moreover, earlier work on the same populations used in our study failed to detect significant effects of seed mass or germination time on days to flower and size at flowering in the first year of reproduction *(4, 9)*. In contrast, rearing populations in a common glasshouse environment to remove maternal influences would have subjected them to bottlenecks and selection for artificial growing conditions.

In June and July 2007 soil at each site was tilled and covered with weed-blocking fabric punctured by 10cm diameter holes for positioning of each seedling of *L. salicaria* at a spacing of 50cm. Two seedlings from each of seventeen families from each of the six populations were randomly assigned to one of the holes. Seeds were germinated in 2cm × 2cm plug trays and staggered by two weeks at each site to coincide with differences in the start of the growing season at each transplant garden. Thus, seeds were planted at the University of Virginia's Blandy glasshouses on May 21 for the southern site and at the University of Toronto's glasshouses on June 4th and June 18th for mid- and northern-latitude sites, respectively. Four weeks after sowing, seedlings were moved outside under shade cloth for four days to harden-off before transplanting into each site.

Reciprocal transplant measurements and analysis

All relevant measurement data (*S1_RecipTransInput.csv*), as well as statistical models and programming code for R *(24)* used in this study and described below are available through the DRYAD database (DOI:LINK).

At the end of the 2007 growing season, we harvested all above-ground structures, dried them to a constant weight in an oven at 35^{o}C, and measured the biomass of all infructescences on each plant. We additionally counted total fruit number for each reproductive individual at both the BEF and KSR sites, but there were no fruits in plants at the Timmins site in 2007. Sepals from viable fruits remain firmly attached to inflorescence stems even after fruits dehisce and seeds disperse, whereas unfertilized flowers are caducous within 48 hours of opening, leaving circular scared receptacles along the stem. As a result, the majority of inflorescence biomass can be attributed to seeds and attached sepals, with a smaller contribution of stem weight. We tested the relationship between infructescence biomass and fruit number using linear models (lm) in R (see *S2_DataPrep.R*).

Infructescence biomass was a highly significant predictor of fruit number at both BEF ($P < 0.001$, $R^2 = 0.952$) and KSR ($P < 0.001$, $R^2 = 0.957$) (Figure S2). The coefficients from these models were used to estimate total fruit set of all reproductive individuals in 2008-2010. We used the KSR model as an approximation for the Timmins site where plants did not produce fruit in the first year. We did this because the longer growing season and drier conditions at BEF resulted in more dehisced fruits and thus a steeper regression slope (i.e. 50.7 vs. 26.6 seeds/g), whereas the Timmins site was both wetter and had a shorter growing season than BEF or KSR, and therefore lost fewer seeds prior to harvest. Importantly, the regression model chosen had no influence on the test for local adaptation, or our estimate of the fitness surface at each site, as these depend on the relative infructescence biomass within each site.

We measured fitness as total reproduction over the duration of the experiment (2007-2010); however, results were similar for reproductive fitness in each year, as these measurements were highly correlated (07-08 $R = 0.808$; 08-09 $R = 0.852$; 09-10 $R = 0.867$).

We used a generalized linear model (see *S3_LocalAdaptTest.R*) to test the effect of common garden site (north, intermediate, or south), population origin (early, intermediate, or late) and the site*origin interaction, with population as a random effect and Poisson error distribution, which is more appropriate for count data in which variance typically scales with the mean. We also allowed for separate among- and within-population variances for each common garden location. We found a highly significant site*origin interaction ($P < 0.001$), indicating that the difference in fitness between each pair of populations depended on the common garden location. Such differences could result from crossing reaction norms, as predicted by local adaptation, but could alternatively result from oblique reaction norms that do not intersect and therefore are not definitive evidence for local adaptation. To test these alternative scenarios, we used a bootstrap model to examine differences between population pairs at each of the common garden sites.

Our bootstrap model (see *S4_Bootstrap.R*) tested the statistical significance of differences among the three pairs of populations at each reciprocal transplant site, and of the same pairs across sites. In each of 10,000 iterations of the model, individuals were resampled, with replacement, from each pair of populations and the mean was recorded. Differences between any two population pairs were considered significant ($P < 0.05$) if there was no overlap in the 95% confidence intervals (CI) of their bootstrap distributions (i.e. a two-tailed test).

Phenotypic selection model

We modeled latitudinal changes in the adaptive landscape as changes in the Gaussian function:

$$W_{i,j} = \bar{W}_j * \exp\left[\frac{-\left(z_{i,j} - \theta_j\right)^2}{2{\sigma_j}^2}\right]$$

In this model the phenotype (z) of the ith individual in the jth population is a point along the primary axis of covariance between flowering time and size (PC1), scaled such that negative or positive values indicate phenotypes that flower early at a small size or later at a larger size, respectively (see *S5_SelectionModel.R*). The absolute fitness of an individual (W_i) with phenotype z_i is determined by its distance from optimum flowering time/size phenotype at a particular latitude (θ_j) and decreases at a rate proportional to the strength of stabilizing selection in that population ($1/\sigma_j^2$). The mean fitness of an individual with the optimum phenotype ($z_{i,j} = \theta_j$) is defined by $\bar{W}_j$, and is limited by the trade-off between flowering time and size. That is, plants that flower early are constrained to be smaller, which reduces reproductive output *(4, 10)*, and this is modeled as a reduction in $\bar{W}_j$.

Differences in parameters θ, σ and $\bar{W}$ define latitudinal changes in the adaptive landscape, which we parameterized for each of the three common garden sites. We first estimated the optimum (θ) flowering time-size combination at each site from a non-linear regression model of PC1 vs. latitude in a common garden experiment of 20 populations spanning the latitudinal range of our common garden sites *(4)*. We then estimated σ^2 as the amount of standing genetic variation for PC1 within populations from latitudinal

regressions in the same study *(4)*. Finally, we estimated $\overline{W}_j$ from linear regressions of seed production with latitude measured in a field survey of natural populations (9). This approach assumes that populations are at selection-migration equilibrium, but should be robust to non-equilibrium scenarios in which stochastic processes (e.g. founder effects) push the mean and variance of PC1 of populations away from the optimum but do not significantly affect the latitudinal regression slopes.

Phenotypic selection analysis

We measured phenotypic selection along the first principal component axis (PC1) of days to first flower and vegetative size at maturity using both *aster* models (*S6_aster_Analysis.R*) *(25, 26)* and fitness splines (*S7_Selection_Splines.R*) *(27)*. We chose *aster* over conventional Lande-Arnold least-squares models of phenotypic selection *(28)* because *aster* explicitly allows for fitness components with different error distributions and thereby improves estimates of the quadratic terms that define the fitness surface curvature *(29)*. Fitness splines allow estimation of more complex fitness surfaces, such as the Gaussian function in our fitness model above, which are not well-described by quadratic functions *(27)*.

We focused on phenotypic selection models because genotypic selection, estimated as the covariance between breeding values for PC1 and fitness *(30–32)*, is insufficient to characterize the complex changes in stabilizing selection along PC1 that we modeled (Figure 1B). Genotypic estimates are often desirable because correlations between phenotypic traits and fitness arising from environmental influences can bias estimates of selection gradients *(33, 34)*. However, this is unlikely in our data because measurements of selection on flowering time and size at the KSR site in a previous study found that selection gradients estimated on individuals and family means were highly concordant *(23)*.

Our composite measure of fitness in the aster analysis included data from 2007-2010 for year-specific survival and reproductive success (binary), and number of fruits given successful reproduction (Poisson). Survival was grouped with reproductive success into a single response variable, because most plants that survived also reproduced, resulting in high collinearity between survival and reproductive probabilities. We used a full quadratic model:

$$W = Site * Origin + Site * PC + Site * PC^2 + \epsilon$$

where W is the composite measure of fitness predicted by the factor seed origin (north, south or mid-latitude) and the linear and quadratic effects of PC1 (PC) of flowering time and vegetative size at maturity – each of which was averaged across 2008-2010 and with separate coefficients for each site. We found a significant Site effect (X^2 = 75,626, $P <$ 0.001, *d.f.* = 6) by excluding the term from the model and comparing the log-likelihood score to that of the full model (Eq. 1) using a likelihood ratio test (LRT). This indicated that fitness surfaces differed significantly among common garden sites, so we estimated fitness landscapes using separate models for each site. Using similar stepwise removal and LRTs we confirmed that the full quadratic model was the best fit in each of the three site-specific models, and then used these separate models to graph the fitness surfaces shown in Figure S3.

Estimates of selection along PC1 could be biased if other ecologically important traits (e.g. frost tolerance, growth rate) are in linkage disequilibrium (LD) with PC1 due to population structure. For example, if northern populations evolved frost tolerance and this significantly increased fitness of northern populations at the northern common garden site, then selection analysis of PC1 at this site could inflate fitness estimates of PC1 phenotypes that flower early at a small size. We found that population origin (north, south, or mid-latitude) had a significant effect on fitness in both the full model and in selection analysis at each site separately (all $P < 0.001$), as expected if populations are locally adapted. However, the latitudinal shift in the fitness peak and strength of stabilizing selection of PC1 remained after including an effect of origin in the *aster* model (compare maxima of Timmins vs BEF in Figure S3), indicating that LD due to population structure is not responsible for this shift.

To directly test the Gaussian selection model (Figure 1B), we measured selection along the first principal component axis (PC1) of days to first flower and vegetative size at maturity using the GAM function in R (*S7_Selection_Splines.R*) to fit fitness splines *(27)*. Fitness in this case was the sum of total seed production over the four field seasons, with zero fitness for non-reproductive individuals. In contrast to *aster* and Lande-Arnold selection analysis, the GAM function is able to fit complex fitness surfaces, providing a better approximation of the Gaussian surface used in our predictive model. Results of the analysis are shown in Figure 1C.

Effect size comparison

We compared the effects on reproductive fitness observed in our reciprocal transplant experiment in each year (2008-2010) with analogous effects of enemy release (ERH) and evolution of increased competitive ability (EICA) in previous meta-analyses *(21, 22)*. We additionally identified two ERH studies that measured effects of natural enemies *(18, 19)*, and one EICA study that compared native and introduced populations *(20)* grown in natural field conditions at two different sites in two different years (1994: Ithaca, NY and 1995: Silwood Park, London, UK). Each year of study was graphed separately to examine variability in effect size between location and year. Means and standard errors of fitness effects were measured as the number of pixels in digitized bar graphs, relative to the axis scale, analyzed with the software package ImageJ *(35)*. This was true for all studies except one meta-analysis *(22)* for which we are grateful to the lead author, Gary Clewley, who provided effect sizes of biocontrol on plant reproductive fitness.

For comparison with our study, we only included measurements of reproductive fitness, which were measured as inflorescence biomass in all studies of *L. salicaria*. Effect sizes of herbivory were calculated as the log-response ratio:

$$LnE = ln\left(\frac{X_E}{X_H}\right)$$

where X is the mean seed production (or infructescence biomass) in treatments excluding (E) or including (H) herbivores. The standard error of this effect size was calculated as the weighted pooled standard deviation (Sp) of each treatment effect, following Borenstein et al. *(36)*:

$$SE = \sqrt{S_p^2 \frac{1}{n_E X_E^2} + \frac{1}{n_H X_H^2}}$$

where n is the number of individuals and S_p^2 is calculated as:

$$\frac{(n_E - 1)S_E^2 + (n_H - 1)S_H^2}{n_E + n_H - 2}$$

Finally, we multiplied SE by 1.96 to calculate approximate 95% C.I. of effect sizes. The log-response ratios for the EICA studies were calculated similarly, but from population means sampled from the introduced or native ranges, rather than individual plants.

We compared these effect sizes (*S8_EffectSizeData.csv*) with fitness differences at the northern and southern common garden sites in each year from 2008-2010 (*S8_EffectSizeYear.R*). Separate bootstrap models were used to generate mean and nonparametric standard errors of the effect of local adaptation (Timmins site), and the fitness cost of reproducing at a small size (BEF site). In each of 10,000 iterations of each bootstrap model, individuals from the northern and southern regions were resampled, with replacement, and the average of each region was used to calculate the log-response ratio.

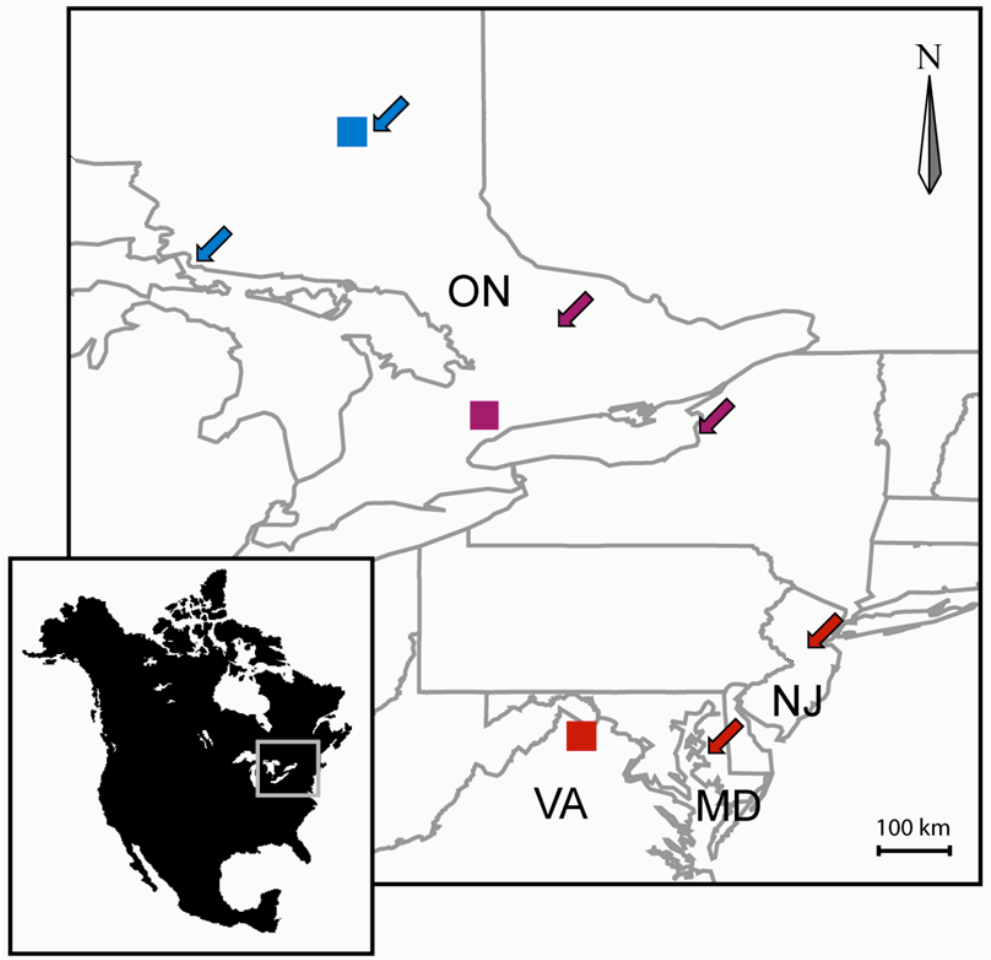

Fig. S1.

Map of eastern North America showing the three common garden locations (squares), and the location of populations of *Lythrum salicaria* used to test for local adaptation (arrows) (ON – Ontario, NJ – New Jersey, MD – Maryland, VA – Virginia).

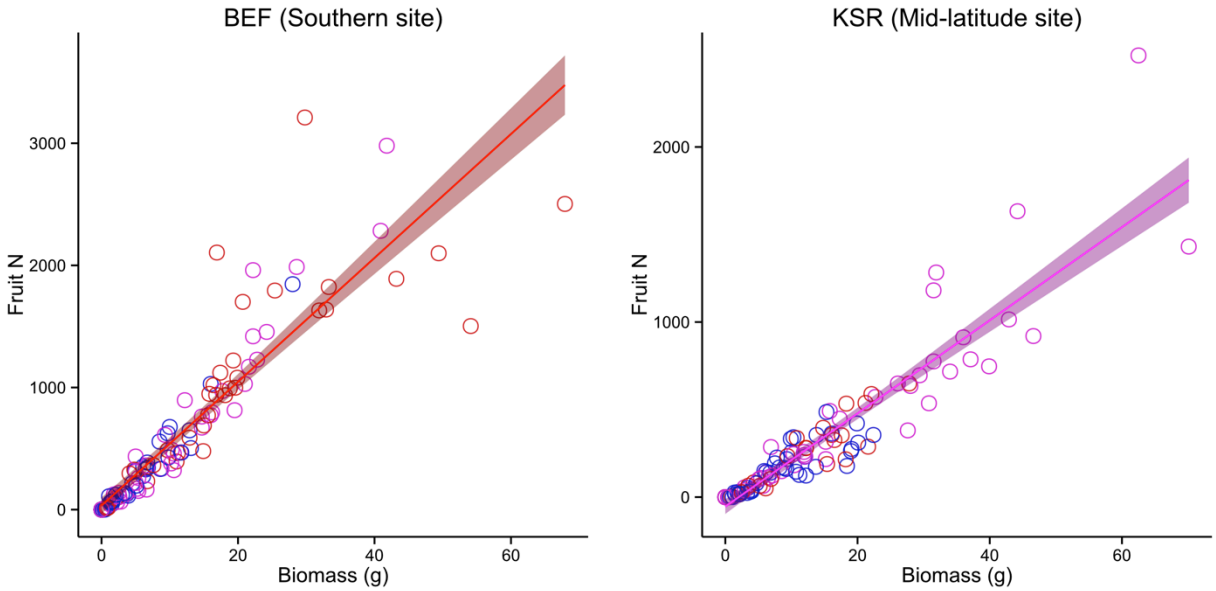

Fig. S2

The relations between fruit number and dry biomass of infructescences of six field-collected populations of *Lythrum salicaria* from southern (red), mid-latitude (purple) and northern sites (blue). Shaded areas show 95% confidence region of regression lines.

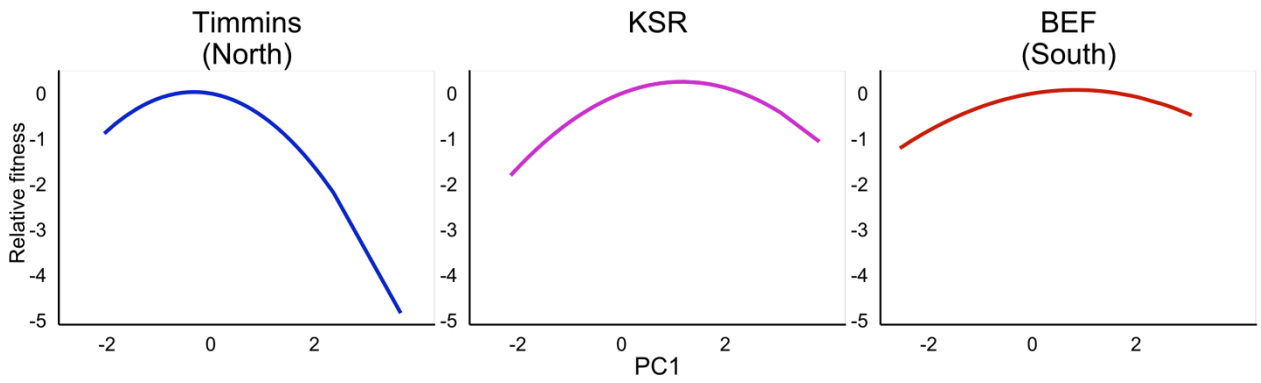

Fig. S3

The relations between fruit number and dry biomass of infructescences of six field-collected populations of *Lythrum salicaria* from southern (red), mid-latitude (purple) and northern sites (blue). Shaded areas show 95% confidence region of regression lines.

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